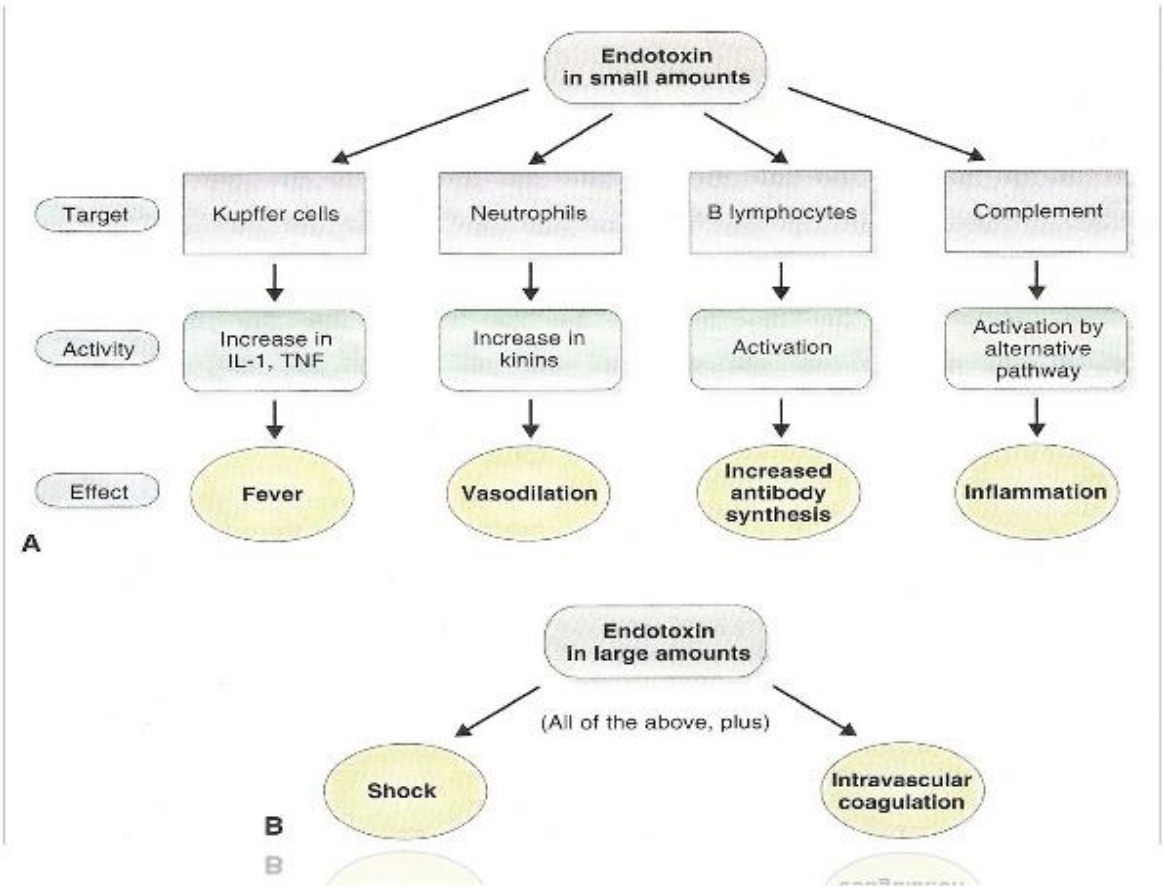
## Microbial toxins

Toxins produced by micro-organisms, including bacteria and fungi. Microbial toxins promote infection and disease by directly damaging host tissues and by disabling the immune system it is **small molecules**, **peptides**, or **proteins** that are capable of causing disease on contact with or absorption by body tissues. Toxin considered first bacterial virulence factors to be identified

**Toxigenesis**, or the ability to produce toxins, is an underlying mechanism by which many bacterial pathogens produce disease.

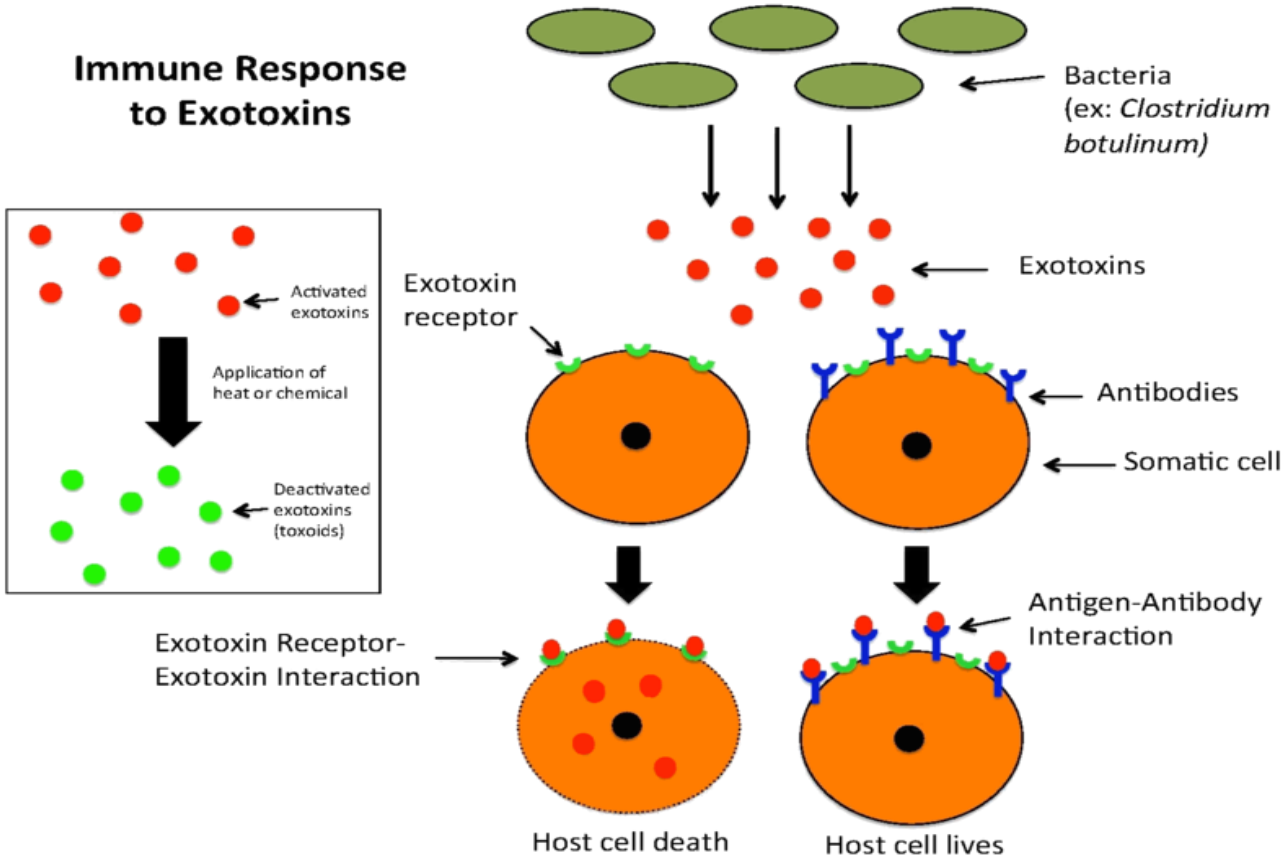
**Toxins are of two types:**

Endotoxins: are cell-associated substances that are structural components of bacteria. Most endotoxins are located in the cell envelope. endotoxin refers specifically to the lipopolysaccharide (LPS) or lipooligosaccharide (LOS) located in the outer membrane of Gram-negative bacteria. Although structural components of cells, soluble endotoxins may be released from growing bacteria or from cells that are lysed as a result of effective host defense mechanisms or by the activities of certain antibiotics.




**Scheme of Immune Response of bacterial endotoxins**

**Exotoxins:** are usually secreted by bacteria and act at a site removed from bacterial growth. However, in some cases, exotoxins are only released by lysis of the bacterial cell. Exotoxins are usually proteins, minimally polypeptides, that act enzymatically or through direct action with host cells and stimulate a variety of host responses. Most exotoxins act at tissue sites remote from the original point of bacterial invasion or growth. However, some bacterial exotoxins act at the site of pathogen colonization and may play a role in invasion.




**Scheme of Immune Response of bacterial exotoxins**

Some of the differences between Exotoxins and Endotoxins

| S.N. | Exotoxins | Endotoxins |
|---|---|---|
| 1 | Excreted by organisms, living cell | Integral part of cell wall |
| 2 | Found in both Gram positive and Gram Negative bacteria | Found mostly in Gram Negative Bacteria |
| 3 | It is polypeptide | It is lipopolysaccharide complex. |
| 4 | Relatively unstable, heat labile (60°C) | Relatively stable, heat tolerant |
| 5 | Highly antigenic | Weakly immunogenic |
| 6 | Toxoids can be madeby treating with formalin | Toxoids cannot be made |
| 7 | Highly toxic, fatal in µg quantities | Moderately toxic |
| 8 | Usually binds to specific receptors | Specific receptors not found |
| 9 | Not pyrogenic usually, Toxin Specific | Fever by induction of interleukin 1 (IL-1) production, S |
| 10 | Located on extrachromosomal genes (e.g. plasmids) | Located on chromosomal genes |
| 11 | Filterable | Not so |
| 12 | It has mostly enzymatic activity | It has no enzymatic activity |

| | | |
|---|---|---|
| 13 | Its molecular weight is 10KDa | Its molecular weight is 50-1000KDa |
| 14 | On boiling it get denatured. | On boiling it cannot be denatured. |
| 15 | Detected by many tests (neutralization, precipitation, etc) | Detected by Limulus lysate assay |
| 16 | Examples: Toxins produced by *Staphylococcus aureus, Bacillus cereus, Streptococcus pyogenes, Bacillus anthrcis*(Alpha-toxin, also known as alpha-hemolysin (Hla)) | Examples:<br>Toxins produced by *E.coli, Salmonella Typhi, Shigella, Vibrio cholera*(Cholera toxin- also known as cholerage |
| 17 | Diseases: Tetanus, diphtheria, botulism | Diseases: Meningococcemia, sepsis by gram negative r |

**Some characters of bacterial toxins:**

**1.** A few bacterial toxins that obviously bring about the death of an animal are known simply as **lethal toxins**, and even though the tissues affected and the target site or substrate may be known, the precise mechanism by which death occurs is not clear (e.g. anthrax LF).

**2.** Some bacterial toxins are utilized as **invasins** because they act locally to promote bacterial invasion. Examples are extracellular enzymes that degrade tissue matrices or fibrin, allowing the bacteria to spread. This includes collagenase, hyaluronidase and streptokinase.

**3.** The pore-forming toxins that insert a pore into eucaryotic membranes are considered as invasins, as well, but they will be reviewed here.

**4.** Some protein toxins have very **specific cytotoxic activity** (i.e., they attack specific types of cells). For example, tetanus and botulinum toxins attack only neurons. But some toxins (as produced by staphylococci, streptococci, clostridia, etc.) have fairly **broad cytotoxic activity** and cause nonspecific death of various types of cells or damage to tissues, eventually resulting in necrosis. Toxins that are phospholipases act in this way. This is also true of pore-forming hemolysins and leukocidins.

**5.** Bacterial protein toxins are strongly **antigenic**. *In vivo,* specific antibody neutralizes the toxicity of these bacterial exotoxins (**antitoxin**). However, *in vitro,* specific antitoxin may not fully inhibit their activity. This suggests that the antigenic determinant of the toxin may be distinct from the active portion of the protein molecule. The degree of neutralization of the active site may depend on the distance from the antigenic site on the molecule. However, since the toxin is fully neutralized *in vivo,* this suggests that other host factors must play a role in toxin neutralization in nature.

**6.** Protein exotoxins are inherently **unstable**. In time they lose their toxic properties but retain their antigenic ones. This was first discovered by Ehrlich who coined the term "toxoid" for this product.

**Toxoids** are detoxified toxins which retain their antigenicity and their immunizing capacity. The formation of toxoids can be accelerated by treating toxins with a variety of reagents including formalin, iodine, pepsin, ascorbic acid, ketones, etc. The mixture is maintained at 37 degrees at pH range 6 to 9 for several weeks.
The resulting toxoids can be used for **artificial immunization** against diseases caused by pathogens where the primary determinant of bacterial virulence is toxin

production. Toxoids are effective immunizing agents against diphtheria and tetanus that are part of the DPT (DTP) vaccine.

**Nomenclature of toxins**

1– Named for host cell attacked:

□□**Neurotoxins , Enterotoxins**

□□**Cytotoxins ( Nephrotoxin ,Hepatotoxin , Cardiotoxin )**

2– Named for producer or disease: cholera, Shiga

3– Named for activity: lecithinase, adenylate cyclase

4-Letter designation: exotoxin A

**A plus B Subunit Arrangement**

Many protein toxins, notably those that act intracellularly (with regard to host cells), consist of two components:

□□one component (**subunit A**) is responsible for the **enzymatic activity** of the toxin

□□the other component (**subunit B**) is concerned with **binding** to a specific receptor on the host cell membrane and transferring the enzyme across the membrane.

□□The enzymatic component is not active until it is released from the native (**A+B)** toxin.

□□Isolated A subunits are enzymatically active but lack binding and cell entry capability.

□□Isolated B subunits may bind to target cells (and even block the binding of the native toxin), but they are nontoxic.

There are a variety of ways that toxin subunits may be synthesized and arranged:

□□**A + B** indicates that the toxin is synthesized and secreted as two separate protein subunits that interact at the target cell surface;

**A-B** or **A-5B** indicates that the A and B subunits are synthesized separately, but associated by noncovalent bonds during secretion and binding to their target

**5B** indicates that the binding domain of the protein is composed of 5 identical subunits.

**A/B** denotes a toxin synthesized as a single polypeptide, divided into A and B domains that may be separated by proteolytic cleavage.

**Attachments and mechanism of toxin entry**

There are at least **two mechanisms of toxin entry into target cells.**

**1- direct entry**, the B subunit of the native (A+B) toxin binds to a specific receptor on the target cell and induces the formation of a pore in the membrane through which the A subunit is transferred into the cell cytoplasm.

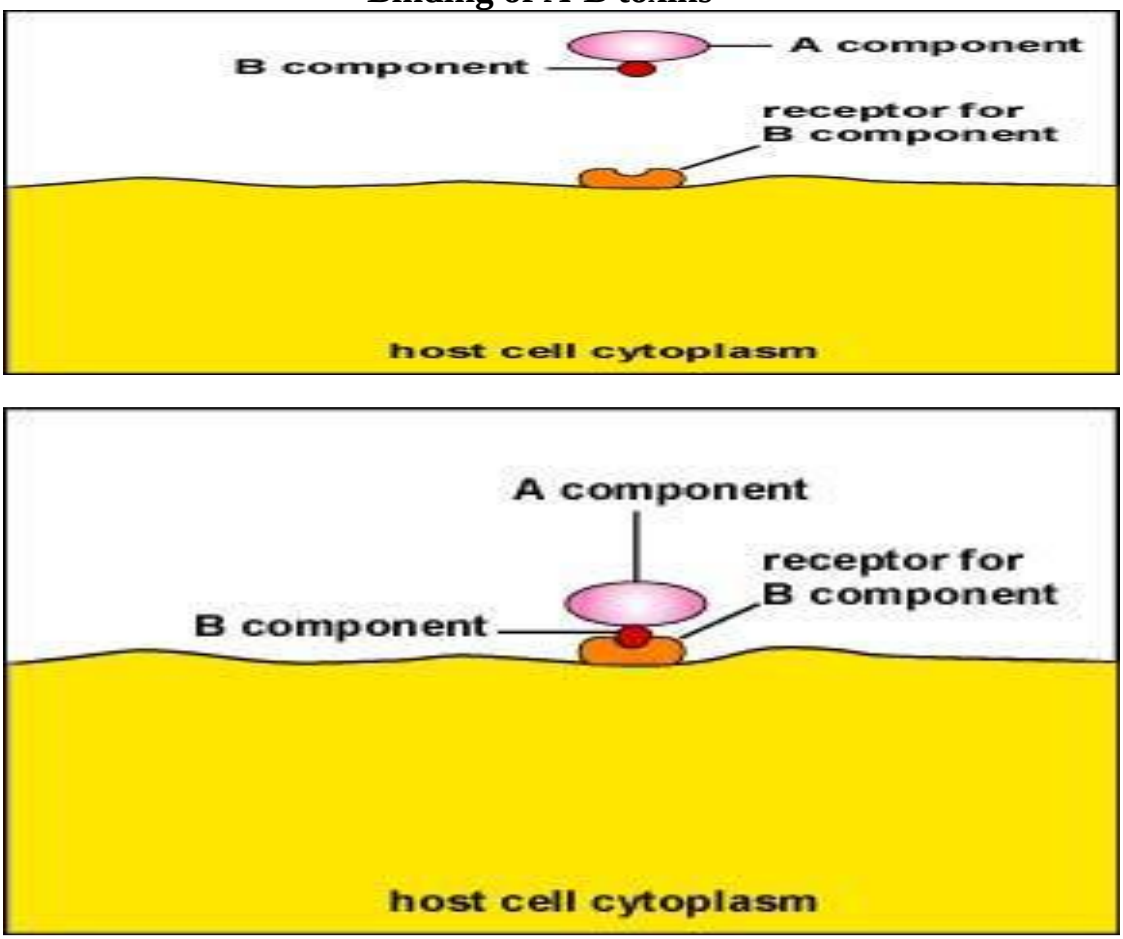
**2- an alternative mechanism**, the native toxin binds to the target cell and the A+B structure is taken into the cell by the process of **receptor-mediated endocytosis** (**RME**).

The toxin is internalized in the cell in a membrane-enclosed vesicle called an **endosome**. H+ ions enter the endosome lowering the internal pH which causes the A+B subunits to separate. The B subunit affects the release of the A subunit from the endosome so that it will reach its target in the cell cytoplasm. The B subunit remains in the endosome and is recycled to the cell surface.

If the B subunit contains a hydrophobic region (of amino acids) that insert into the membrane (as in the case of the diphtheria toxin), it may be referred to as the

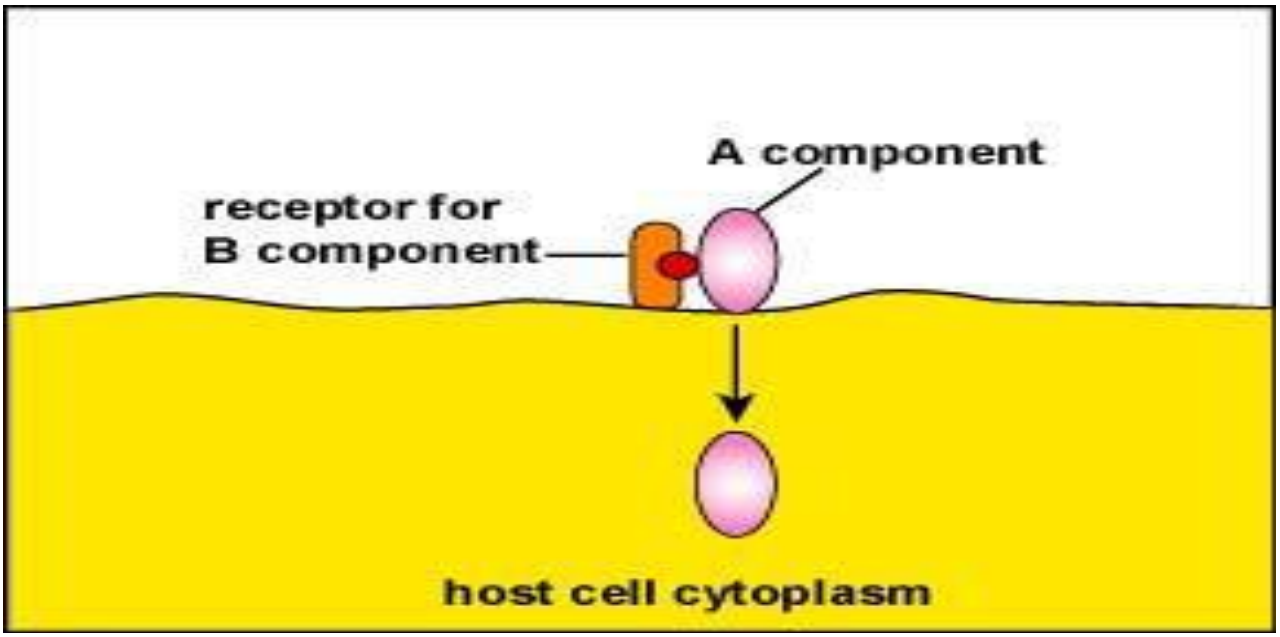
T (translocation) domain of the toxin.

**Binding of A-B toxins**




The B (binding) component of the exotoxin binds to a receptor on the surface of a susceptible host cell.

**Entry of A Component of A-B Toxins by Direct Passage through the Host Cell's Membrane**




**Entry of A-B Toxins by Endocytosis**