Lecture (4)

AB Structure–function properties of exotoxins: -

Most bacterial exotoxins possess AB structure–function properties. Exotoxins are organized into one of several general types of AB organization.

The AB5 exotoxins are composed of six proteins that are noncovalently associated as an oligomer. Cholera toxin is the prototype for the AB5 exotoxin. The A domain of cholera toxin constitutes the ADP-ribosyltransferase domain, whereas the B5 domain is composed of five identical proteins, forming a pentamer. This is organized into a ring structure, on which the A domain is positioned. The five proteins that make up the B domain may be identical, as is the case for cholera toxin and the heat-labile enterotoxin of E. coli, or may be different proteins that form a nonsymmetrical ring structure, as observed with the B oligomer of pertussis toxin.

The third class of AB exotoxin is composed of proteins that are not associated in solution, but that do associate following the binding and processing of the B domain to the host cell. C2 toxin is an example of this class of A-B exotoxin. C2 toxin is a bipartile exotoxin composed of a protein that encodes the catalytic A domain and a separate protein that encodes the B domain. The A domain protein of C2 toxin ADPribosylates actin. The B domain protein of C2 binds to sensitive cells and is nicked

by a eukaryotic protease. The processed B components oligomerize and are then capable of binding either of the A domain proteins. A new class of toxin organization has recently been recognized in which the A domain is a protein and the bacterium is directly responsible for its delivery into the cell. The bacterium binds to the eukaryotic cell and uses a type III secretion apparatus to deliver cytotoxins, also called effector proteins, into the intracellular compartment of the cell.

CONVERSION OF EXOTOXINS INTO TOXOIDS: -

A. Chemical detoxification of bacterial exotoxins: -

Shortly after the determination that toxic components were associated with bacterial pathogens, several studies showed that cell extracts or cell cultures of a pathogen could be treated with chemical denaturants, such as formalin, to produce nontoxic immunogenic material that could prevent the disease associated with that pathogen. In the case of diphtheria toxin and tetanus toxin, chemical modification with formalin produced toxoids that were used as acellular vaccines in large-scale immunizations. This resulted in a remarkable decrease in the incidence of both diphtheria and tetanus within the populations that were immunized. In areas where these toxoids are not administered, diphtheria and tetanus remain clinically important diseases. In addition to formalin, other chemicals have been used to detoxify bacterial exotoxins, including glutaraldehyde and hydrogen peroxide. In contrast, the chemical toxoiding of other exotoxins, such as cholera toxin and pertussis toxin, has been more difficult because the treatment of these toxins with denaturants often results in a reduction of immunogenicity. Thus, there is

a need to develop alternative strategies for eliminating the cytotoxicity of certain exotoxins without compromising their immunogenicity.

B. Genetic detoxification of bacterial exotoxins: -

Developments in genetic engineering have provided an opportunity to produce recombinant forms of bacterial exotoxins that possess greatly reduced toxicity, but retain immunogenicity. The use of genetic engineering to develop a toxoid of pertussis toxin has been successful. The whole-cell pertussis vaccine is composed of a chemically treated preparation of Bordetella pertussis, which is effective in the elicitation of a protective immune response after mass immunization. However, the whole-cell pertussis vaccine is acutely reactive when administered to children. Genetically engineered forms of pertussis toxin have been produced that possess essentially no catalytic activity or cytotoxicity, but that maintain native conformation and elicit a protective immune response when used as an immunogen. These recombinant noncytotoxic forms of pertussis toxin have been engineered with multiple mutations in their active site, virtually eliminating the risk of reversion to a cytotoxic form. Similar strategies are being applied to other bacterial exotoxins with the goal of engineering acellular vaccine candidates.

Endotoxins

Gram-negative bacteria have lipopolysaccharide (LPS) in the outer membrane of their cell wall that, under certain circumstances, is toxic to specific hosts. This LPS is called an endotoxin because it is bound to the bacterium and is released when the microorganism lyses. Some is also released during bacterial multiplication. The toxic component of the LPS is the lipid portion, called lipid A. Lipid A is not a single macromolecular structure but appears to be a complex array of lipid residues. The lipid A component exhibits all the properties associated with endotoxicity and gram-negative bacteremia.

Besides the preceding characteristics, bacterial endotoxins are:-

1. Heat stable.
2. Toxic only at high doses (milligram per kilogram amounts).
3. Weakly immunogenic.
4. Generally similar, despite source.
5. Usually capable of producing general systematic effects: fever (are pyrogenic), shock, blood coagulation, weakness, diarrhea, inflammation, intestinal hemorrhage, and fibrinolysis (enzymatic breakdown of fibrin, the major protein component of blood clots).

The characteristics of endotoxins and exotoxins are contrasted in table 1.

The main biological effect of LPS is an indirect one, being mediated by host molecules and systems rather than by LPS directly. For example, endotoxins can initially activate Hageman Factor (blood clotting factor XII), which in turn activates up to four humoral systems: coagulation, complement, fibrinolytic, and kininogen systems.

Gram-negative endotoxins also indirectly induce a fever in the host by causing macrophages to release endogenous pyrogens that reset the hypothalamic thermostat. One important endogenous pyrogen is the cytokine interleukin-1. Other cytokines released by macrophages, such as the tumor necrosis factor, also produce fever.

Recent evidence indicates that LPS affects macrophages and monocytes by binding to special plasma proteins called LPS-binding proteins. The LPS-LPS-binding protein complex then attaches to receptors on monocytes, macrophages, and other cells. This triggers several events, including the production of cytokines IL-1, IL-6, and tumor necrosis factor. As mentioned previously, IL-1 and tumor necrosis factor induce fever. These cytokines also promote other endotoxin effects: complement activation, coagulation, prostaglandin formation, and so forth.

Table (1) Characteristics of Exotoxins and Endotoxins

Characteristic	Exotoxins	Endotoxins
Chemical composition	Protein, often with two components (A and B).	Lipopolysaccharide complex on outer membrane; lipid A portion is toxic.
Disease examples	Botulism, diphtheria, tetanus.	Gram-negative infections, meningococcemia.
Effect on host	Highly variable between different toxins.	Similar for all endotoxins.
Fever	Usually do not produce fever.	Produce fever by release of interleukin-1.
Genetics	Frequently carried by extrachromosomal genes such as plasmids.	Synthesized directly by chromosomal genes.
Heat stability	Most are heat sensitive and inactivated at 60–80°C.	Heat stable.
Immune response	Antitoxins provide host immunity; highly antigenic.	Limited antibodies produced; weakly immunogenic.
Location	Usually excreted outside the living cell.	Part of outer membrane of gram-negative bacteria.
Production	Produced by both gram-positive and gram-negative bacteria	Found only in gram-negative bacteria; Released on bacterial death and some liberated during growth.
Toxicity	Highly toxic and fatal in microgram Quantities.	Moderate toxicity.
Toxoid production	Converted to antigenic, nontoxic toxoids; toxoids are used to immunize (e.g., tetanus toxoid)	Toxoids cannot be made.

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