Unit 1

Normal Microflora of the Human Body and Host Pathogen Interaction

NORMAL HUMAN FLORA

HUMAN MICROBIOME

- The human microbiome (or **human micro biota**) is the aggregate of microorganisms that reside on the surface and in deep layers of skin, in the saliva and oral mucosa, in the conjunctiva, and in the gastrointestinal tracts. They include bacteria, fungi. Some of these organisms perform tasks that are useful for the human host. However, the majority have no known beneficial or harmful effect. Those that are expected to be present, and that under normal circumstances do not cause disease, but instead participate in maintaining health, are deemed members of the ***normal flora***

OVERVIEW OF HUMAN-MICROBIAL INTERACTIONS

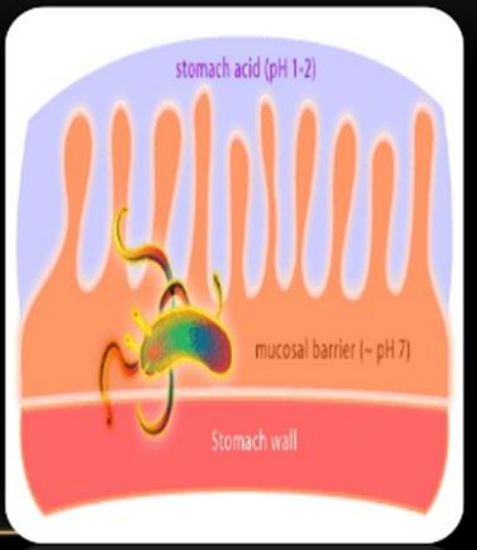
- **Microbial flora of the healthy human host**

1) The reasons for understanding the normal flora of the healthy human body
   - ▶ Normal flora vs. human body
   - ▶ Some normal flora: opportunistic pathogens
     when injury occurred,
     when resistance of body decreased,
     when moved to another site

2) Origin of the normal flora
3) Relationship between normal flora and human host
4) Distribution and occurrence of the normal flora

COMMENSAL RELATIONSHIP.

- Sometimes the relationship between a member of the normal flora an its host cannot be deciphered. Such a relationship where there is no apparent benefit or harm to either organism during their association is referred to as a **commensal relationship.** Many of the normal flora that are not predominant in their habitat, even though always present in low numbers, are thought of as commensal bacteria. However, if a presumed commensal relationship is studied in detail, parasitic or mutualistic characteristics often emerge.



NORMAL FLORA ARE MUTUALISTIC

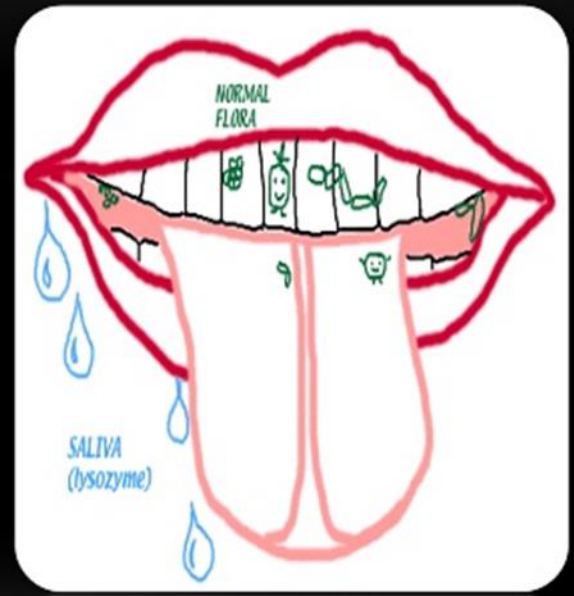
- Much is not known about the nature of the associations between humans and their normal flora, but they are thought to be dynamic interactions rather than associations of mutual indifference. Both host and bacteria are thought to derive benefit from each other, and the associations are, for the most part, **mutualistic.** The normal flora derive from their host a steady supply of nutrients, a stable environment, and protection and transport. The host obtains from the normal flora certain nutritional and digestive benefits, stimulation of the development and activity of immune system, and protection against colonization and infection by pathogenic microbes.

WHEN WE GET COLONIZED WITH NORMAL FLORA

- A human first becomes colonized by a normal flora at the moment of birth and passage through the birth canal. In utero, the fetus is sterile, but when the mother's water breaks and the birth process begins, so does colonization of the body surfaces. Handling and feeding of the infant after birth leads to establishment of a stable normal flora on the skin, oral cavity and intestinal tract in about 48 hours.

FACTORS INFLUENCING NORMAL FLORA

1. Local Environment (pH, temperature, redox potential, O2, H2O, and nutrient levels…).
2. Diet
3. Age
4. Health condition (immune activity…)
5. Antibiotics,…..etc



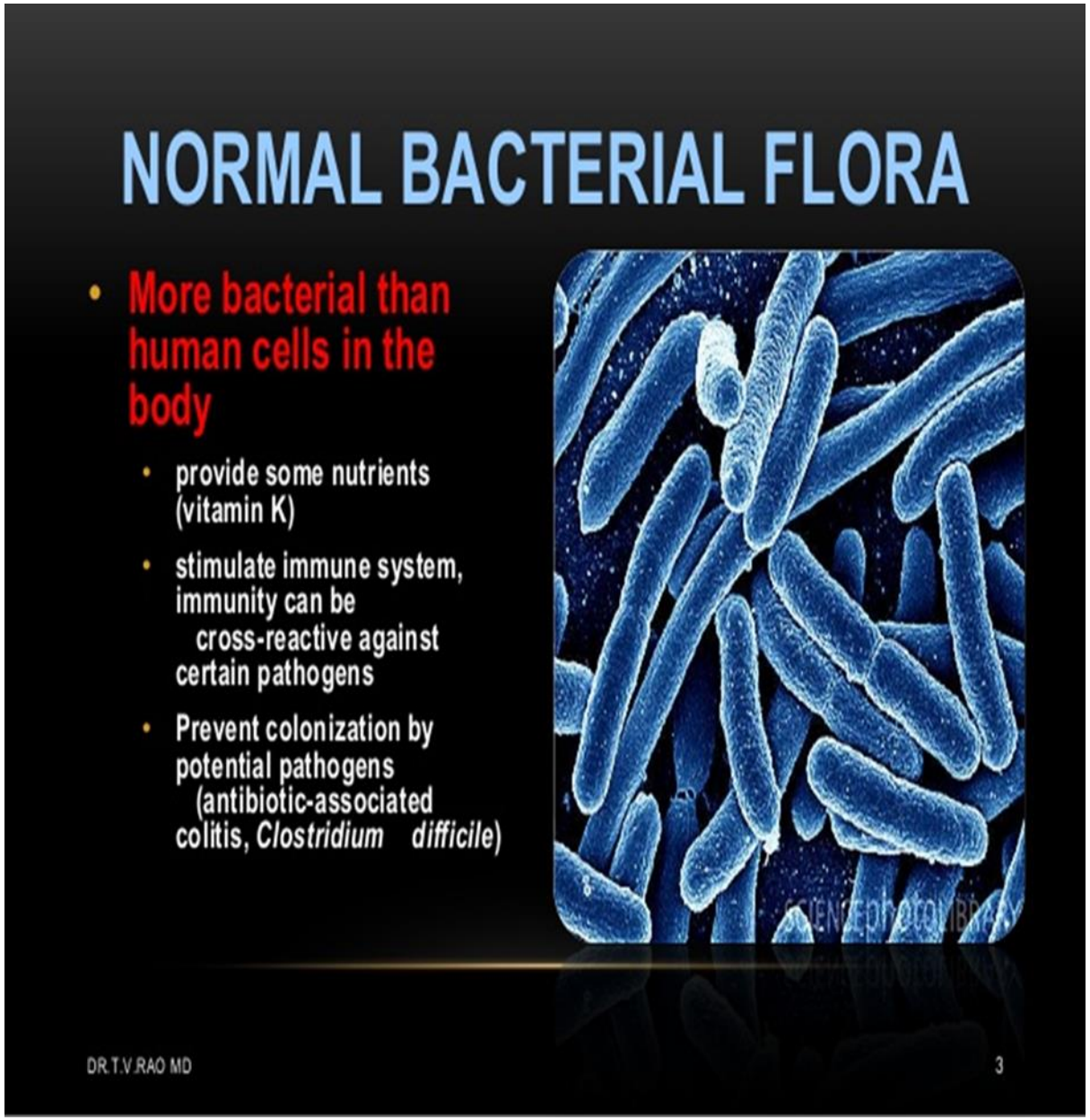
NORMAL FLORA AND HUMAN HOST

1. Use of germfree animals
2. Use of antimicrobial agents
3. Knowledge of certain characteristics of normal flora organisms

Gnotobiotic- Animals that are germ free or that live in association with one or more known organisms

CHARACTERISTICS OF NORMAL FLORA ORGANISMS

Adherance
Desquamation



DISTRIBUTION AND OCCURRENCE OF THE NORMAL FLORA

Bacteria make up most of the normal flora of the human body.
Various fungi (mainly yeasts) and protozoa may also inhabit the body
Whether viruses can be considered as true normal flora is not very clear.

Echo viruses- enteric cytopathogenic human orphans

NORMAL FLORA OF THE SKIN

- The most important sites are:

1. Axilla
2. Groin
3. Areas between the toes

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Factors discouraging skin colonization

- Dryness
- Low pH
- Inhibitory substances

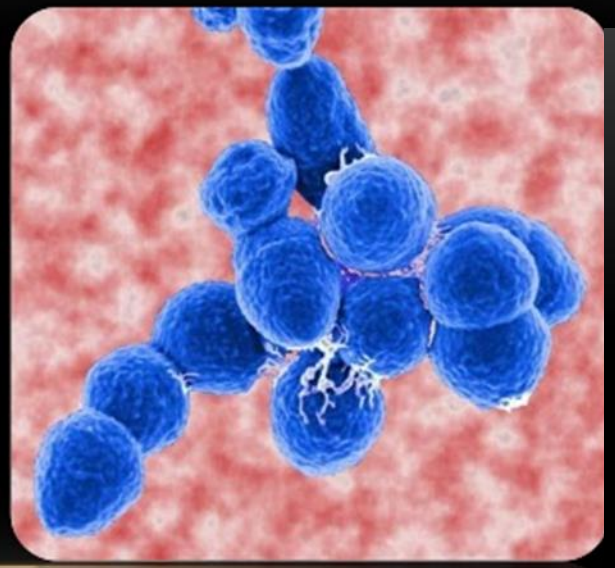
Staphylococcus epidermidis
Corynebacteria
Propionibacteria acnes

Acne vulgaris

NORMAL FLORA OF THE RESPIRATORY TRACT

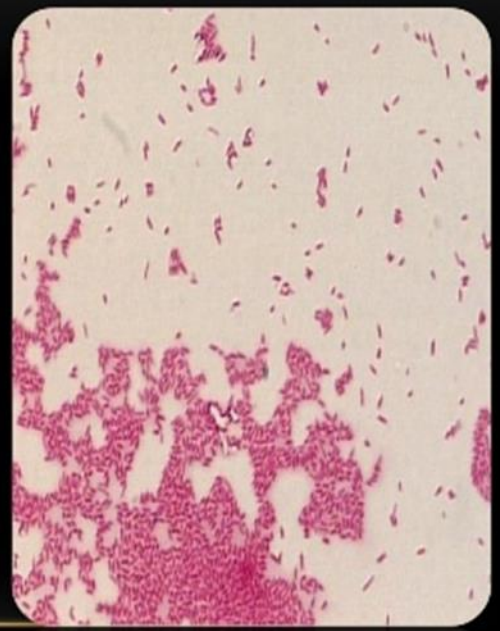
B) The upper respiratory tract (nasopharynx).

1. Non-hemolytic streptococci
2. Alpha-hemolytic streptococci
3. *Neisseria* sp.
4. *Streptococcus pneumoniae*
5. *Streptococcus pyogenes*
6. *Hemophilus influenzae*
7. *Neisseria meningitidis*



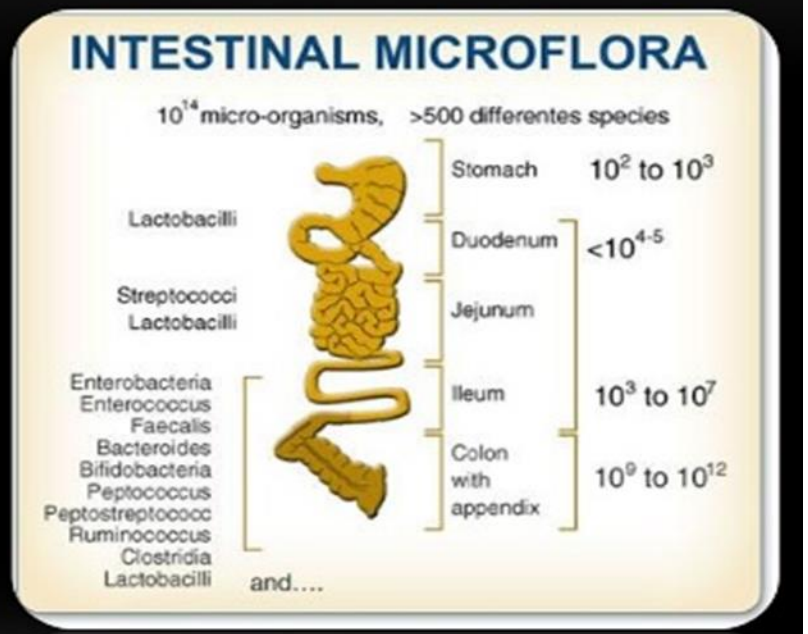
NORMAL FLORA OF THE RESPIRATORY TRACT

- **C) The lower respiratory** tract (trachea, bronchi, and pulmonary tissues):
- Usually sterile.
- The individual may become susceptible to infection by pathogens descending from the nasopharynx e.g.
- ***H. influenzae***
- ***S. pneumoniae*).**



THE FLORA OF THE LARGE INTESTINE (COLON)

1. Enterococci
2. Clostridia
3. lactobacilli
4. Bacteroides
5. Bifidobacterium (*Bifidobacterium bifidum*)
6. *Escherichia coli*
7. Methanogenic bacteria
8. *Viridans streptococci*
9. *Staphylococcus sp.*
10. *Proteus sp.*
11. *Candida albicans* (Yeast)
12. *Mycoplama sp.*



FLORA OF THE GENITOURINARY TRACT

S. epidermidis
Streptococcus faecalis
Corynebacteria

The adult female genital tract has a very complex normal flora.

Lactobacilli, *Candida albicans*

Types of Infections

- **Acute infection-** has a short and relatively severe course. Ex- Streptococcal pharyngitis
- **Chronic infection-** has a long duration. Ex- Tuberculosis
- **Fulminating infection-** occurs suddenly and with severe intensity. Ex- Cerebrospinal meningitis
- **Localized infection-** restricted to a limited area of the body. Ex- Urinary tract infection
- **Generalized infection-** Affects many or all parts of the body. Ex- Blood infection like typhoid fever.
- **Mixed or polymicrobial infection-** more than one kind of microorganism contribute to the infection. Ex- Gas gangrene
- **Primary infection-** an initial localized infection that decreases resistance and thus paves the way for further invasion by the same or other microorganisms. Ex- Viral influenza
- **Secondary infection-** infection that is established after a primary infection has caused a decreased resistance. Ex- Pneumococcal pneumonia
- **Subclinical infection-** may result in only a very minor amount of damage to the host so no detectable clinical symptoms are observed.
- **Bacteremia**- indicating the presence of bacteria in the blood
- **Septicemia**- indicating the presence of bacteria and their growth products in the blood.
- **Nosocomial infection-** an infection acquired while in the hospital.

Lethal Dose (LD)- number of organisms required to kill the test animal.
LD_{50}- number of organisms which when administered to a number of laboratory animals will kill 50% of them.
The virulence of a pathogen is usually measured by determining its LD_{50} dose.
LD_{50} of *Vibrio cholera* is 5-10 but for *E. coli* it is 10^3 organisms.

LD_{100} or minimal lethal dose- number of organisms which when administered to a number of laboratory animals will kill 100% of them.

Reservoir of infection- source of an infectious agent.

Contagious or communicable disease- a pathogen will move with ease from one individual to the next. People who come in contact with someone suffering from a contagious disease are at risk of contracting that disease unless they are immune.

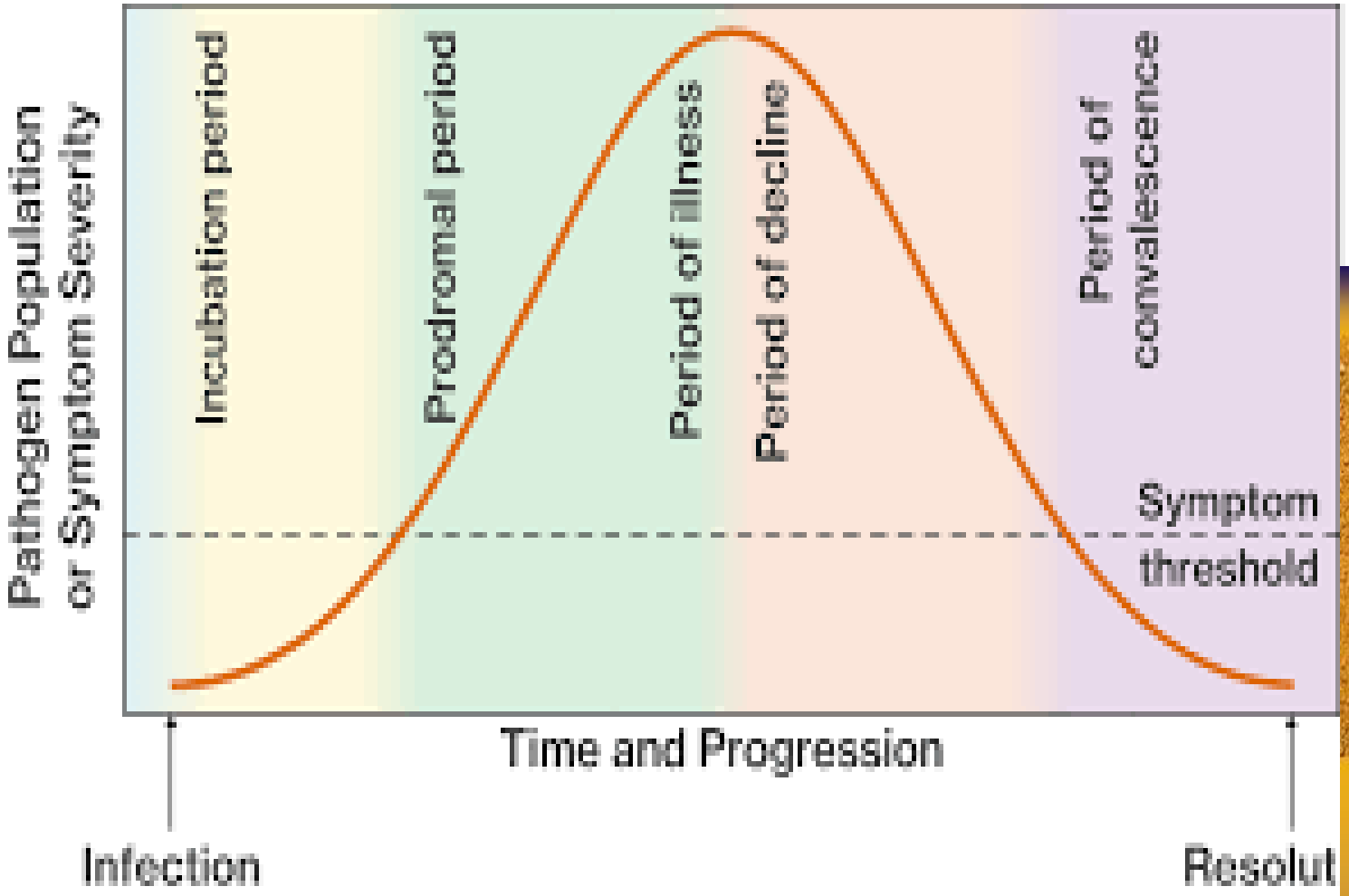
Carriers- they do not develop disease symptoms but are reservoirs of infectious agents.

Endemic- a disease constantly present in a population in relatively low numbers. Ex- chicken pox is endemic in United Kingdom

Epidemic- outbreaks of disease in which unusually high number of individuals in a population are infected. Ex- Cholera, malaria, dengue

Pandemic- an outbreak of disease that affects large numbers of people in a major geographical region.

Signs are objective changes such as rash or fever that a physician can observe.
Symptoms are subjective changes in body function such as pain or loss of appetite that are experienced by the patient.
A characteristic group of signs and symptoms constitutes a **disease syndrome**.



STAGES OF INFECTIOUS DISEASE

- Infectious diseases tend to occur in stages including (in typical order)
  - Incubation period
  - Prodromal phase (not typical)
  - Invasive phase
  - Decline phase
  - Convalescence period
  - Sequelae (not typical)

Phases of Infectious Disease

- **Incubation period** – time between infection and the appearance of signs and symptoms.
- **Prodromal phase** – mild, nonspecific symptoms that signal onset of some diseases.
- **Clinical phase** – a person experiences typical signs and symptoms of disease.
- **Decline phase** - subsidence of symptoms.
- **Recovery phase** – symptoms have disappeared, tissues heal, and the body regains strength.

Nosocomial Infections

Hospital acquired infections also called nosocomial infections are defined as infections developing in patients after admission to the hospital, which were neither present nor in incubation at the time of hospitalization.

Semmelweiss (1861)- hand washing with chlorinated lime.
Joseph Lister (1867)- antiseptic surgery

Factors Influencing Infection

- Age
- Infected patients
- Drug-resistance
- Susceptible patients
- Surgical procedure

Sources of Infection

A. **Endogenous**- autoinfection or autochthonous infection
B. **Exogenous**- from other people or immediate objects in the environment
1. Contact with other patients and staff
2. Environmental Sources
(a) Inanimate objects- equipment (beds pans), blankets, medical equipment (endoscopes, catheters), floors, food and water. Coliforms are common in this environment
(b) Hospital air- Gram positive cocci
(c) Surfaces- surfaces contaminated by patient's secretions, excretions, blood, etc. animals and insects are also sources of hospital infection

Mode of Transmission

1. Contact route
(a) Hands or clothing
(b) Inanimate objects
2. **Air-borne route**
(a) Droplet of respiratory infection is transmitted from person to person by inhalation
(b) Dust from bedding, floors, exudate dispersed from a wound during dressing. *P. aerug*inosa, *S. aureus*
(c) Aerosols produced by nebulizers, humidifiers and air conditioning apparatus transmit certain pathogens (coliforms, *Legionella*) to the respiratory tract if water in the instrument becomes contaminated.

3. Oral route
4. Parenteral route- blood donation or tissue donation. Hepatitis B and HIV

Common Hospital Acquired Infection

1. Urinary tract infection- 40% . *E. coli, Candida albicans*, *Enterococcus, Klebsiella*
2. Infection of the lower respiratory tract- 15-20%. *Haemphilus influenzae, Streptococcus pneumonia, S. aureus*
3. Wound and skin sepsis- 18% *S. aureus, E. coli*, Proteus spp, *Enterococcus*
4. Gastrointestinal infections- Salmonella spp.

Prevention

- ✓ Administration of antibiotics and antiseptic therapy
- ✓ Infectious patient should be isolated
- ✓ Proper sterilization and disinfection of the inanimate objects
- ✓ Disinfection of excreta and infected material
- ✓ The transmission route is to be controlled by regular washing of hands, disinfection of equipments and change of working clothes
- ✓ Susceptible host protected by vaccination

Pathogenicity

Pathogenicity- capacity to initiate disease
To be a pathogen the organism must have the properties of- **Transmissibility**, **infectivity** and **virulence**

Types of Bacterial Pathogen- (a) **Opportunistic pathogens**- Coagulase negative staphylococci, *E. coli*

(b) **Primary pathogens**

Virulence Determinants-that facilitate pathogenesis

Ex- although 6 separate serotypes of encapsulated *Haemophilus influenza* are recognized serious infection is almost exclusively associated with isolates of serotype b.

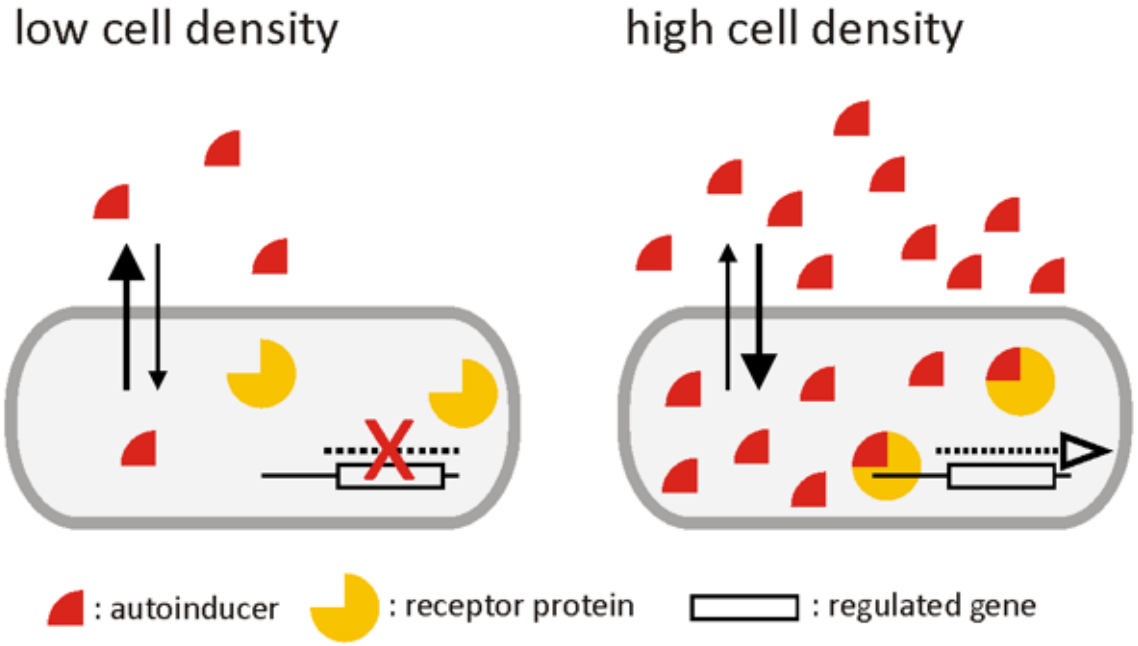
Different strains of *E. coli* can cause several different diseases like gastro-intestinal diseases, urinary tract infections, septicaemia etc.
Virulence determinants encoded by genomic DNA sequences, plasmids, bacteriophages and transposons have been reported.

Quorum Sensing

Genetic studies have shown that expression of several different virulence determinants in a single bacterium are sometimes regulated in a coordinated fashion. Iron limitation the situation encountered in host tissues is one environmental stimulus which coordinately increases production of many bacterial proteins including virulence determinants such as haemolysin of *E. coli*. In other bacteria, like *S. aureus* and *P. aeruginosa* some virulence determinants are expressed exclusively or maximally during the stationary phase of growth. Expression of these factors is associated with production of inducer molecules or pheromones in the bacterial culture which accumulate as the bacteria grow until a threshold level is reached and gene expression is triggered a process known as **quorum sensing**.

The ability to regulate production of virulence determinants may save energy in situations in which expression is not required

Quorum sensing may be important in establishing a sufficiently large population of bacteria in tissue to guarantee survival of the infecting organism.



Establishment of infection

Potential pathogens may enter the body by various routes called the **portals of entry**.

Portals of entry- (a) respiratory tract
(b) gastro-intestinal tract
(c) urinary or genital tract
(d) direct tissue entry through insect bites or by accidental or surgical trauma to the skin.

Opportunistic pathogens act as ready source of infection in the compromised host.

For many primary pathogens transmission to a new host and establishment of infection is very complex.
Bordetella pertussis require direct contact with infectious material.
Treponema pallidum and *Neisseria gonorrhoeae* require direct person to person mucosal contact for transmission.

Salmonella, Shigella, Campylobacter –primary source is environmental and infection follows ingestion of contaminated food or water.

Reservoir of infection

Colonization

Colonization- establishment of a stable population of bacteria in the host
Adhesion is essential for colonization

Importance of adhesion- (a) to avoid host defense mechanisms
(b) For invasive bacteria it is an essential preliminary to penetration through tissues

Successful colonization also requires that bacteria are able to acquire essential nutrients in particular iron for growth.

Adhesion

Adhesion involves surface interactions between specific **receptors** on the mammalian cell membrane (usually carbohydrates) and **ligands** (usually proteins) on the bacterial surface.

The presence or absence of specific receptors on mammalian cells contributes significantly to tissue specificity of infection.

Non specific surface properties such as surface charge and hydrophobicity also contribute to the initial stages of the adhesion process.

Fimbrial Adhesins

Fimbriae are thin, rigid rod like structures
Fimbriae are involved in mediating attachment of some bacteria to mammalian cell surfaces
Different strains or species of bacteria may produce different types of fimbriae which can be identified on the basis of **antigen composition**, **morphology** and **receptor specificity**.

Mannose sensitive fimbriae and mannose insensitive fimbriae

The antigenic composition of fimbriae can be complex.
2 fimbrial antigens called **Colonization factor antigens (CFA)** I and II have been identified in enteropathogenic *E. coli* strains.
CFA II consists of 3 distinct fimbrial antigens designated as **Coli surface (CS) antigens** 1, 2 and 3.
Another *E. coli* strain E8775 has been found to produce 3 other CS antigens, CS4, CS5 and CS6
Pyelonephritogenic *E. coli* produce a group of adhesins called **X-adhesins**, 2 fimbrial types designated **S**and **M** on the basis of receptor specificity.
Evolutionary significance of such heterogeneity?
For some fimbriae the association with infection is clear. Ex- K88 fimbrial antigen is clearly associated with the ability of *E. coli* K88 to cause diarrhea in pigs.
Type I fimbriae
N-methylphenylalanine fimbriae

Non- fimbrial Adhesins

1. Filamentous haemglutinin of *Bordetella pertussis*
2. Mannose resistant haemagglutinin of *Salmonella* serotype
3. Fibrillar haemagglutinin from *Helicobacter pylori*
4. Outer membrane proteins are involved in the adherence of *N.gonorrhoeae* and enteropathogenic *E. coli* to the cell surfaces
5. Exopolysaccharides present on the surface of some Gram –positive bacteria are also involved in adhesion. Ex- *Streptococcus mutans*
6. Actinomycetes may adhere to other oral bacteria- co-aggregation
7. Teichoic acid and surface proteins of coagulase –negative staphylococcus mediate adherence of the bacterium to prosthetic devices and catheters
8. Flagella act as adhesins in *Vibrio cholerae* and *Campylobacter jejuni*

Binding to Fibronectin

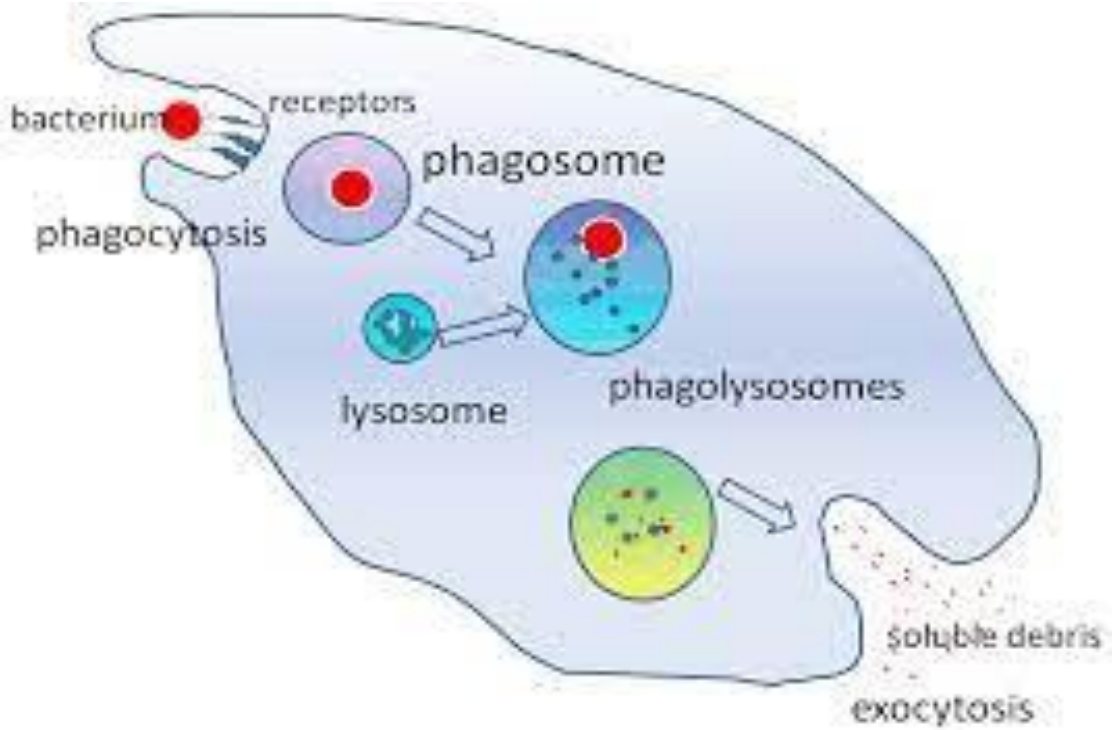
- Fibronectin is a complex multifunctional glycoprotein found in plasma and associated with mucosal cells surfaces where it promotes numerous adhesion functions.
- Many pathogenic bacteria bind fibronection at the bacterial surface.
- *Streptococcus pyogenes*- lipoteichoic acid
- *S. aureus*
- *T. pallidum*

Consequences of adhesion

- ❑ Prevents loss of pathogen from the host
- ❑ Induces structural and functional changes in mucosal cells and these may contribute to disease.
- ❑ Induces changes in bacterial protein synthesis

Invasion

Some bacteria exert their pathogenic effects without penetrating the tissues of the host.
Examples of organisms that are able to invade and survive within host cells are *Salmonella, Shigella, Escherichia*, *Campylobacter, Neisseria*.
It helps to avoid humoral host defence mechanism
Provides a niche rich in nutrients
Devoid of competition from other bacteria.
For some bacteria like *Neisseria meningitides* penetration through or between epithelial cells allows dissemination from the initial site of entry to other body sites.



Uptake into host cells

Many intracellular pathogens use normal phagocytic entry mechanisms to gain access

Shigella invade colonic mucosal cells but rarely penetrate deeper into the host tissues.

Salmonella proceed through the superficial layers of the gut and invade deeper tissues

Role of cell receptors

The availability of specific receptors defines the type of host cells that are involved.
Mycobacterium tuberculosis adhere to complement receptors on the surface of phagocytic cells.
The receptor for *Yersinia pseudotuberculosis* belongs to a family of proteins termed **integrins**.

Avoidance of host defence mechanisms

Pathogenic bacteria have evolved ways to avoiding or neutralizing highly efficient clearance systems.
Since most of the interactions between the bacterium and the immune effectors involve the bacterial surface, resistance to these effects is related to the molecular architecture of the bacterial surface layers.

1. Capsules

Many bacterial pathogens avoid phagocytosis by production of an extracellular capsule.
Most capsules are polysaccharides composed of sugar monomers that vary among different bacteria.
Polysaccharide capsules reduce the efficiency pf phagocytosis in a number of ways-

i. The hydrophilic nature of the capsule may hinder uptake by phagocytosis
ii. Capsule prevent efficient opsonization of the bacterium by complement or specific antibody.
iii. Capsule tend to be weakly immunogenic.

2. Streptococcal M protein

Present in *Streptococcus pyogenes*
It is not a capsule but functions in a similar manner to prevent complement deposition at the bacterial surface.
It binds fibrinogen and fibrin and hinders the access of complement activated by the alternative pathway.

3. Resistance to killing by phagocytic cells

Some pathogens not only survive within macrophages and other phagocytes but may actually multiply intracellularly.
Different organisms use different strategies for survival.
M. tuberculosis is thought to resist intracellular killing by preventing phagosome-lysosome fusion.
Staph. aureus and *N. gonorrhoeae* produce catalase that protect them against toxic oxygen radicals.
The smooth lipopolysaccharide of many bacterial pathogens is thought to contribute to their resistance to the effects of bactericidal cationic peptides present in the phagosome.

4. Immunoglobulin A proteases

Pathogens that cause diseases on mucosal surfaces produce a protease that specifically cleaves immunoglobulin A (IgA) which is the principal antibody type produced at these sites.
Nearly all the pathogens causing meningitis possess an IgA protease and a polysaccharide capsule.

5. Antigenic variation

- Variation in surface antigen composition during the course of infection provides a mechanism of avoidance of specific immune responses directed at those antigens.
- This strategy is most highly developed in blood- borne parasitic protozoa.
- *N. gonorrhoeae* shows antigenic variation in an outer membrane protein known as PII and in its fimbriae.
- The capacity for variation in surface antigens means that antibody produced in response to infection by one strain of a pathogen may not protect against subsequent challenge with a different strain of that bacterium.
- This makes development of vaccines based on inhibition of attachment difficult.

6. Serum resistance

To survive in the bloodstream, bacteria must be able to resist lysis as a result of deposition of complement on the bacterial surface.

In the Enterobacteriaceae resistance is primarily due to the composition of the lipopolysaccharide (LPS) present in the outer membrane.

Smooth colonial variants are more resistant then rough colonial variants.

In *N. gonorrhoeae* complement binds but forms an aberrant configuration in the bacterial outer membrane so that it is unable to effect lysis.

7. Iron acquisition

In order to multiply in body fluids or on mucous membranes bacteria must obtain iron.
Pathogens have evolved very efficient mechanisms for scavenging iron from mammalian iron-binding proteins.
E. coli, K. pneumoniae produce extracellular iron chelators called **siderophores**.
N. meningitides, H. influenzae, have receptors for transferrin or lactoferrin or both on their surfaces.
Bacteriodes species remove iron by proteolytic cleavage of the chelator.
Listeria monocytogenes causes the reduction of iron which reduces the affinity for the chelator.

Bacterial Toxins

- **TOXINS:** Are major determinants of virulence.
- Usually virulent strains of the bacterium produce the toxin while non virulent stains do not.
- **Toxigenesis:** The ability to produce toxins, is an underlying mechanism by which many bacterial pathogens produce disease

- There are two types of bacterial toxins.
- ENDOTOXINS: LIPOPOLYSACCHARIDES - Are associated with the cell walls of gram –ve bacteria
- EXOTOXINS : PROTEINS - Released into the extracellular environment of pathogenic bacteria

Features of endotoxins

- **Endotoxin:**
- Integral part of the cell wall of Gram-negative bacteria. Released on bacterial death and in part during growth.
- Formed only by Gram-negative bacteria
- Lipopolysaccharides. Lipid A portion is responsible for toxicity
- No specific receptor.
- Moderately toxic. Fatal to animals in large doses.
- Relatively heat stable. Toxicity is not destroyed above 60°C for hours.
- Weakly antigenic. Antibodies are protective.
- Not converted to toxoid.
- Synthesis directed by chromosomal genes.
- Usually produce fever in the host by release of interleukin-1 and other mediators

- The physiological effects of LPS toxins include fever, circulatory changes and other general symptoms such as weakness and non localized aches.

Mechanism of endotoxins

Begins with CD14 binding of receptors on Macrophages that:

- 1. Induces cytokine production: IL-1, IL-6, IL-8, TNF, PAF
- 2. Activation of complement cascade (C3a, C5a or alternate pathway)
- 3. Activation of coagulation cascade (Hageman factor; Factor XII)

- All endotoxins can produce the same signs and symptoms:
  - Chills, fever, weakness, general aches, blood clotting and tissue death, shock, and even death Can also induce miscarriage.
- The biological activity is associated with the lipopolysaccharide(**LPS**) . the toxicity is associated with the lipid component (**lipid A**) and the immunogenicity is associated with the **polysaccharide** components.
- Endotoxins are heat stable. But certain powerful oxidising agents such as super oxides, peroxide and hypochlorite are neutralise them.

Although all Gram-negative bacteria have LPS in their cell wall it is not toxic unless it is released from the outer layer of the cell.

Release of endotoxin into the circulatory system can result in **septic shock** characterized by a life threatening severe drop in blood pressure and multiple organ failure.

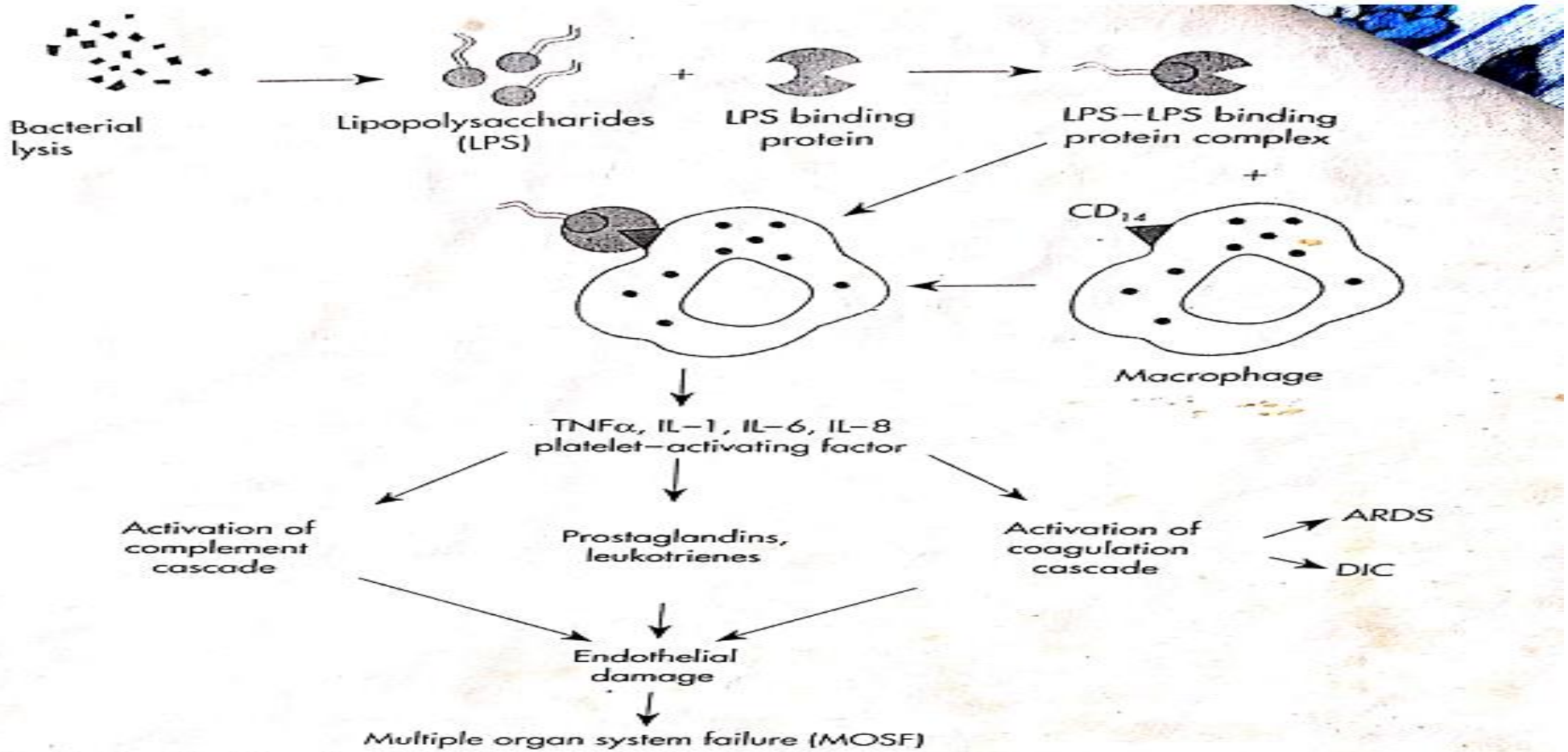


Fig. 13-21 Endotoxin—Underlying Biochemical Events. LPS triggers a series of biochemical mediators that lead to cytokine release, endothelial damage, acute respiratory distress syndrome, and multiple organ system failure. ARDS, Acute respiratory distress syndrome; DIC, disseminated intravascular coagulation.

Features of Exotoxins

Exotoxins:

- Excreted by living cells
- Produced by Gram-positive and Gram-negative bacteria
- Polypeptides
- Usually bind to specific receptors on cells
- Highly toxic. Fatal to animals in very small doses
- Relatively heat labile. Toxicity destroyed over 60°C
- Highly antigenic. Stimulate formation of antitoxin. Antitoxin neutralizes the toxin Converted to toxoid by formalin. Toxoid is nontoxic but antigenic used to immunize, e.g. tetanus toxoid
- Usually controlled by extra-chromosomal genes, e.g. plasmids, phage gene
- do not produce fever in the host

Protein toxins can be classified in various ways-

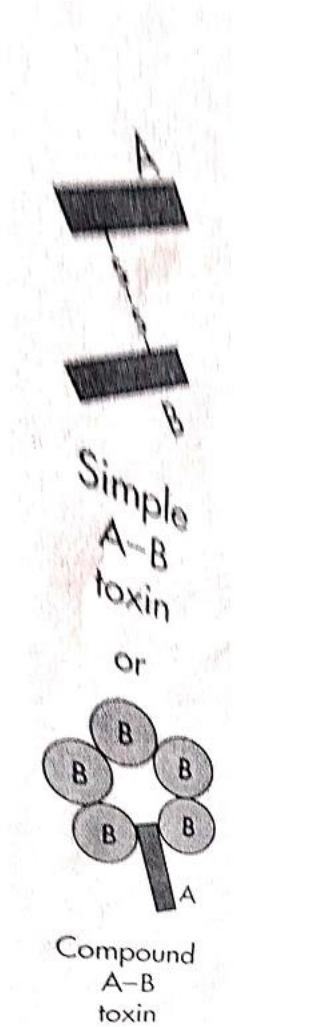
- It can be classified according to the disease they cause like diphtheria toxin, botulinum toxin, etc.
- They can be classified according to the cells they affect like neurotoxins, enterotoxins, cytotoxins and hemolysin.
- Bacterial exotoxins are also classified based on their mechanisms of action such as-

a. **A-B toxins**
b. **Membrane disrupting toxins**
c. **Superantigens** (toxins that stimulate cytokine release by T cells)

The toxin produced by *Staphylococcus aureus* which is responsible for toxic shock syndrome is an example of a superantigen toxin.
Superantigens form many bridges between antigen presenting cells ant T cells, leading to excessively high levels of IL-2 release.
Nausea, vomiting and fever are all physiological responses to high levels of IL-2 in the bloodstream.

A+B subunit arrangement

- Many protein toxins, notably those 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.



1. BACTERIAL PROTEIN TOXINS

A + B Subunit Arrangement of Protein Toxins

- Many protein toxins, consist of two components:
  1. Subunit A: responsible for the enzymatic activity of the toxin.
  2. Subunit B: 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 toxin.
- Isolated A subunits are enzymatically active and but lack binding and cell entry capability.
- Isolated B subunits may bind to target cells (and even block the binding of the native A+B toxin), but they are nontoxic.

Tertiary structure of the pertussis toxin produced by *Bordetella pertussis*. Pertussis toxin is a member of the A-B bacterial toxin superfamily. It is a hexameric protein comprising five distinct subunits,

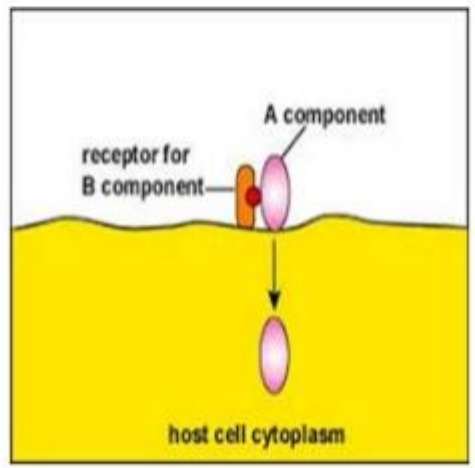
Attachment and Entry of Toxins

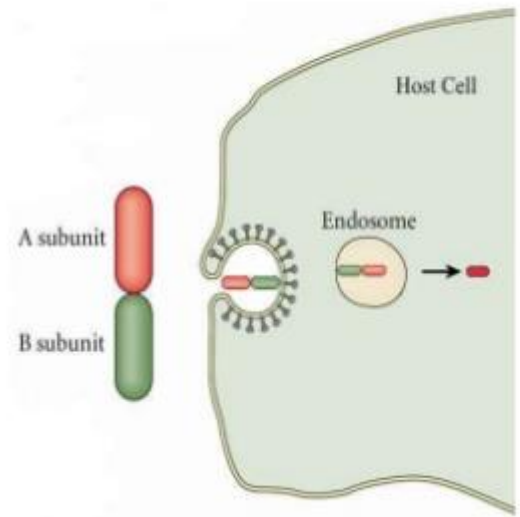
There are at least **two mechanisms of toxin entry into target cells.**

- **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.

- **Alternative mechanism** : receptor-mediated endocytosis (**RME**).

Attachment and entry of toxin





Within the host cell, most activated A portions of A-B toxins remove the ADP-ribosyl group from NAD^+ and add that functional group to a target protein (ADP ribosylation).

Target protein + NAD^+  Nicotinamide + ADP-ribosyl-protein

ADP-ribosylation inactivates the target protein or alters its normal functional activities.
For example- the A region of diphtheria toxin catalyzes the ADP-ribosylation of **elongation factor-2** which is essential for elongation of the polypeptide during translation.

CHARACTERISTICS OF BACTERIAL ENDOTOXINS AND EXOTOXINS		
PROPERTY	ENDOTOXIN	EXOTOXIN
Chemical nature	Lipopolysaccharide (mw = 10kDa)	Protein (mw = 50-1000kDa)
Relationship to cell	Part of outer membrane	Extracellular, diffusible
Denatured by boiling	No	Usually
Antigenic	Yes	Yes
Form toxoid	No	Yes
Potency	Relatively low (>100ug)	Relatively high (1 ug)
Specificity	Low degree	High degree
Enzymatic activity	No	Usually
Pyrogenicity	Yes	Occasionally

A.S. HOZA

Diphtheria Toxin

Diphtheria results from the action of a protein toxin produced by strains of *Corynebacterium diphthe*riae **harboring a temperate phage**.

Only lysogenized cells of *C. diphtheriae* produce diphtheria toxin proteins.

Diphtheria toxin is released from the bacterial cells as a protein composed of 2 polypeptide chains: A and B

Fragment B is required to bind to the eukaryotic cell membrane for fragment A to gain access to the cytoplasm of the cell.

Fragment A catalyzes the transfer of adenosine diphosphoribose (ADPR) from nicotonamide adenine dinucleotide (NAD^+) to eukaryotic elongation factor 2 (EF2) which functions in protein synthesis.

Thus diphtheria toxin effectively inhibits protein synthesis in the host cells.

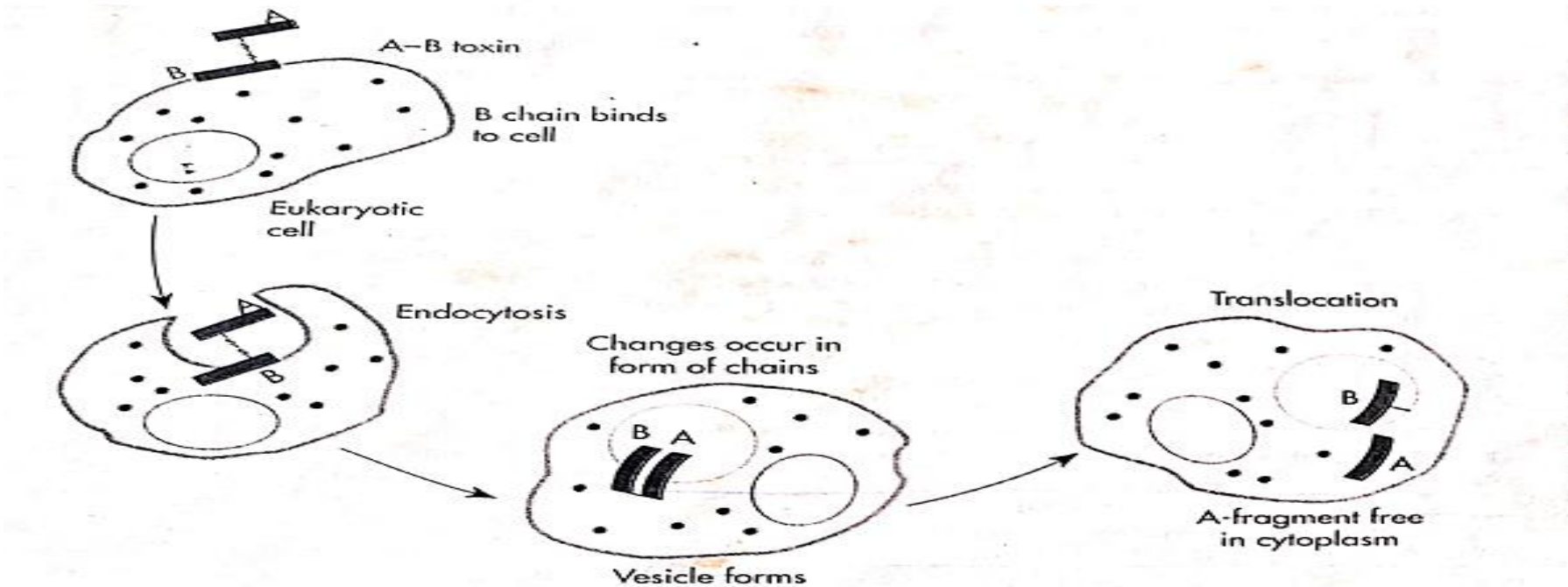


Fig. 13-30 Diphtheria Toxin—Structure and Activation. Diphtheria toxin is an A-B toxin. It enters a target cell by endocytosis. Within a human cell the toxin is cleaved and the activated A portion moves into the cytoplasm where it blocks protein synthesis.

- The bacteria generally do not invade the tissues of the respiratory tract.
- *C. diphteriae* is normally transmitted via droplets from an infected individual to a susceptible host, establishing a localized infection on the surface of the mucosal lining of the upper respiratory tract.
- There is generally a localized inflammatory response, pharyngitis in the vicinity of the bacterial multiplication in the upper respiratory tract.
- In severe infections with *C. diphtheriae* symptoms include low-grade fever, cough, sore throat, difficulty in swallowing and swelling of the lymph glands.
- Complications from diphtheria can block respiratory gas exchange and result in death due to suffocation.
- The widespread use of diphtheria vaccine has greatly reduced the incidence of this disease.
- In immunized individuals, infection with toxigenic strains of *C. diphtheria* is generally restricted to a localized pharyngitis with no serious complications.
- The immunological treatment is augmented by antibiotics such as erythromycin to eliminate the bacterial infection.

Botulinum Toxin

- Botulinum toxin is produced by *Clostridium botulinum*
- It is a neurotoxin because they bind to nerve synapses, blocking the release of acetylcholine from nerve cells of the central nervous system and causing the loss of motor function.
- It is an A-B toxin
- It binds to **specific sialic acid-containing glycoproteins or glycolipids** found in those neurons.
- The active A portion of the toxin is a **zinc requiring endopeptidase** that cleaves specific proteins called **synaptobrevins** that occur in the secretory vesicles of neurons.
- The toxin appears to form a channel in the membrane of the neuron, leading to its **inability to release the neurotransmitter acetylcholine.**
- The inability to transmit impulses through motor neurons can cause respiratory failure resulting in death.

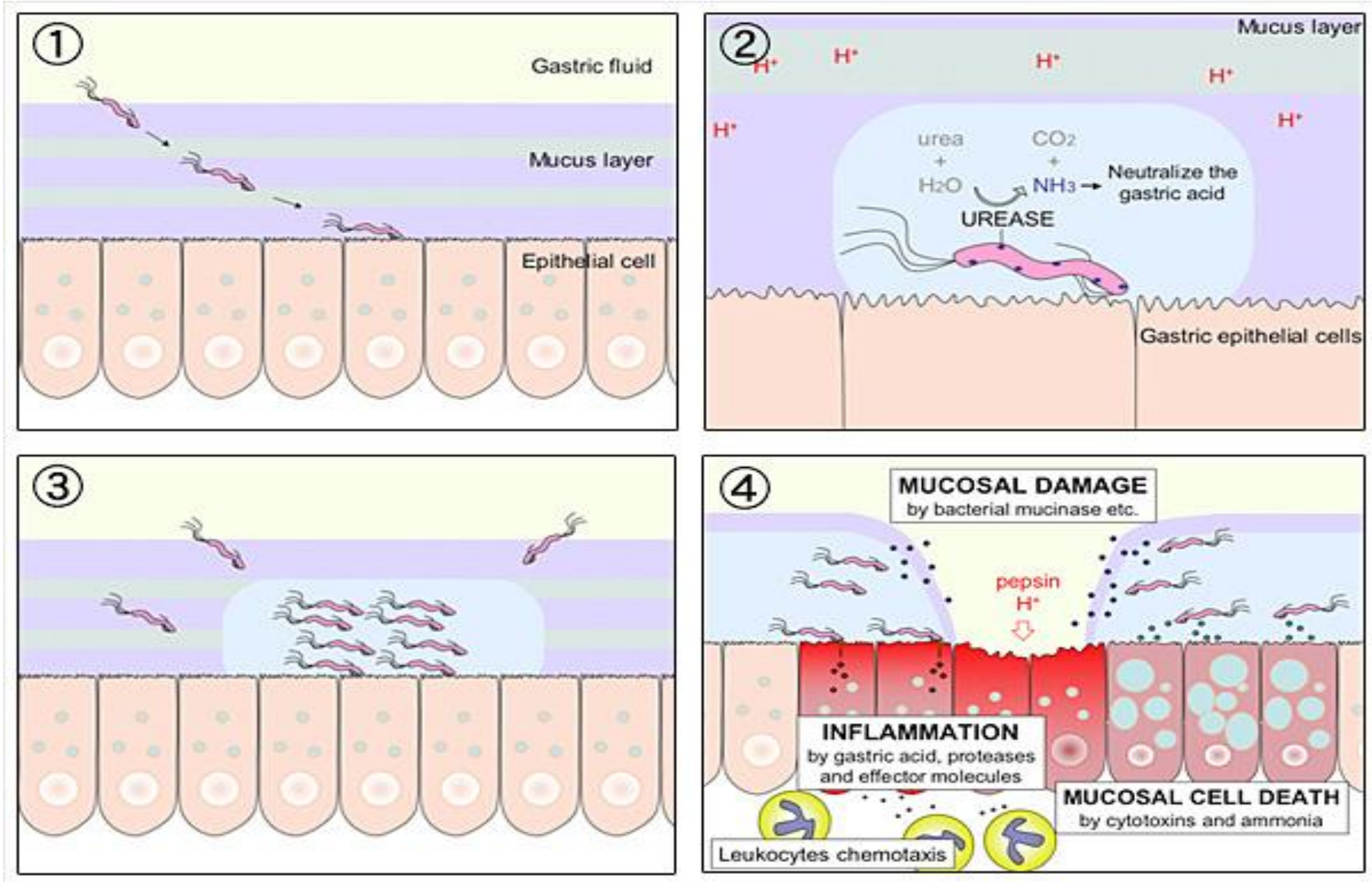
- There are **seven types of botulinum** toxin, designated A through G.
- Types **A, B** and **E** cause food poisoning of humans.
- Symptoms of botulism can appear 8-48 hours after ingestion of the toxin.
- Early symptoms are nausea, vomiting, headache and double vision.
- Subsequently the toxin causes severe impairment of nervous system functions resulting in **flaccid paralysis.**
- The use of trivalent ABE antibodies is useful in treating this disease.

Shiga Toxin

- The toxin produced by *Shigella dysenteriae* is called 'Shiga toxin' and it is a neurotoxin.
- **It interferes with the circulatory vessels that supply blood to the central nervous system** rather than affecting the nerve cells directly.
- Any neurological effects of the Shiga toxin are thus secondary to the primary action of the toxin on the vascular circulatory system which is targeting the endothelial cells of the kidney.
- Shiga toxin also acts as a cytotoxin and enterotoxin.
- **Shiga toxin is an A-B toxin that is released when cells *of S. dysenteriae* lyse**.
- The B portions of Shiga toxin bind to cell surface glycolipids.
- Shiga toxin **does not catalyze ADP-ribosylation** but it rather blocks host cell protein synthesis by **cleaving the N-glycosidic bonds of a single specific adenosine residue in 28S rRNA,** a component of the 60S ribosomal subunit of the eukaryotic human cell.
- Cleavage of the 28SrRNA at this site prevents binding of aminoacyl-tRNAs and thus halts protein synthesis.
- There is severe diarrhea and fluid loss during infections with *Shigella dysenteriae*.
- Damage to the circulatory system also causes kidney failure, a disease condition known as hemolytic uremic syndrome.

Helicobacter Cytotoxins

- *Helicobacter pylori can produce cytotoxins that cause formation of peptic ulcers.*
- *Chronic infections* with *H. pylori* may cause stomach cancer.
- The organism produces urease, an enzyme that breaks down ureas into ammonia.
- Ammonia neutralizes gastric acids, permitting survival of *H. pylori* in the stomach and the upper region of the small intestine.
- The cytotoxins cause formation of vacuoles within mucosal cells lining the stomach and small intestine.
- Killing of cells in the mucosal lining removes the protection against gastric acid and that exposure to HCl and pepsin in the gastric fluid causes ulcer formation.
- Duodenal ulcers occur in men between ages 20 and 50.
- Gastric ulcers are common in middle-aged and elderly men, especially those who are malnourished, alcoholics and chronic users of aspirin.


①
Gastric fluid
Mucus layer
Epithelial cell
②
Mucus layer
H+
urea + H2O
CO2 + NH3
Neutralize the gastric acid
UREASE
Gastric epithelial cells
③
④
MUCOSAL DAMAGE
by bacterial mucinase etc.
pepsin
H+
INFLAMMATION
by gastric acid, proteases and effector molecules
MUCOSAL CELL DEATH
by cytotoxins and ammonia
Leukocytes chemotaxis

Tetanospasmin

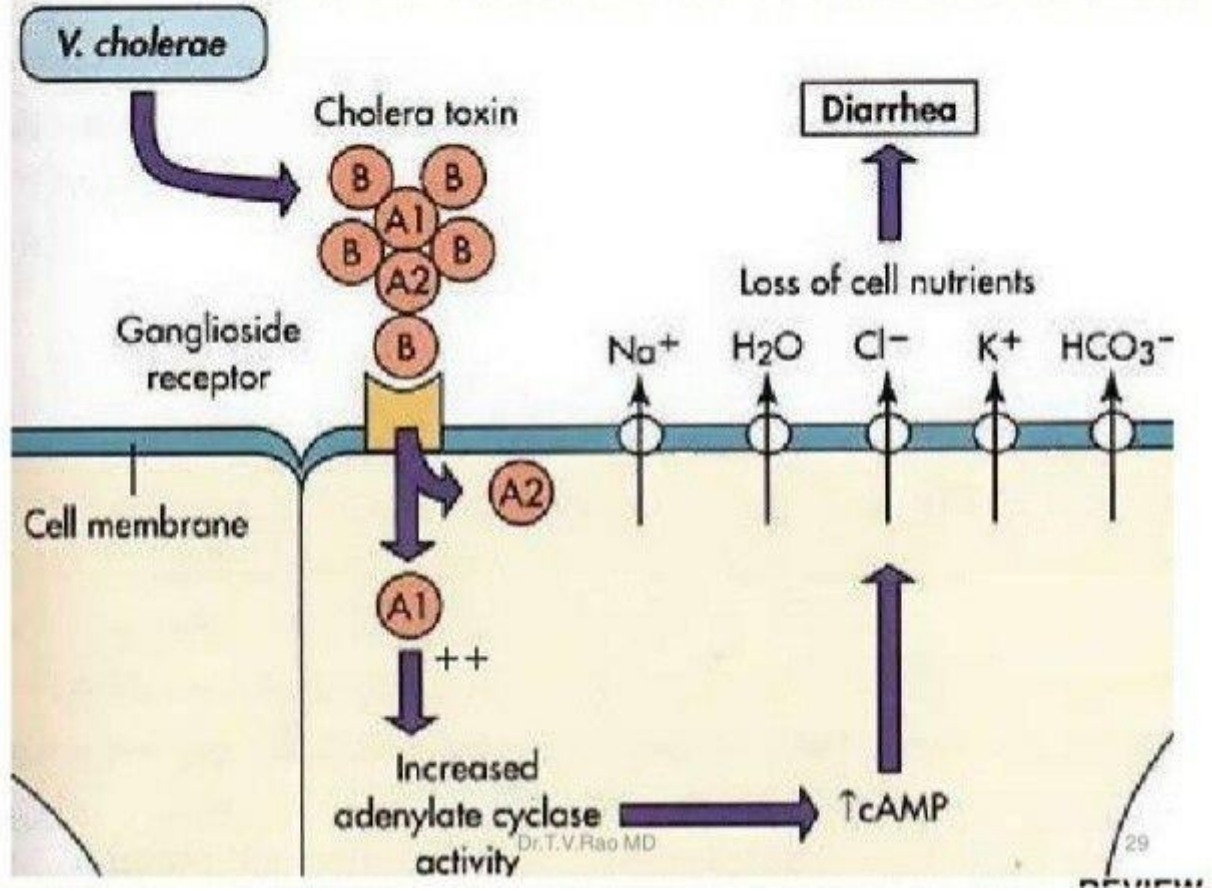
The neurotoxin tetanospasmin, produced by *Clostridium tetani* interferes with the peripheral nerves of the spinal cord.
Tetanospasmin inhibits the ability of the nerve cells to properly transmit signals to the muscle cells, causing the symptomatic spastic paralysis.
Like the neurotoxin of *C. botulinum*, the neurotoxin of *C. tetani* also paralyzes motor neurons but it act only on the nerves of the central nervous system.
Tetanus toxin is also an A-B toxin that is activated by protease cleavage.
There are conserved amino acid sequences between tetanospasmin and botulinum toxin.
Also the active portion of both toxins are zinc requiring endopeptidase.
Tetanus toxin inhibits the release of **glycine or γ -aminobutyric acid** from the inhibitory neurons (interneurons) in the anterior horn of the spinal cord.
Spastic paralysis occurs because muscles are unable to relax between nerve impulses.
Tetanus is sometimes referred to as **lockjaw** because the muscles of the jaw and neck contract convulsively so that the mouth remains locked closed making swallowing difficult.

C. tetani is widely distributed in soil.
Transmission to humans normally occurs as a result of a puncture wound that inoculates the body with the spores of *C. tetani*.
If anaerobic conditions develop at the site of the wound, the endospores of *C. tetani* germinate and the multiplying bacteria produce neurotoxin.
C. tetani is noninvasive and multiplies at the site of inoculation only.
The neurotoxin however spreads systemically causing the symptoms of this disease.
If untreated tetanus is frequently fatal.
Tetanus can be treated by the administration of tetanus antitoxin to block the neurotoxin.
The disease can be prevented by immunization with tetanus toxoid.

Cholera Toxin

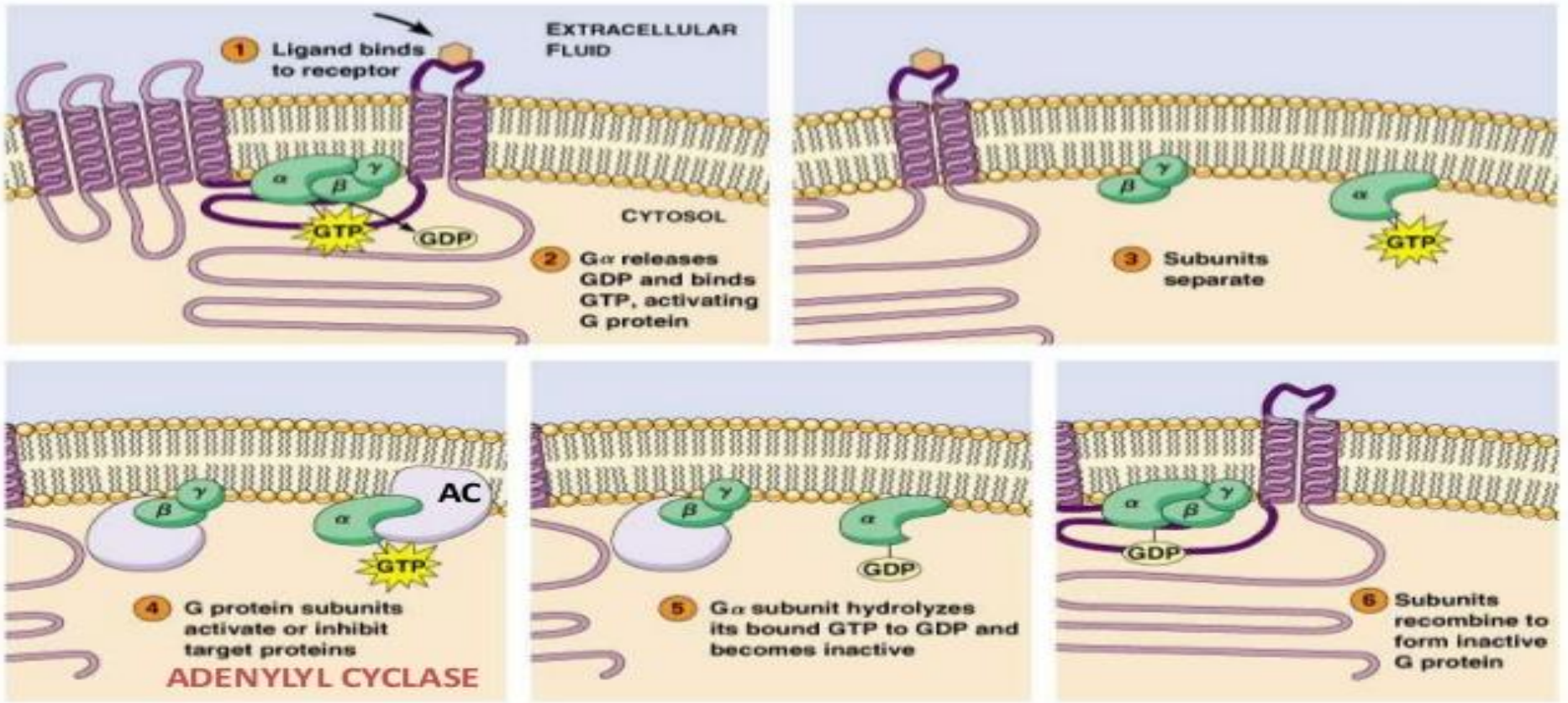
- The toxin produced by *Vibrio cholera* causes cholera.
- This toxin called cholera toxin or choleragen is an enterotoxin.
- Growth of this bacteria within the gastrointestinal tract leads to severe diarrhea and life-threatening shock due to electrolyte imbalance.
- Cholera toxin is an **A-B toxin** that has **5 B** subunits and **one A** Subunit (composed of portions A1 and A2).
- The subunits are assembled in the periplasm and the intact toxin is excreted through the outer membrane.
- The A1 portion of the A subunit triggers **increased adenylcyclase act**ivity that is responsible for the disease symptoms.
- Excreted toxin binds to **G_{M1} gangliosides** on host mucosal cells.
- Activation of the toxin involves protease cleavage of the A portion to form subunits A1 +A2.
- The A1 subunit is translocated by an unknown mechanism into the host cell cytoplasm where it **ADP-ribosylates a membrane protein, G_s.**
- G_s regulates adenylate cyclase by controlling the amount of cAMP in the host cell.
- ADP-ribosylation of G_s prevents this regulation, so cyclic AMP rises to high levels, disrupting the functions of ion pumps and creating an ion imbalance that leads to diarrhea.

Mechanism of Action of Cholera Toxin



Adenylatecyclase is activated by GTP bound to its regulatory subunit.
Hydrolysis of adenylatecyclase-GTP complex inactivates adenylcyclase.
Choleragen transfers ADP-ribosyl to the adenylcyclase regulatory subunit, inhibiting its ability to hydrolyze GTP.
Adenylcyclase therefore remains in the active state and continues to make cyclic AMP.
The resulting elevated concentrations of cyclic AMP cause the release of inorganic ions including chloride and bicarbonate ions from the mucosal cells that line the intestine into the intestinal lumen.
The change in the ionic balance resulting from the action of this toxin causes the movement of large amounts of water into the lumen in attempt to balance the osmotic pressure.
This leads to severe dehydration that sometimes results in the death of infected individuals.
The rapid loss of fluid from the cells of the gastrointestinal tract associated with this disease often produces shock.
If untreated the mortality rate is high.
The symptoms of cholera include nausea, vomiting, abdominal pain, diarrhea with ‘rice water stools’ and severe dehydration followed by collapse, shock and in many cases death.
V. cholerae itself does not invade the body and is not disseminated to other tissues.
Treatment of cholera centers on replacing fluids and maintaining electrolyte balance.
Treatment with tetracycline generally reduces the duration of the disease.

Activation of G Proteins



Escherichia coli Enterotoxin

There are several different pathogenic strains *of E. coli* that are classified based on their virulence.

1. **Enterotoxigenic *E. coli* (ETEC)**
2. **Enteropathogenic *E. coli* (EPEC)**
3. **Enterohemorrhagic *E. coli* (EHEC)**
4. **Enteroinvasive *E. coli* (EIEC)**

Enterotoxigenic *E. coli* (ETEC)

- ETEC strains produce two enterotoxins (**LT** and **ST**).
- ETEC strains cause persistent diarrhea in infants , particularly in developing countries.
- LT-1 , the toxin associated with human disease has 75% amino acid identity with cholera toxin.
- It has the same structure, (five B and one A subunits).
- It has the same mechanism (ADP-ribosylation of G_s) and interacts with the same receptor (G_{M1}).
- Like cholera toxin, the heat labile enterotoxins produced by *E. coli* **stimulate adenylcyclase activity in the small intestine epithelium.**
- This in turn results in increased permeability of the intestinal lining, which causes loss of body fluids electrolytes.
- Genes for LT and ST are carried on plasmids along with the genes for CFAs.

- ST is a family of small peptide toxins.
- STa is the main contributor to ETEC diarrhea.
- STa acts as a hormone analog and **stimulated host cell guanylate cyclase**, resulting in an increase in intracellular cGMP levels.

- Enterotoxin producing strains of *E. coli* are capable of causing mild to severe forms of enterocolitis.
- In most cases, enterotoxin producing strains of *E. coli* do not invade the body through the gastrointestinal tract; rather, heat-labile and heat-stable toxins released by cells growing on the surface lining of the gastrointestinal tract cause diarrhea.
- The disease syndrome is often called **traveler's diarrhea**.
- From United states to Mexico and vice versa, people often suffer severe diarrhea as a result of ingestion of strains of *E. coli* foreign to their own microbiota.
- Therefore not drinking the water in different locales may prevent this form of enterocolitis.
- Adults in regions where ETEC strains are prevalent develop immunity, which is why only visiting travelers usually get sick.

Enteropathogenic & Enterohemorrhagic *E.coli* (EPEC) & (EHEC)

- In some cases, enteropathogenic strains of *E.coli* invade the body through the mucosa of the large intestine to cause a serious form of dysentery.
- Enterohemorrhagic *E. coli* strains such as *E. coli* O157:H7 invade the body through the mucosa of the large intestine and cause severe form of diarrhea and also cause hemolytic uremic syndrome (HUS) which can result in kidney failure.
- EHEC strains are similar to EPEC strains except that EHEC strains produce a toxin similar to Shiga toxin (Shiga-like toxin).
- Shiga- like toxin may be responsible for the bloody diarrhea in HUS associated with EHEC strains.
- Shiga- like toxin of EHEC strains is encoded by a gene located on a temperate phage.

Enteroinvasive *E.coli* (EIEC)

- ✓ Enteroinvasive *E. coli* (EIEC) strains cause a disease identical to that caused by *Shigella* spp but do not produce Shiga toxin.
- ✓ Invasive strains of *E. coli* are primarily associated with contaminated food and water in Southeast Asia and South America.
- ✓ The ability to invade the mucosa of the large intestine depends on the presence of a specific K antigen in enteropathogenic serotypes of *E. coli*.
- ✓ HUS is not a complication of EIEC infection.
- ✓ The location of genes for virulence of EIEC occur on a large virulence plasmid