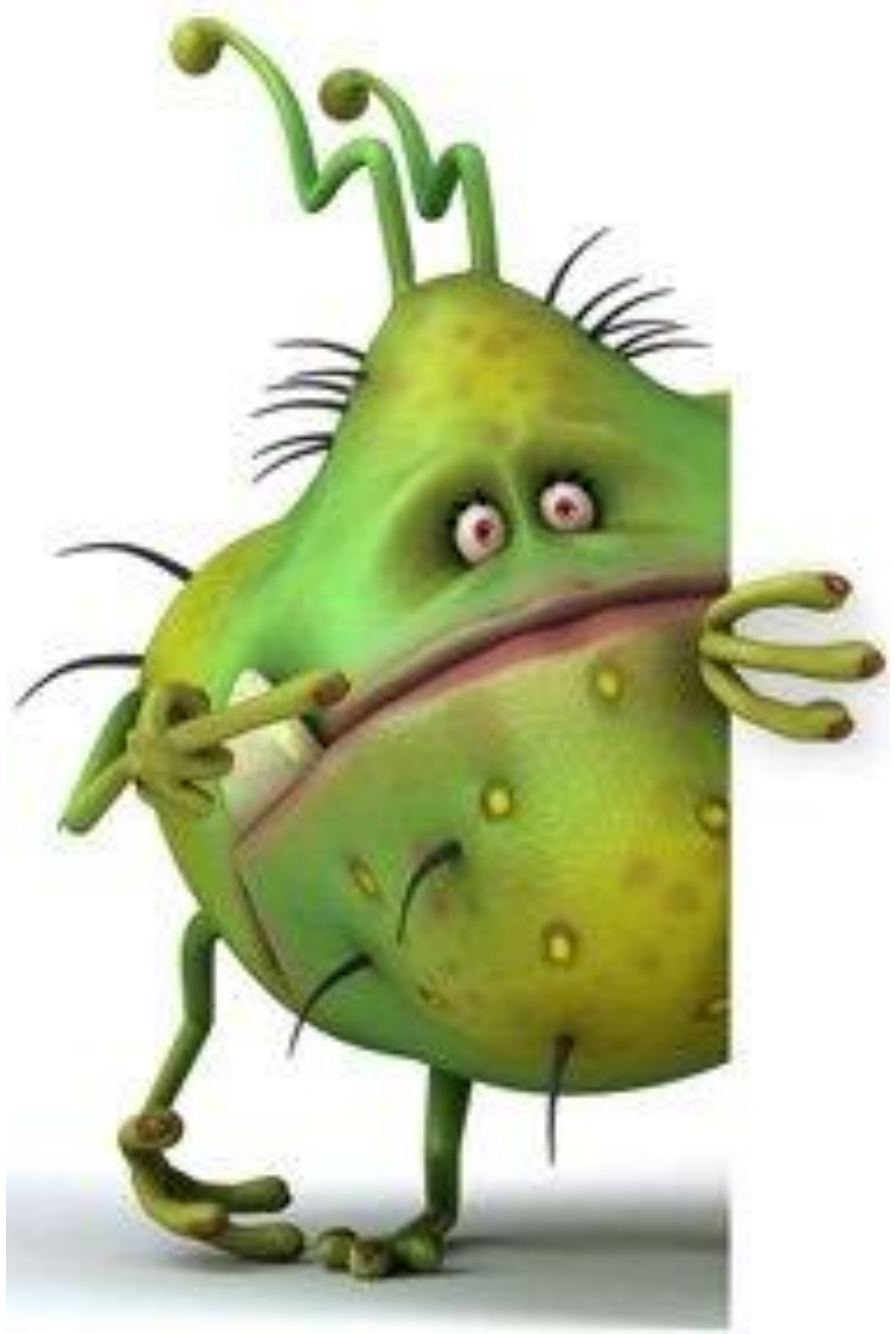

Microbial Toxins (BIOC 422)
Bacterial Toxins (Introduction)

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Bacterial Cell Wall Structure

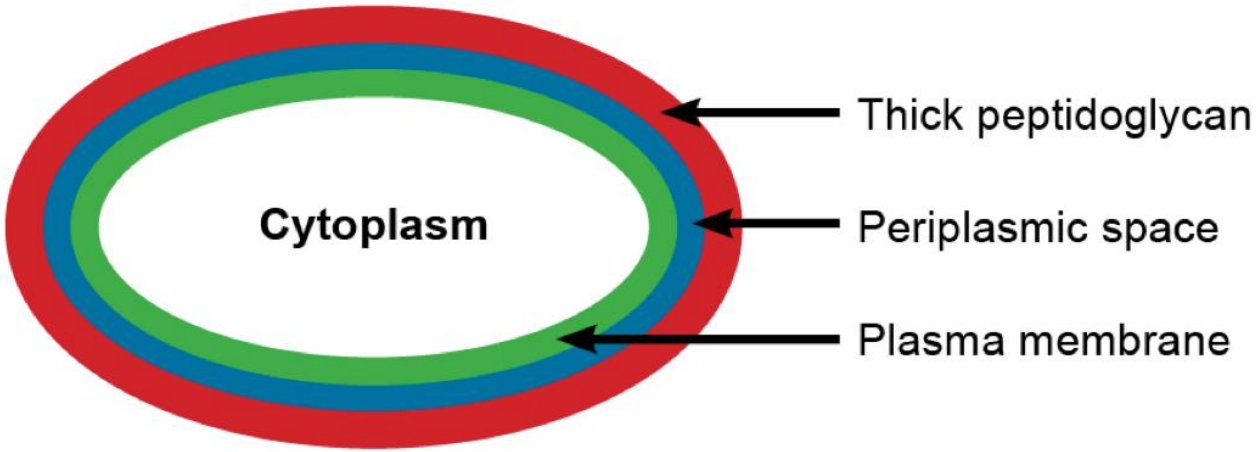
To remember the differences in the cell wall of gram positive & negative organisms - think of a boring, *long powerpoint presentation.*
Long ppt will be your mnemonic guide =D

Lipopolysaccharide
Outer membrane
Negative
Gram?

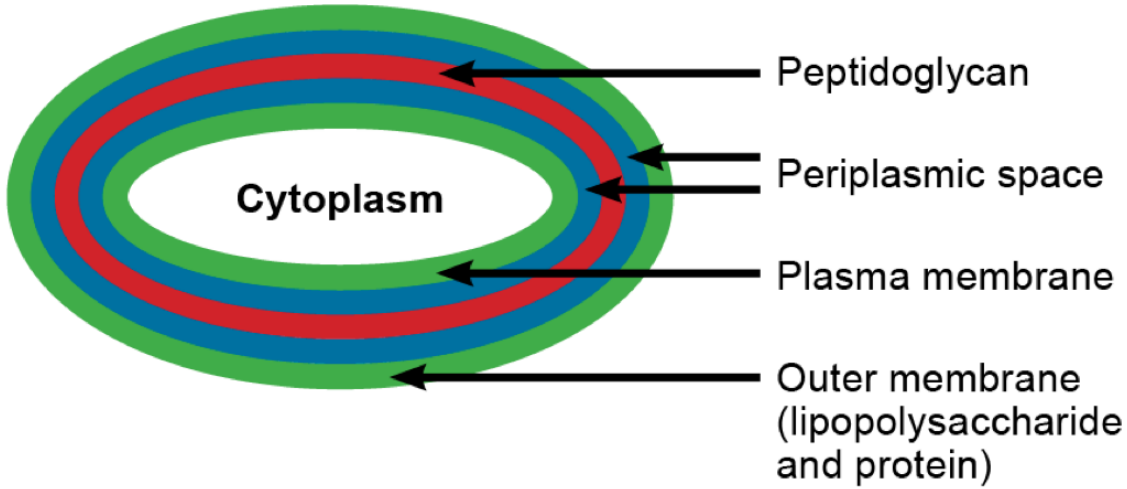
Positive
Peptidoglycan (thick)
Teichoic acid

Character	Gram positive	Gram negative
Number of layers	One	Two
Thickness	Thick(20-80nm)	Thin (8-10 nm)
Outermembrane	Absent	Present
Periplasmic space	Present in some	Present in all
Chemical composition	Petidoglycan,Teichoic acid and lipotechoic acid	Lipopolysaccharide,lipoporteins and peptodoglycan
Porins proteins	Absent	Present
Lipid	Less	More
Peptidoglycan	More	Less
Permeablilty of molecules	More penetrable	Less penetrable
Resistance to molecules	Less	More

GRAM-POSITIVE



GRAM-NEGATIVE



Bacterial Toxins

How Bacterial Toxins damage the host cells?

There are four basic ways in which bacteria can damage a host:

1) Use host cell's nutrients

2) Binding to and invading host cells (direct damage) (Most bacterial damage is carried out by toxins)

3) Induce hypersensitivity reactions (allergies)

4) Production of toxins (Toxigenicity)
   May be exotoxins or endotoxins

How Bacterial Toxins damage the host cells?

1- Use host cell's nutrients

❖Examples: Iron

What are some of the biological importance of iron in human cells?

- Oxygen transport and storage (hemoglobin, myoglobin)
- Electron transport and energy production (cytochromes and dehydrogenases//electron transport chain)
- Metabolism and detoxification (cytochromes//drugs, xenbiotics)
- ….etc.

How Bacterial Toxins damage the host cells?

1- Use host cell's nutrients

❖Examples: Iron

✓Iron in the host's cell is tightly bound to iron transport proteins such as hemoglobin.

✓Iron is required for the growth of most pathogenic bacteria, but the concentration of free iron is low in the host's cells (tightly bound).

✓When iron is needed by a pathogen, **Siderophores** are released into the medium.

*Siderophores: are small, iron chelating molecules.

✓ Siderophores take the iron away from iron transport proteins and form Iron- Siderophores Complex.

✓This complex binds to the bacterial surface and is used by the bacteria.

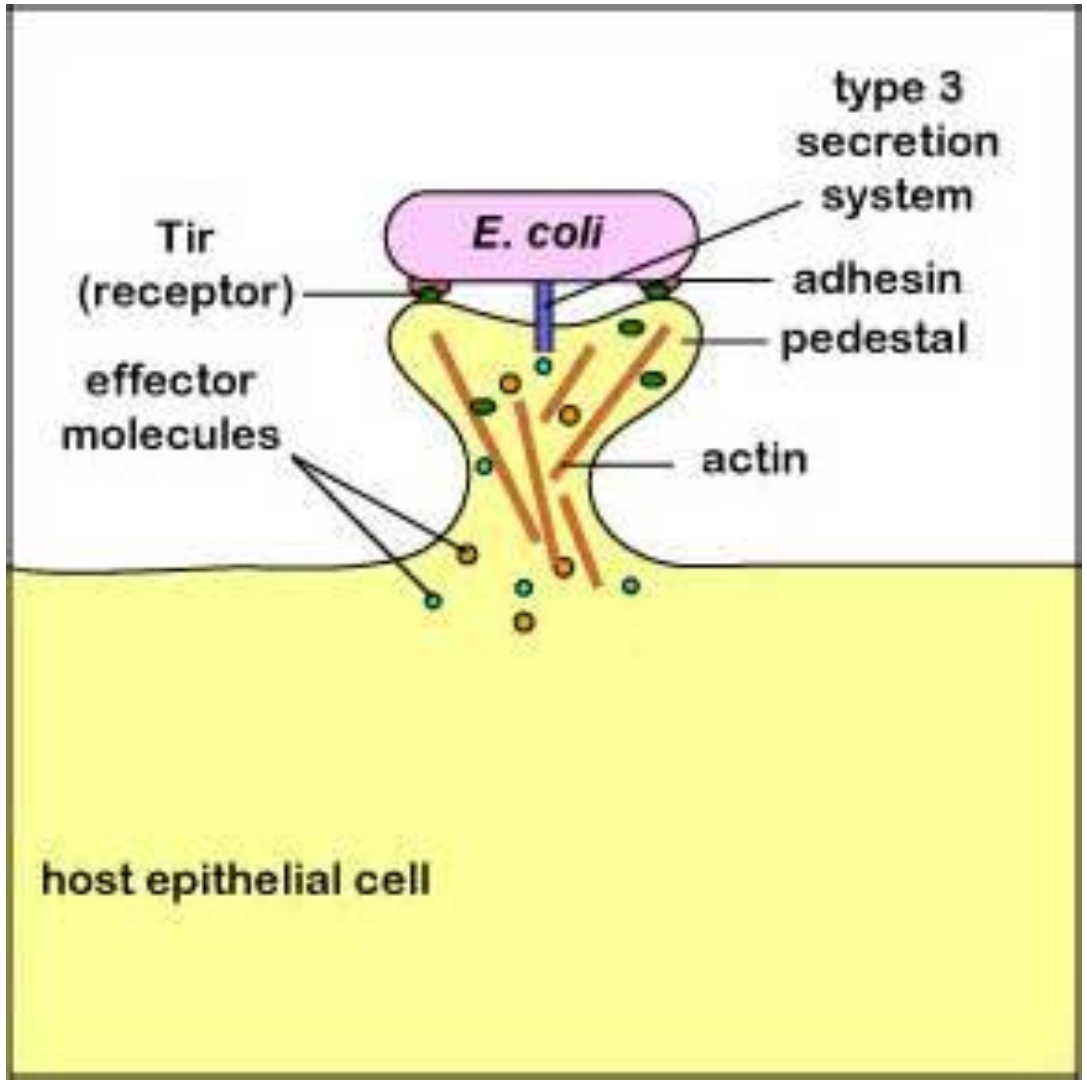
How Bacterial Toxins damage the host cells?

2- Direct damage

- Some bacteria can induce the host cells to engulf them (*E. coli, Shigella, Salmonella,* and *Neisseria gonorrhoeae*) to ensure infection and destruction of the host cell.
  - Pathogens can produce enzymes that disrupt normal tissue and allow for further invasion into the tissues.
- Multiply inside the host's cell by using nutrients and produce waste products.
- As pathogens metabolize and multiply in the cells>>>cells usually rupture!
- the microbes then released and spread to other tissues in great numbers.

Disrupt host cell function

Produce waste products


type 3
secretion
system
E. coli
Tir
(receptor)
adhesin
pedestal
effector
molecules
actin
host epithelial cell

How Bacterial Toxins damage the host cells?

3- Induce hypersensitivity reactions (allergies)

- The immune system plays an important role in protecting the body from microorganisms and other foreign substances.

>> when the body senses a foreign substance (antigen), the immune system is triggered.

>> it has two different types of immune responses:

Innate immunity:	Acquired Immunity:
▪ is present at birth and does not have to be learned through exposure to an invader. ▪ non-specific defence mechanisms that come into play immediately or within hours of an antigen's appearance in the body. ▪ has no memory of the encounters. ▪ The white blood cells involved in innate immunity are (Monocytes, Neutrophils, Eosinophils, Basophils & Natural killer cells)	▪ is not present at birth. It is learned. ▪ It is built overtime as you are exposed to various infections ▪ it generates special chemicals (antibodies) that neutralize the harmful toxins produced by the pathogen. ▪ The major cells of acquired immunity are T lymphocytes and B lymphocytes

How Bacterial Toxins damage the host cells?

3- Induce hypersensitivity reactions (allergies)

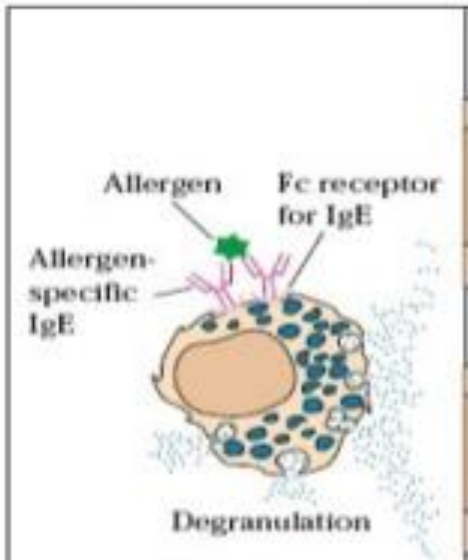
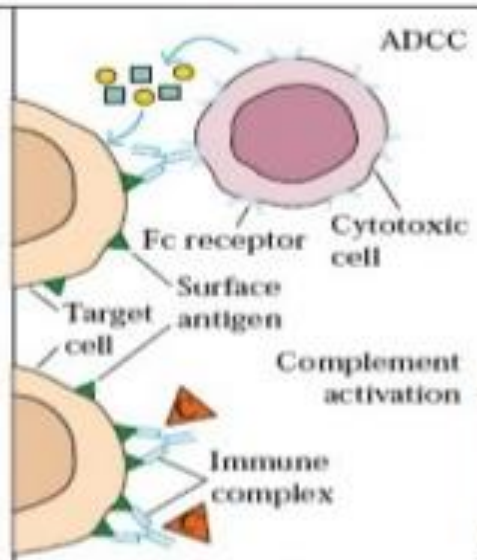
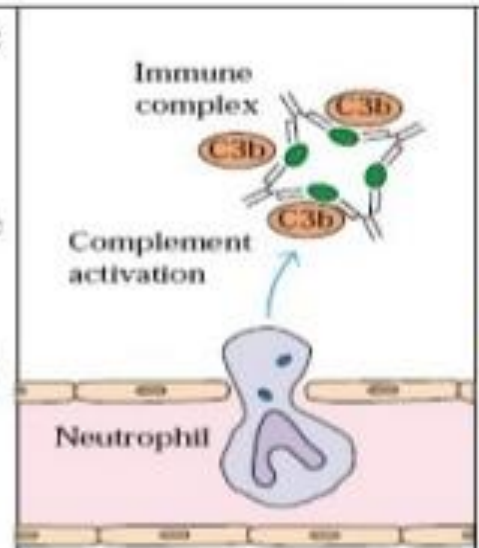
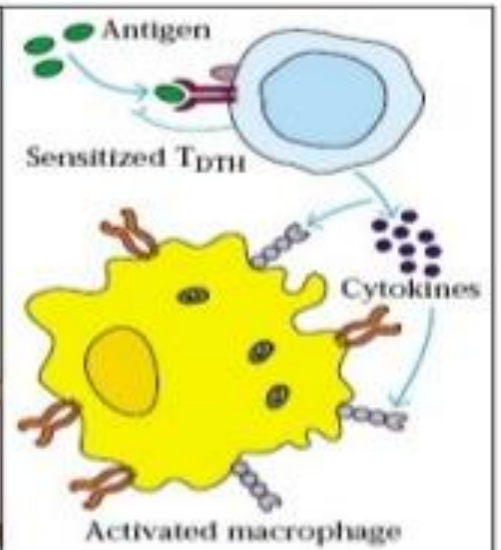
- **Normally,** the immune system fights harmful agents such as bacteria and toxins.

- **But in some cases**, the acquired immune system begins to mark otherwise harmless compounds “known as: allergens” and produce an unnecessary defensive mechanism.

  >> the body , causing an allergic reaction .
  >> if the activity of the immune system is excessive or over-reactive, a hypersensitivity reaction develops.
  **autoimmune diseases are sometimes subset of hypersensitivity.

- ❖ *Hypersensitivity:* is a set of undesirable reactions produced by the normal immune system, including allergies and autoimmunity. These reactions may be damaging, uncomfortable, or occasionally fatal.
- ❖ *Bacterial allergy:* a specific hypersensitivity to a particular bacterial antigen. Example: *Mycobacterium tuberculosis;* it is dependent on previous infection with the specific organism.

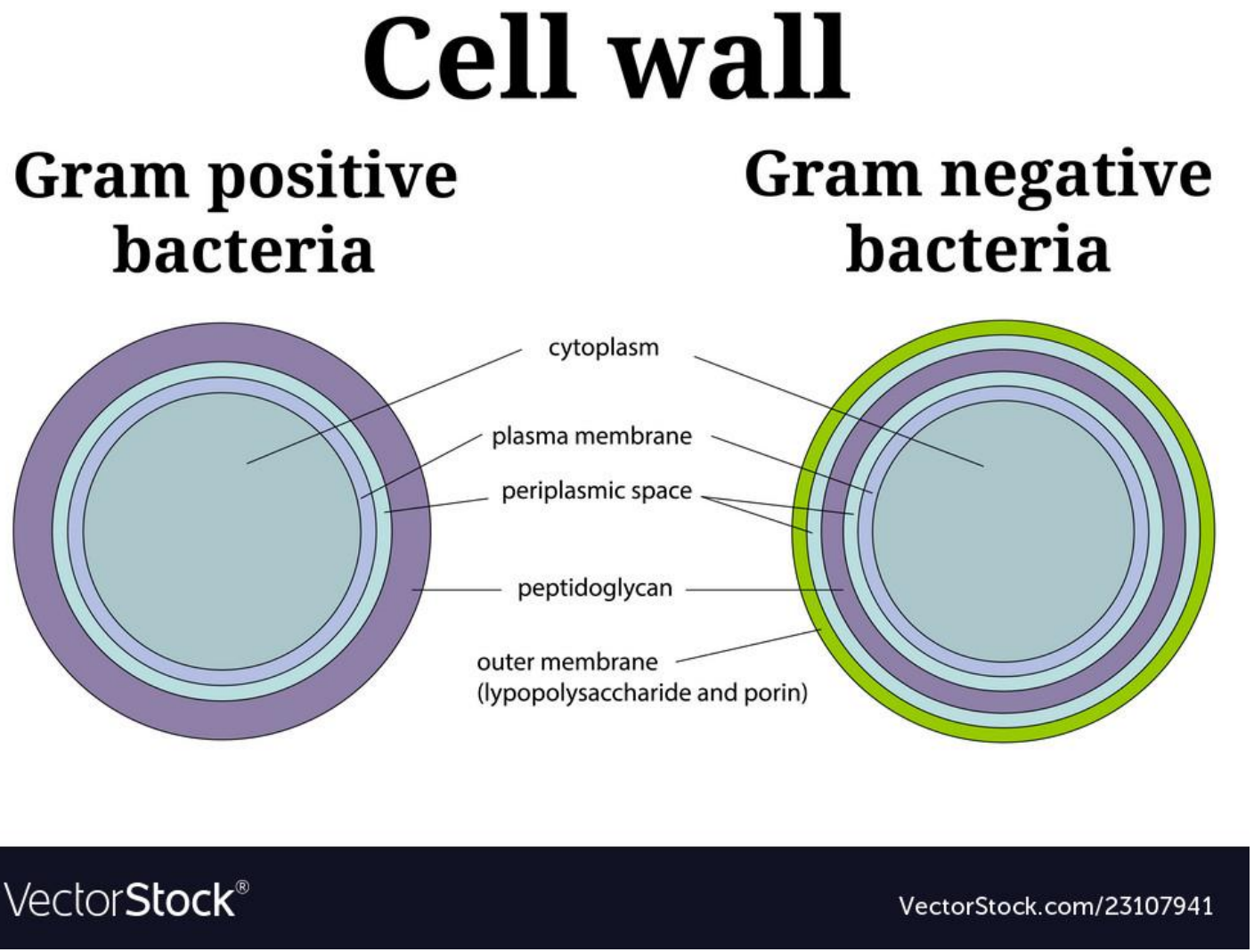
Hypersensitivity Reactions Conclusion:

 Type I	 Type II	 Type III	 Type IV
IgE-Mediated Hypersensitivity	IgG-Mediated Cytotoxic Hypersensitivity	Immune Complex-Mediated Hypersensitivity	Cell-Mediated Hypersensitivity
Ag induces crosslinking of IgE bound to mast cells and basophils with release of vasoactive mediators	Ab directed against cell surface antigens meditates cell destruction via complement activation or ADCC	Ag-Ab complexes deposited in various tissues induce complement activation and an ensuing inflammatory response mediated by massive infiltration of neutrophils	Sensitized T_H1 cells release cytokines that activate macrophages or T_C cells which mediate direct cellular damage
Typical manifestations include systemic anaphylaxis and localized anaphylaxis such as hay fever, asthma, hives, food allergies, and eczema	Typical manifestations include blood transfusion reactions, erythroblastosis fetalis, and autoimmune hemolytic anemia	Typical manifestations include localized Arthus reaction and generalized reactions such as serum sickness, necrotizing vasculitis, glomerulnephritis, rheumatoid arthritis, and systemic lupus erythematosus	Typical manifestations include contact dermatitis, tubercular lesions and graft rejection

How Bacterial Toxins damage the host cells?

4- Production of toxins (Toxigenicity)

- **Bacterial toxigenesis** is an underlying mechanism by which many bacterial pathogens produce disease.



❖ At a chemical level, there are two main types of bacterial toxins:

I. Endotoxins (The cell-associated toxins) **a toxin present inside a bacterial cell that is released when it disintegrates**

II. Exotoxins (the extracellular diffusible toxins) **a toxin released by a living bacterial cell into its surroundings**

Some important facts

- Many bacterial toxins are **proteins**. The toxins are usually liberated from the organism by lysis, but some are shed with outer membrane proteins in outer membrane vesicles.
- An important non-protein toxin is **lipopolysaccharide (LPS) or endotoxin**, which is a constituent of the cell wall of gram negative bacteria. Toxins may damage the eukaryotic cell membrane by combining with some structural component, or otherwise alter its function.
- Many toxins combine with **specific receptors** on the surface membrane, frequently glycoproteins or gangliosides, and penetrate the cell to reach their **intracellular target**.
- A common mechanism of entry is **absorptive endocytosis**. Many protein toxins have an A-B structure, **B being a polypeptide** which binds to the receptor and **A being an enzyme**.
- Many toxins are **activated**, either when produced by the bacterium or when bound to the membrane receptor, by **proteases**.

How Bacterial Toxins damage the host cells?

4- Production of toxins (Toxigenicity)

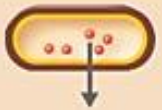
- Toxigenicity: is the ability to produce toxins.
- Toxigenesis: It is an underlying mechanism by which many bacterial pathogens produce disease.
- Toxins: Poisonous substances produced by microbes.
- Toxemia: Presence of toxins in the blood.
- Toxin effects: May include fever, cardiovascular problems, diarrhea, shock, destruction of red blood cells and blood vessels, and nervous system disruptions.
- Of 220 known bacterial toxins, 40% damage eukaryotic cell membranes.

How Bacterial Toxins damage the host cells?

4- Production of toxins (Toxigenicity)

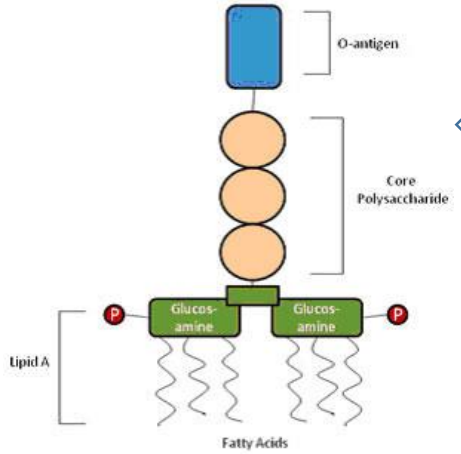
Exotoxins vs. Endotoxins

Table 15.3 Exotoxins and Endotoxins

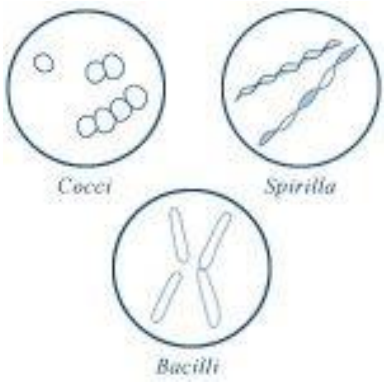
Property	Exotoxin	Endotoxin
		
Bacterial Source	Mostly from gram-positive bacteria	Gram-negative bacteria
Relation to Microorganism	Metabolic product of growing cell	Present in LPS of outer membrane of cell wall and released with destruction of cell or during cell division
Chemistry	Proteins, usually with two parts (A-B)	Lipid portion (lipid A) of LPS of outer membrane (lipopolysaccharide).
Pharmacology (Effect on Body)	Specific for a particular cell structure or function in the host (mainly affects cell functions, nerves, and gastrointestinal tract)	General, such as fever, weaknesses, aches, and shock; all produce the same effects
Heat Stability	Unstable, can usually be destroyed at 60–80°C (except staphylococcal enterotoxin)	Stable, can withstand autoclaving (121°C for 1 hour)
Toxicity (Ability to Cause Disease)	High	Low
Fever-Producing	No	Yes
Immunology (Relation to Antibodies)	Can be converted to toxoids to immunize against toxin; neutralized by antitoxin	Not easily neutralized by antitoxin; therefore, effective toxoids cannot be made to immunize against toxin
Lethal Dose	Small	Considerably larger

★

Toxoid: a chemically modified toxin from a pathogenic microorganism, which is no longer toxic but is still antigenic and can be used as a vaccine.



Endotoxin vs Exotoxin



- **Manily gram positive bacteria produce exotoxins** but it could produced sometimes by gram negative bacteria, while endotoxins are produced mainly by **gram negative bacteria**.
- Endotoxins **cannot** act as enzymes, but exotoxins **can** act as enzymes.
- Endotoxins are less toxic than exotoxins. (**Why?**)
- Exotoxins are **specific** to particular bacterial strain while endotoxins are not.
- Endotoxins are **poor antigens**, whereas exotoxins are **highly antigenic**.
- By stimulating the immune system exotoxins produce antitoxins to neutralize the toxin while endotoxins do not produce antitoxins.

http://www.differencebetween.com/difference-between-endotoxin-and-vs-exotoxin/

What is the difference between toxoid and antitoxin?

- antitoxin: is an antibody with the ability to neutralize a specific toxin.

- **Antitoxin, any of a group of *antibodies* formed in the body (animals or humans) as a response to the introduction of poisonous products, or toxins. By introducing small amounts of a specific toxin into the healthy body, it is possible to stimulate the production of antitoxin so that the body's defences are already established against invasion by the bacteria or other organisms that produce the toxin. (used as a treatment).**

- Toxoid : a chemically modified toxin from a pathogenic microorganism, which is no longer toxic but is still antigenic and can be used as a vaccine.

- **A toxoid is a bacterial toxin whose toxicity has been weakened or suppressed either by heat or chemical (e.g. formaldehyde), or genetically engineered while other properties, typically immunogenicity, are maintained. Toxoids are used in vaccines as they induce an immune response to the original toxin or increase the response to another antigen. For example, the tetanus toxoid is derived from the tetanospasmin produced by *Clostridium tetani* and causing tetanus.**

4- Production of toxins (Toxigenicity)

- **During infection**, pathogenic microbes actively remodel host cells and tissues to create a more suitable niche for withstanding the rigors of the host environment such as the generation of protein toxins that **modulate important functions of both immune and non-immune cells**.
- The potency of intracellularly acting toxins is derived, in part, from their mode of action; most are **enzymes that catalyze the covalent modification of specific molecular targets**.

How do toxins make it into target cells?

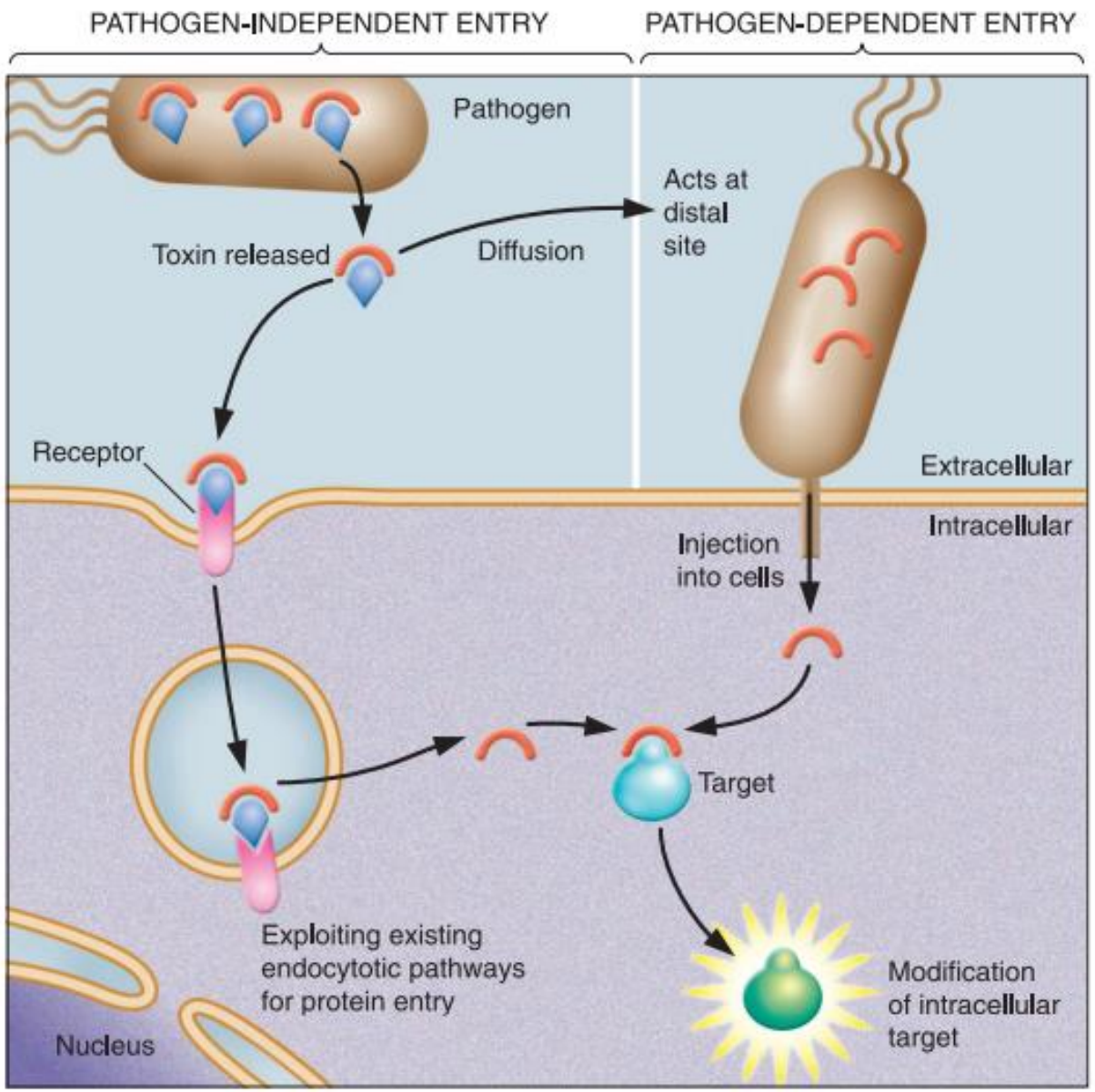
➢ To overcome the membrane barrier, there are at least two mechanisms of toxin entry into target cells:

>> pathogens directly inject toxins into host cells (pathogen-dependent entry)

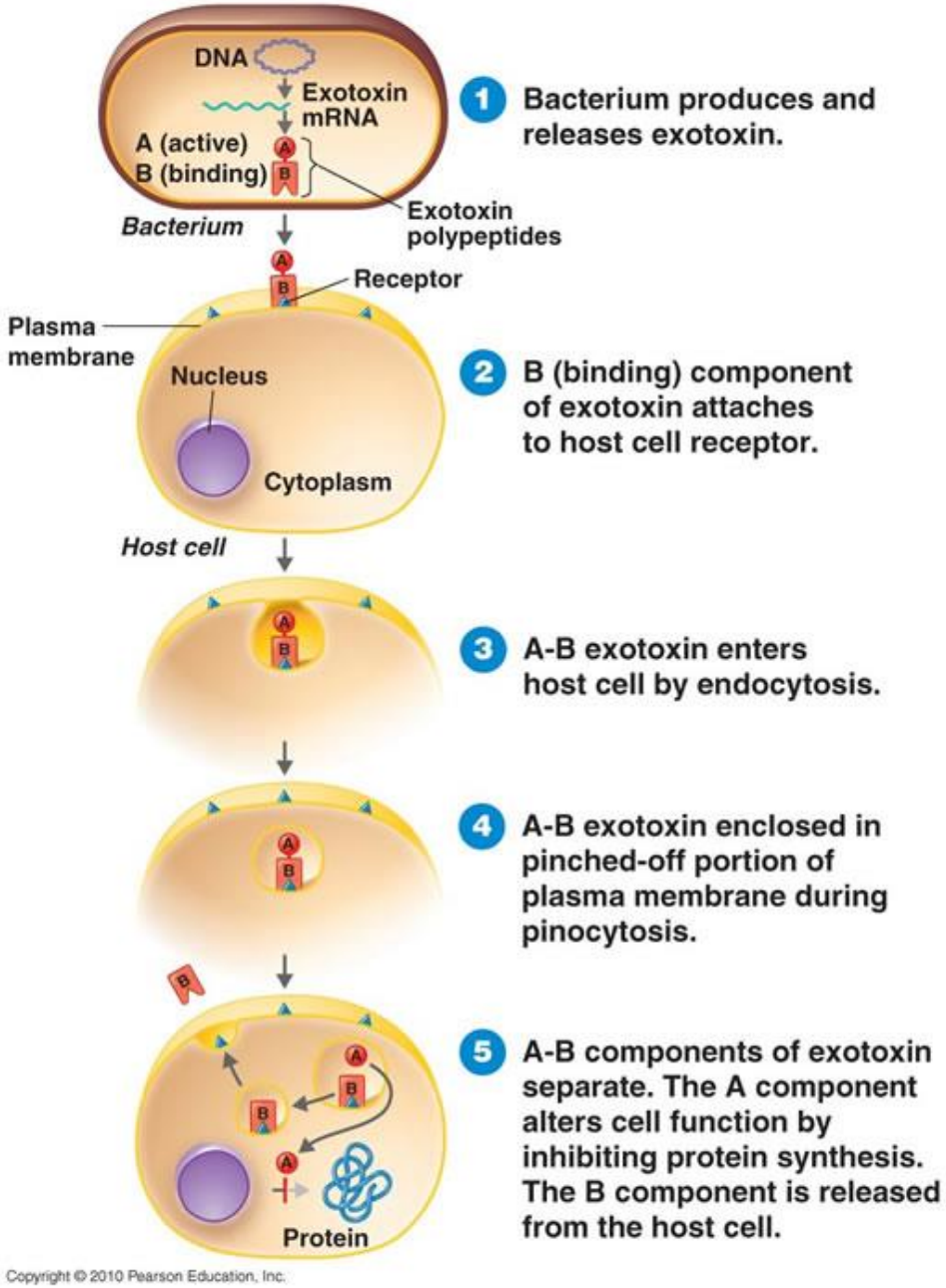
>> alternatively, release AB toxins, which must then enter host cells independent of the bacterium (pathogen-independent entry)

How do toxins make it into target cells?

RELEASE AB TOXINS



DIRECTLY INJECT TOXINS



(PATHOGEN-INDEPENDENT ENTRY)

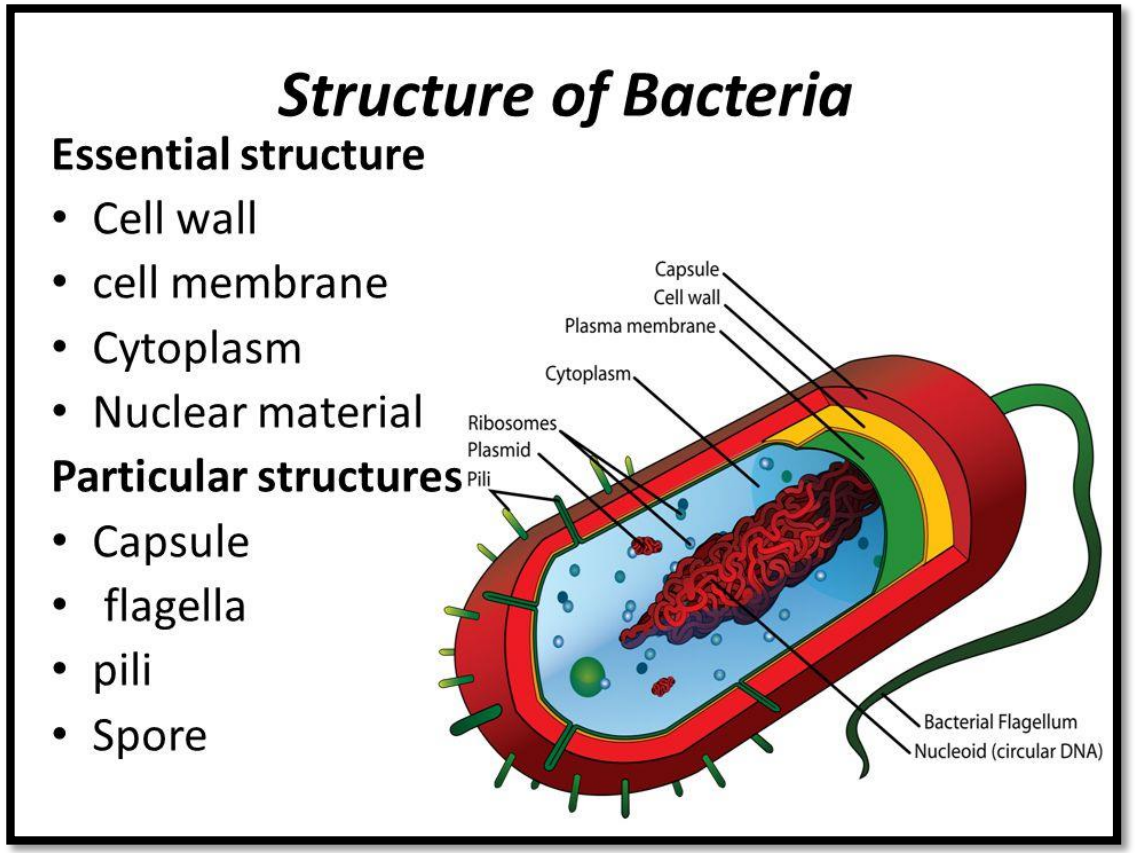
RELEASE AB TOXINS

4- Production of toxins (Toxigenicity)

Online textbook of bacteriology by Kenneth Todar, PhD
http://textbookofbacteriology.net/antiphago.html

Intercellular bacterial survival:

1. Ability of Pathogens to Avoid or Overcome Phagocytes.
2. Inhibition of Phagocytic Engulfment.
3. Survival Inside of phagocytes.
4. Products of Bacteria that Kill or Damage Phagocytes



4- Production of toxins (Toxigenicity)

Intercellular bacterial survival:

1. Ability of Pathogens to Avoid or Overcome Phagocytes (avoid to contact with phagocytes);

- Pathogens may invade or **remain confined in regions inaccessible to phagocytes**. Certain internal tissues (e.g. the lumens of glands, the urinary bladder) and surface tissues (e.g. unbroken skin) are not patrolled by phagocytes.
- Some pathogens are able to **avoid provoking an overwhelming inflammatory response**. Without inflammation the host is unable to focus the phagocytic defences.
- Some bacteria or their products **inhibit phagocyte chemotaxis**. For example, Streptococcal streptolysin (which also kills phagocytes) suppresses neutrophil chemotaxis, even in very low concentrations. Fractions of *Mycobacterium tuberculosis* are known to inhibit leukocyte migration. The *Clostridium ø* toxin also inhibits neutrophil chemotaxis
- Some pathogens can cover the surface of the bacterial cell with a component which is seen as "self" by the host phagocytes and immune system.
    - For example, pathogenic ***Staphylococcus aureus*** produces cell-bound coagulase and clumping factor which clots fibrin on the bacterial surface. **Group A streptococci** are able to synthesize a capsule composed of hyaluronic acid. Hyaluronic acid is the ground substance (tissue cement) in connective tissue

4- Production of toxins (Toxigenicity)

Intercellular bacterial survival:

2. **Inhibition** of Phagocytic Engulfment

- Some bacteria employ strategies to avoid engulfment (ingestion) if phagocytes do make contact with them. Many important pathogenic bacteria bear on their surfaces substances that **inhibit phagocytic adsorption or engulfment**.

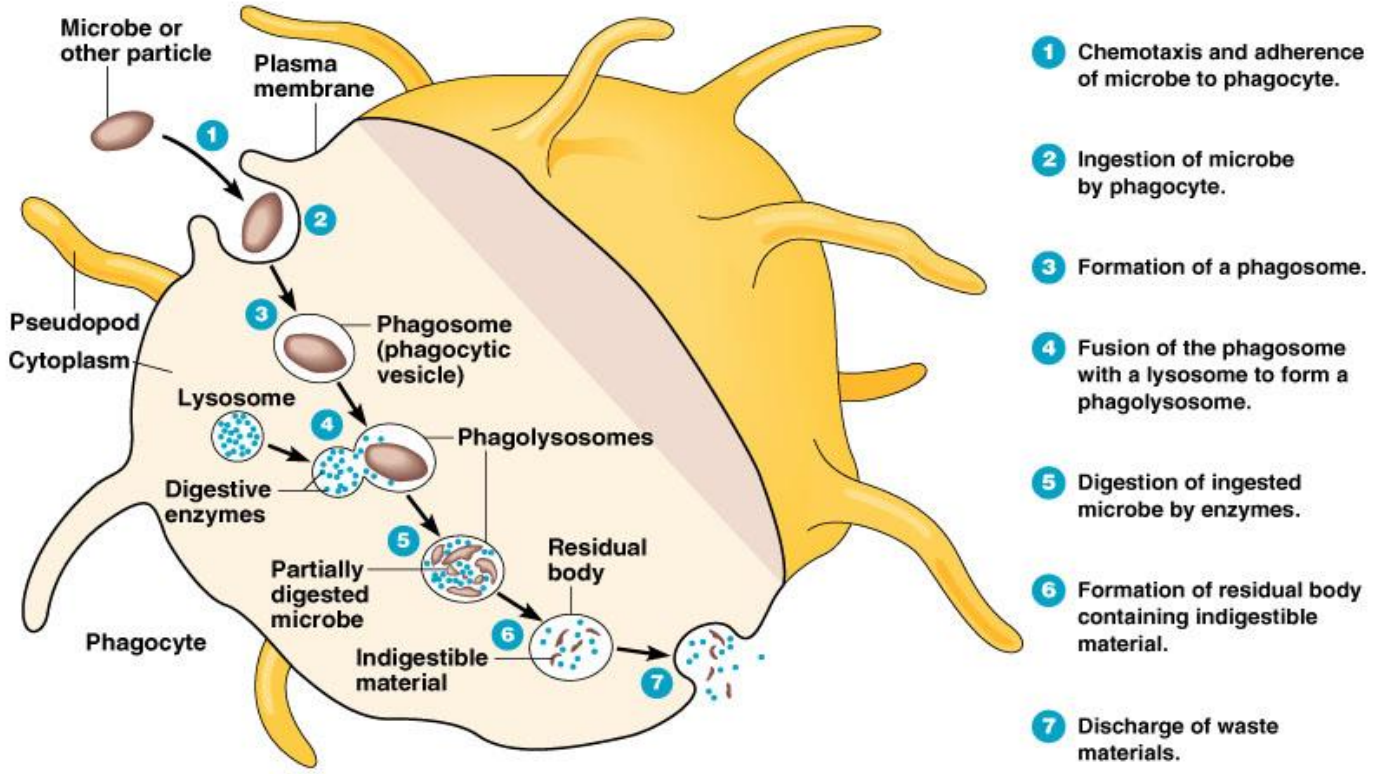
- Resistance to phagocytic ingestion is usually **due to a component of the bacterial cell surface** (cell wall, or fimbriae, or a capsule).

4- Production of toxins (Toxigenicity)

Intercellular bacterial survival (Cont.):

3. Survival Inside of phagocytes (interfere with the bactericidal activities of the host cell)

- Inhibition of fusion of the phagocytic lysosomes (granules) with the phagosome (The bacteria survive inside of phagosomes because they prevent the discharge of lysosomal contents into the phagosome environment), or survival inside the phagolysosome, or escape from the phagosome (e.g. phospholipase A).



(a) Phases of phagocytosis

4- Production of toxins (Toxigenicity)

Intercellular bacterial survival (Cont.):

4. Products of Bacteria that Kill or Damage Phagocytes

>> One obvious strategy in defence against phagocytosis is **direct attack** by the bacteria upon the professional phagocytes.

>> Phagocytes may be killed by a pathogen before or after ingestion.

Other Anti-phagocytic Strategies Used by Bacteria; For example, a pathogen may have a mechanism to inhibit the production of phagocytes or their release from the bone marrow.

4- Production of toxins (Toxigenicity)

- Some important definitions:

Pathogenicity: the ability of an organism to cause disease by overcoming the defence mechanism.

Virulence: the degree of pathogenicity.

Antitoxin: antibodies produced against toxins (exotoxins!).