Principles of Microbiology & Immunology

SEMESTER 1

Koch's other Achievements

- Koch and co-workers also developed basic microbiological cultivation techniques:
    - The use of agar and Petri dishes:
        - It produced pure cultures, which were essential for the success of his work with infectious diseases.
    - Perfected methods for sterilisation, disinfection and filtration
    - Discovered the causative agents of tuberculosis, cholera and many other important human diseases
    - Established a set of rules (postulates) to link a specific microorganism with a particular disease.

Koch's Original Postulates

	Postulates	Experimentation
1	The microorganism must be present in every case of the disease but absent from healthy hosts.	Koch developed a staining technique to examine human tissue – *Mycobacterium tuberculosis* could be identified in diseased tissue.
2	The suspected microorganisms must be isolated and grown in a pure culture.	Koch grew *M. tuberculosis* in pure culture on coagulated blood serum.
3	The same disease must result when isolated microorganisms is inoculated into a healthy host.	Koch injected cells from pure culture into guinea pigs – guinea pigs subsequently died of *tuberculosis*.
4	The same microorganism must be isolated again from diseased host.	Koch isolated *M. tuberculosis* in pure culture on coagulated blood serum from dead guinea pig.

- This was not feasible for many reasons:
    - Some pathogens were a part of normal microbiota.
    - Some pathogens cannot be grown or cultured.
    - Some pathogens grow or cause disease only in humans.
    - Sometimes more than one pathogen is involved in causing a disease.
        - E.g. Polymicrobial Diseases (I.e. periodontal disease).
    - It doesn't take into account of the immune status of the individual.
        - E.g. Very young or elderly individuals, or immunocompromised.

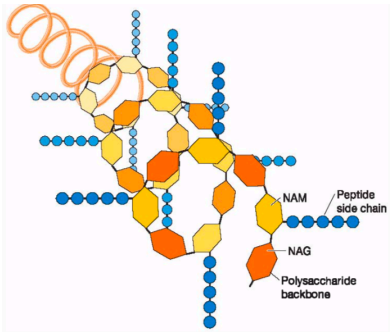
Koch's Molecular Postulates

- Emphasis on virulence genes (VG):
    - Genes which encode the characteristics that allow microorganisms to cause disease.

Molecular Postulates
VG must be found in pathogenic strains of microbes.
VG must be expressed during an infection or disease.
Mutation (or deletion) of the VG decreases pathogenicity.
Replacement/restoration of the VG mutation/deletion restores pathogenicity.

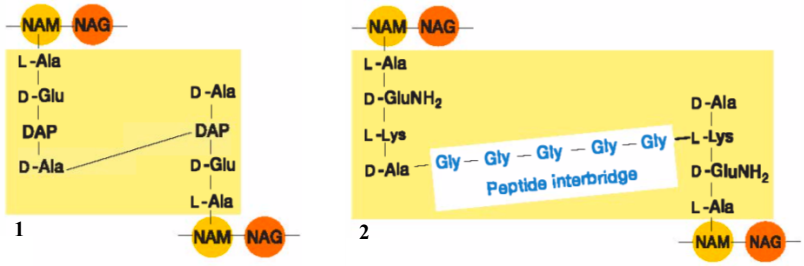
Peptidoglycan

- Forms an enormous mesh-like tensile structure.
- The helical peptidoglycan strand consists of two alternating sugar derivatives: N-Acetyl-Glucosamine (NAG) and N-Acetyl-Muramic acid (NAM) and several different amino acids.
    - The amino acids form a short peptide consisting of four alternating D- and L-amino acids.
    - This peptide is connected to NAM as shown below.



- The peptidoglycan 'mesh' is formed by linking the sugars of the helical peptidoglycan subunits together through cross-linking.
- Individual peptidoglycan strand can be cross-linked to another strand directly in two ways:

1. Direct linkage – Covalent bond between terminal **D-Ala** and **DAP**.
2. Peptide interbridges – Pentaglycine between terminal **D-Ala** to **L-Lys**.



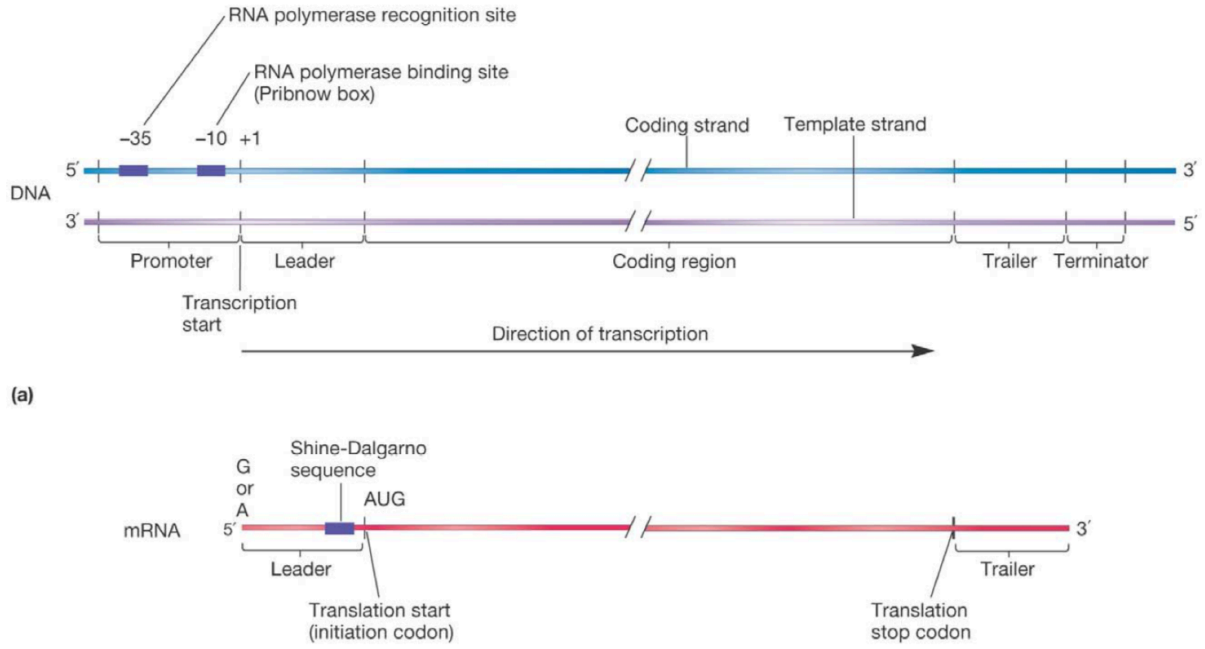
- Level of cross-linking varies between bacteria:
    - GP bacteria: More cross-linking in peptidoglycan → Less porous.
    - GN bacteria: Less cross-linking in peptidoglycan → More porous.

Mechanism of Gram Staining

1. Filtration – fix onto glass slide – emulsify with water then dry out with heat block.
2. Crystal violet – This gets into bacterial cell and makes it purple.
3. Iodine treatment – Binds to crystal violet and forms complex in bacterial cell wall.
4. Decolourisation – Exposure to alcohol or acetone will have different effects depending on the bacteria:
    a. **GP**: The pores around the crystal violet complex will shrink and retain the colour.
    b. **GN**: Due to more porous peptidoglycan layer, it will become colourless.
5. Counterstain with safranin
    a. Crystal violet iodine complex will stay in gram positive – Cell → Purple.
    b. Crystal violet iodine complex will be removed in gram negative – Cell→ Pink

Gene Structure

- Gene: Polynucleotide sequence that codes for a functional product – proteins, tRNA, rRNA, sRNA.
- Bacterial gene has 5 main regions:
    - Promoter: RNA polymerase recognition/binding site – It orients RNA polymerase, so it is ready for transcription.
        - It is NOT transcribed or translated.
        - There is variation in promoter sequences, which allows for variation in expression (covered later).
        - There are 2 binding regions, one at -35 and -10 → Called Pribnow box.
            - The numbers indicate 'upstream of ATG start codon' of the gene.
    - Leader: A region of DNA located between the Promoter and the coding region.
        - It is transcribed but NOT translated.
        - It directs the ribosome to bind there. I.e. It gives ribosome something to grab on to.
        - It contains the transcription start site.
    - Coding Region: Both transcribed and translated into protein.
        - It is bounded by the start and stop codons which mark translational start/stop.
    - Trailer: Region immediately following the stop codon.
        - It is transcribed, but it signals the end of the protein during translation → stops the ribosome
        - It is required for proper coding and expression of gene.
    - Terminator: Signals RNA polymerase to stop transcription → RNA polymerase dissociates from DNA.



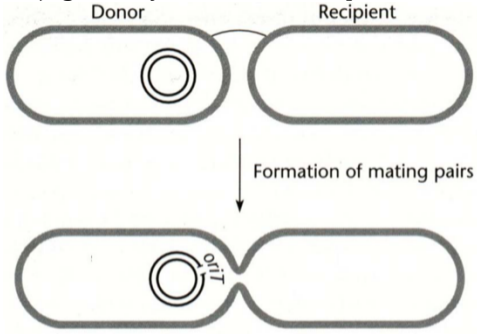
Mutation and Repair

- Despite DNA polymerase III holoenzyme's ability to proofread, wrong bases are sometimes added into the complementary strand → Not always perfect.
    - Error rate: 1 in a million base pairs.
- There are many repair mechanisms to remove mistakes but aren't always 100% effective.
- The consequence of this error may be catastrophic, beneficial or silent.
- This type of error is spontaneous and nature → So, it is named, Spontaneous mutations.
- Induced Mutations: Exposure to a mutagen (any agent that damage DNA) can induce mutations.

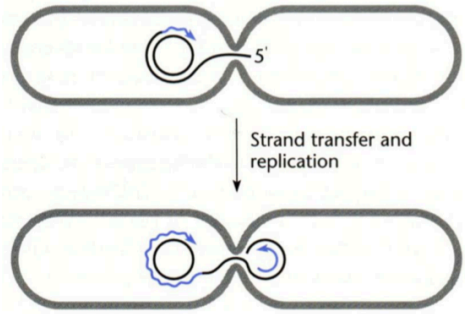
Conjugation

- Some plasmids can transfer themselves and other DNA elements from one cell to another in a process called conjugation.
- In conjugation, we have a donor cell that contains conjugative plasmids (or the cell containing the plasmid capable of conjugation) and we have a recipient cell that receives the conjugative plasmids.
    - A recipient cell that has received DNA as a result of conjugation is called transconjugant.
- Conjugative plasmids can be either broad or narrow host range.
    - Broad host range plasmids are known as promiscuous plasmids.
    - I.e. Plasmids that can move between different species.
- The process of conjugation, or the mechanism of DNA transfer works like this:

1. The donor cell produces a pilus (encoded by the plasmid tra (for transfer) genes).
2. The pilus contacts a potential recipient that does not contain the plasmid.
3. Retraction of the pilus brings the cells into close contact and a pore forms in the adjoining cell membranes.
    - However, not all conjugation systems involve a pilus in mating pair formation.



4. Formation of the mating pair signals the plasmid to begin transfer.
5. The rolling circle replication is generally the mechanism by which conjugative plasmids transfer themselves from one cell to another.
    - As the complementary DNA strand is being synthesized, it moves through the pore



Transformation

- Transformation is the uptake of naked DNA (DNA that is in the environment surrounding a bacterial cell) by the bacteria.
- It is a random process → any part of the genome can be taken up.
- Can be controlled/used in the laboratory.
- Bacteria that are able to take up naked DNA are called competent.
- A bacterium that has taken up DNA is called a transformant.

T Cell Help for CD8 T cells

- Helper T cells can become activated and then upregulate B7 and produce IL-2 cytokines to help activate CD8 T cells.
    - DCs activates CD4+ T cells, which in turn enhance the co-stimulatory activity of DCs.
    - DCs with enhanced B7 expression activates CD8+ T cells directly.
    - Activated CD4+ T cells will then secrete IL-2, which directly acts on activated CD8+ T cell.
- Thus, cytotoxic T cells rely on the IL-2 cytokine to proliferate and become an effector cell.

T Cell Help for Macrophages

- Infected macrophages express CD40 and MHC Class II + Antigen.
- Helper T Cells bind to macrophage via TCR (and CD40L co-stimulation) and it results in the release of cytokine IFN-γ.
- Macrophage becomes activated and lysozymes fuse with phagosomes exposing intravesicular bacteria to lysosomal enzymes.

What Type of Immune Response?

- We need different responses for different types of microbes and location; E.g.:
    - Viruses → Cell-mediated Immune Response
    - Bacteria → Humoral Immune Response + Macrophages
    - Worms → Humoral Immune Response (IgE) + Basophiles + Eosinophils
- The cells of the innate immune response can provide cues that direct the subsequent adaptive immune response (via different cytokines).
- For example:
    - Once a CD4 T cell is activated (from naïve CD4 T cell), it is initially known as T_H0 cell.
    - Depending on the cytokines produced by the innate cells, T_H0 cells can be directed into different types of T Helper Cells:
        - T_H1 → Strong cell-mediated responses (e.g. help to activate macrophages, CD8 T cells).
            - T_H1 cells produce IL-2 (→ Activate CD8 T cells), or IFN-γ (→ Activate Macrophage and/or induce isotype switching in B cells to IgG).
        - T_H2 → Strong humoral (antibody) responses.
            - T_H2 cells produce IL-4, IL-5, IL-6 which stimulate different things in B cells.
    - If innate cells produce IFN-γ / IL-12 / IL-18 cytokines, the T_H0 → T_H1
    - If innate cells produce IL-4 cytokines, the T_H0 → T_H2
- During a Viral Infection:
    - Innate cells will generally produce IFN-γ around T_H0 cells, which turns T_H0 cells into T_H1 cells.
    - The T_H1 cells would then produce their cytokines for anti-viral response, proliferation of CD8 T cells, and cause B cells to switch their antibody isotype to IgG (an opsonising antibody).
- During a Bacterial Infection:
    - The innate cells may produce a little bit of IFN-γ → Some T_H1 cells (for macrophage activation and IgG antibody → enhance phagocytosis).
    - But it will mostly produce IL-4 to turn T_H0 cells into T_H2 cells for neutralising antibody production.
- During a Parasite Infection (Worms):
    - The innate cells will produce IL-4 to activate T_H0 cells into T_H2 cells.
    - The T_H2 cells would then produce IL-4 cytokines to induce isotype switching in B cells to produce IgE antibodies (→ important for parasite infection).
        - It can also cause degranulation of mast cells, eosinophils and basophils.

Colonisation of the Host (Step 2)

- In order for a bacterium to be pathogenic, it needs to colonise the host.
- Colonisation is the process whereby pathogens establish themselves within the host.

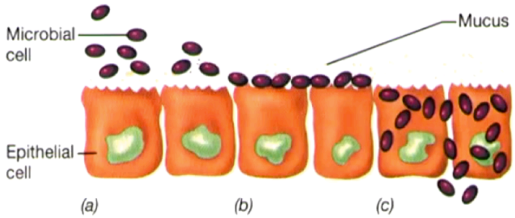
    - They must overcome the physical barriers and innate immune response.
    - Must out-compete the normal microbiota.
    - It normally requires adherence of the bacteria to a mucosal surface.
        - This can require the use of structures on bacterial cell surface to mediate adherence.
        - The mechanism of adherence is normally both host-specific and tissue-specific.
            - Host-specific: A particular bacterial pathogen is not going to be able to cause infection, for instance, in every mammal known.
                - I.e. it may cause disease in humans, but not others.
            - Tissue-specific: If it causes disease in an organism, it may only cause infection in one particular type of surface in their body, but not multiple types of surfaces.

Adherence to Host Cells

- Adhesion is mediated by external structures on the surface of bacteria called adhesins:

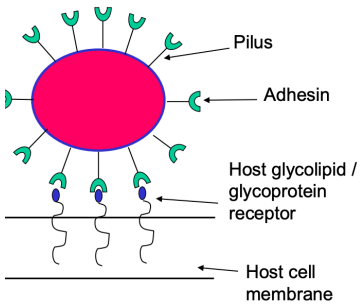
    - Pili (Fimbriae): Establish 'loose' or non-intimate adhesion.
    - Afimbrial adhesins: Establish 'close' or intimate adhesion.

- One type of pathogen may produce multiple adhesins.
- The process of adhesion is as followed:



(a) 'Loose' attachment of the bacteria to the mucosal surface.
(b) 'Close' adhesion of bacteria to epithelial cells.
    - Gastrointestinal epithelial cells are interchangeably used with enterocytes.
(c) Invasion of epithelial cells or through the tight junctions between cells.
    - It is important to recognise that this step does not happen with all bacteria (only some do).
    - So, many bacterial pathogens will remain in the lumen or the surface of our gut.
    - I.e. Not all bacteria will colonise us and have step (c) as the end result; many bacteria that colonise in us, will not go past step (a) or (a) and (b); but still cause disease.

Pili / Fimbriae



- These structures facilitate loose adherence to the host cell. They are:

    - Complex structures.
    - Rod shaped, hollow cylinder.
    - Ordered helical array of protein subunits of pilin.

- Tip of the pilus mediates adhesion:

    - The tip may be composed of pilin protein (the same that make up the entire pili) or other proteins.
    - Confers host cell / tissue specificity → The tip proteins will bind to certain carbohydrates on the surface of host cells, such as glycolipids or glycoproteins (these are what we call host cell receptors)

- May be all over the cell (peritrichous) or polar (located at one end only).

Avoid Detection by Antibodies (Evade Host Defences – Step 3)

- In addition to avoiding complement fixation, pathogens can also avoid detection by antibodies:

1. Remain inside host cells: Strategy used by viruses and some bacteria.
   - By remaining inside host cells, there is less chance of being recognised by soluble components of the immune system such as antibodies, but also cellular recognition.
     - They must **NOT** display antigen on host cell surface.
     - Because as soon as the host cell displays pathogen's antigen, it will be recognised as foreign by the immune system.
   - They must be able to survive in harsh intracellular environments.
     - There are some immune system recognition pathways which operate within the cells that can recognise and be triggered by bacterial and viral infections etc.
     - So, these pathogens either have to be able to shut down those processes or to be able to get out of that environment.

2. Host mimicry: Pathogens may possess a component like protein or structure on their bacterial cell surface that is highly similar to or even the same as the components of our own cells.
   - As they are similar or same as our cells, the immune system won't mount an immune response against it.
   - E.g. Neisseria meningitidis type B has capsules made of sialic acid which is a common component of human cell surface sugars (glycocalyx) or gangliosides.
     - There is extremely poor immune response to type B capsule because they are similar.
     - Thus, allows bacteria to flourish in the blood stream → high population.
       - Overtime they will begin to die and because they are gram negative, they will release huge amounts of LPS (endotoxin) → septic shock.

3. Colonise privileged site: Bacterial infection in areas with poor access for immune system:
   - Sterile site – e.g. CSF.
   - Also, other privileged site can be created by the bacteria.
     - E.g. Granuloma can result after a bacterial infection is established (M. tuberculosis).
     - When immune cells surround the area of bacteria growth to constrain and control it, they might become calcified and form an impenetrable area for immune cells.
   - Also, Bacteria can form and reside in biofilms → An important mechanism that enables them to establish an infection while resisting soluble factors like antibodies.
     - Pseudomonas aeruginosa – a ubiquitous, water loving, opportunistic pathogen.
       - It causes infection in lung by forming biofilm.
     - Salmonella Typhi – GI pathogen that causes typhoid fever → life threatening fever.
       - It forms biofilms in the gall bladder
       - People who survive this infection can be lifelong carriers and constantly shed it in their faeces.
       - Now there is a vaccine, but in the past people had to remove their gallbladder.

4. Antigenic Variation (or phase variation): Some bacteria can change its antigenic surface (or the type of antigen displayed on their surface), so, it goes through cyclic patterns of recognition and non-recognition.
   - Majority will display one type of antigen on their surface, but there will be some cells in that population that will express change antigenic surface through mutation, recombination, gene switching.
   - E.g. Plasmodium falciparum, some bacterial pathogens and viruses do this.

5. Destroy antibodies: Pathogens can also destroy antibody molecules to avoid detection by antibodies.
   - Remember that IgA functions as a dimer (even though it is produced as monomers initially) and they are secreted on mucosal surfaces and breast milk.
   - Some bacteria possess enzymes called specific IgA protease that cleaves the hinge of IgA antibodies and cause IgA antibodies to lose their function.
   - It is an uncommon method, but still possible.

Avoid Phagocytosis (Evade Host Defences – Step 3)

- Pathogens have multiple ways to subvert phagocytosis:

1. Kill phagocyte with a toxin: Bacteria kills the phagocyte before it kills it.
    - Staphylococcus aureus → produce haemolysin that punctures hole in the phagocyte membrane surface.
    - Salmonella Typhimurium (Not same as Typhi) → Induces apoptosis of phagocyte.
2. Prevent opsonisation: They can do this by:
    - Binding to antibodies in the wrong way → preventing complement activation.
        - E.g. Protein A of staphylococcus aureus.
    - Prevent antibodies from binding to their surface through a capsule.
    - Coat themselves with host protein → hide from immune system.
    - E.g. Streptococcus pyogenes break down C5a which is a chemoattractant for phagocytes.
3. Prevent contact with phagocyte (capsule): Capsule prevents direct binding of phagocyte and opsonisation by antibodies and complement.
4. Inhibit phagolysosome fusion: Pathogen prevents maturation of phagosome to phagolysosome.
    - Pathogen avoids exposure to toxic lysosomal components by this process.
    - Because they block fusion of phagosome with lysosome, the phagosome fails to acidify.
        - E.g. Salmonella Typhimurium invades cells and replicates within a vacuole. The pathogen prevents fusion of lysosome with its vacuole.
5. Escape into the cytoplasm: Some can lyse themselves out of phagosomes and escape into the cytoplasm of the host cell and this allows the pathogen to avoid toxic lysosomal contents.
    - E.g. Listeria monocytogenes – following invasion by zipper mechanism, Listeria lyses out of phagolysosome into the cytoplasm and replicates.
6. Organism resists killing by producing anti-oxidants and / or inhibiting the respiratory burst.
    - Pathogens may produce certain catalase that breaks down products of respiratory burst.
    - Some may also prevent activation of respiratory burst.
        - E.g. Salmonella Typhimurium prevents assembly of NADPH oxidase on the phagosomal membrane.

Multiply and Disseminate

- In terms of multiplying, and growth for dissemination, there are major impediments (hindrance) which the bacteria may encounter:
    - Normal microbiota → acts as competitive force for colonisation space and nutrients → protecting against foreign invaders.
    - Pathogenic bacteria are chemoheterotrophs, so they need to secure a source of nutrients in the environment to grow, multiply and cause disease.
        - Iron is an essential nutrient for microbes (without it, they can't grow), but iron is often sequestered away in insoluble forms or in conjunction with high affinity proteins (e.g. Hb).
- Dissemination:
    - Pathogens must escape the effects of normal microbiota / immune system.
    - Then, they might be free to travel to immune privileged or other body sites via different mechanisms → Open up new sources of nutrients.
        - In terms of moving around the body, they often move around by infecting macrophages.

A-B Toxin - Compound

- An example of compound A-B toxin is the Cholera toxin produced by Vibrio cholerae and it causes severe diarrhoea in infected people.
    - The disease is a toxin mediated disease, but it requires the colonisation of GI tract.
    - It is a waterborne disease → Infection from drinking water contaminated with faeces → Faecal-oral transmission route.

- How this toxin works:
    - AB_5 toxin binds via the B subunit to the host cell membrane and is internalised into a vacuole where the low pH cleaves off the toxigenic unit, and it is actually the A_1 subunit that possesses the toxigenic activity and A_2 subunit is the bystander.
    - The A1 subunit causes ADP-ribosylation of GTPase present in the host cell.
        - A1 subunit is an ADP-ribosyltransferase.
        - GTPase regulates adenylate cyclase activity in the host cell.
    - ADP-ribosylation of GTPase locks GTPase into a constitutively 'on' form → Resulting in:
        - Increased levels of adenyl cyclase activity and levels of cAMP in cell → Ultimately results in secretion of ions into lumen → Fluid loss.

A-B Toxins (Neurotoxins) – Compound

- Instead of targeting cells that may be in mucous membrane in respiratory tract or the gut; neurotoxins target neurons or cells in neural pathways → affect ability of muscles to contract or relax.
- An example of a neurotoxin is Botulinum toxin produced by soil dwelling organism called, Clostridium botulinum. It is a spore forming organism and this implies how it contaminates and cause infections.
    - This toxin causes a disease called Botulism, but it is not common now as we have better food practices. But, if we do see it, it is often associated with infants who have not yet established a protective microbiota in their gut and had contaminated honey.
    - The bacteria can contaminate / colonise anaerobic environment such as tinned food.
    - The bacteria that produces this toxin does not have to be living at the time the food is consumed.
        - The bacteria could have lived in the food. I.e. produced the toxin and died.
        - Ingestion of toxin is sufficient to cause the disease.
    - This toxin binds to a receptor called synaptotagamin II on host cells and leads to flaccid paralysis.

- The Botulinum toxin is a neurotoxin that acts on the neuromuscular junctions in the body.
- Normally, neurons produce and release acetylcholine into the junction to stimulate the function of muscles.
    - ACh is produced internally in the cell and is transported via vesicles towards axon terminals and it needs to undergo protein interaction with SNARE proteins → fuses with membrane and release its ACh.

- However, in the presence of Botulinum toxin, the toxin is taken up by the vesicles by receptor mediated endocytosis.
    - The active subunit once it has been taken up by the vesicle, is cleaved off the B unit and translocates across the vesicle membrane and into the cytoplasm of the cell.
    - It then cleaves off the SNARE proteins on the membranes of axon terminal, and consequently, vesicles containing ACh are no longer able to bind to the inner surfaces of membrane and release ACh into synaptic junction → No contraction of muscles → flaccid paralysis.