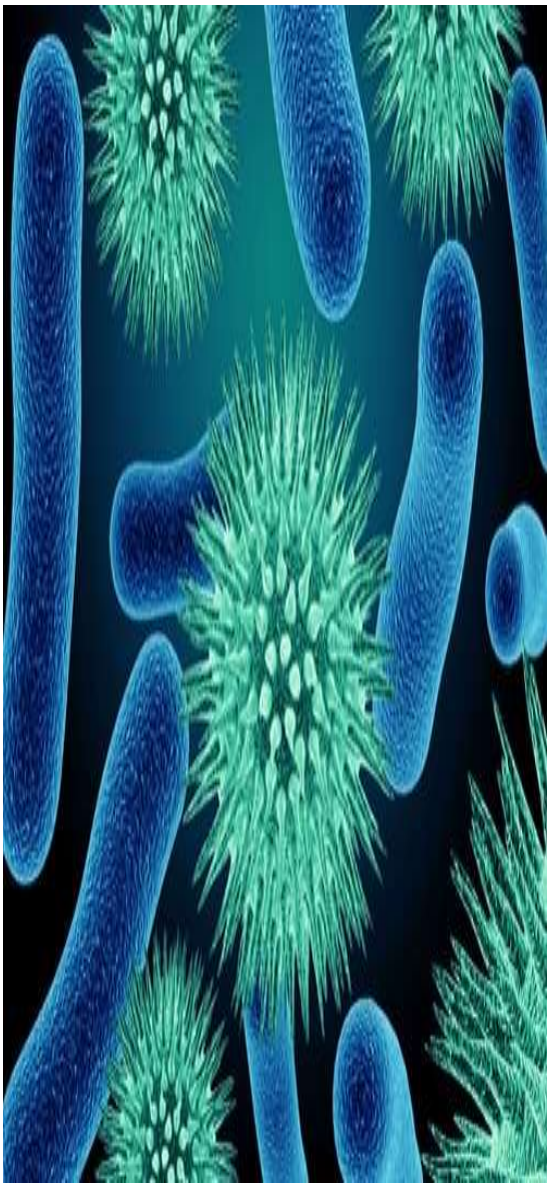

People's Democratic Republic of Algeria

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University 08 May 1945-Guelma-

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Host-Pathogen Interaction

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Course handbook, pedagogical support document

Host-Pathogen Interaction

Master I : Applied Immunology

Dr. Hami Manel

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FUNDAMENTAL TEACHING UNIT I (FTUI)

(Credits: 4; Coefficients: 2)

Subject 1: Host-Pathogen Interaction, Semester: 2

Teaching Objectives:

The objective is to provide students with theoretical training on the interactions/relationships existing between pathogenic microorganisms for animal cells and the immune system as a whole. To analyze through several models of host-infectious agent relationships, the mechanisms involved by microorganisms to infect the host and evade the host's immune defenses: antigenic variation, genetic polymorphism, manipulation of the immune response.

Recommended Prerequisite Knowledge: Microbiology, virology, parasitology, immunology.

Subject Content :

Chapter 1: Positive Interactions: Symbiosis and Commensalism

- Human commensal flora: quantitative and qualitative aspects, their role, imbalances, and their consequences
    - Symbiotic interaction
    - Adaptation of bacteria to environmental stressors

Chapter 2: Negative Interactions: Pathogenicity

- Bacteria-host interactions
- Notions of virulence and pathogenicity.
    - Bacterial attachment to host cells, biofilm formation.
    - Cellular invasion and intracellular survival mechanisms.
    - Secretion systems and cellular effectors.

Chapter 3: Bacterial Vectorization.

Chapter 4: Experimental Models for Identifying Virulence Factors.

Chapter 5: Strategies for Evasion by Infectious Agents Mechanisms of antibiotic resistance Evading the host's immune system

Chapter 6: Control of Bacterial Infections

SummaryList of Figures

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Introduction :

The interaction between the host and pathogens is a complex and crucial field of microbiology and immunology. It encompasses the mechanisms by which pathogenic microorganisms such as bacteria, viruses, parasites, and fungi seek to colonize and multiply within the host organism, while the host attempts to eliminate or neutralize them to prevent disease. These interactions often trigger immune responses to defend the host against infection.The mechanisms of interaction include the attachment of pathogens to host cells, cellular invasion, the production of toxins and other virulence factors, as well as the host's immune response. Robert Koch's postulates have been of considerable support in understanding the pathogenicity of these agents, opening a new chapter in the knowledge of infectious diseases. Understanding the mechanisms by which pathogens induce infectious diseases also requires mastering concepts of virulence, host defense, and evasion of the immune system. Despite the arsenal of defense mechanisms acquired by the human body and other higher mammals over millions of years, which can be divided into natural and specific immunity, infectious diseases result from the inefficiency and failure of defenses to eliminate the infectious threat. Furthermore, the emergence of new pathogens, particularly viruses, and the resurgence and resistance to drugs of previously known and fought agents, make medical microbiology and immunity current and future fields of interest. these interactions is essential for developing strategies for the prevention, diagnosis, and treatment of infectious diseases. This often involves a thorough analysis of the molecular, cellular, and immunological mechanisms involved in the host-pathogen interaction.

In this course material, we will explore the various aspects of the host-pathogen interaction, highlighting its importance in understanding the pathogenesis of infectious diseases and in the development of new therapeutic and preventive approaches.

Chapter I : Positive Interactions

Introduction :

Human beings evolve within a complex ecosystem. The relationship they maintain with various microorganisms is the result of a long evolution, which has taken several modalities. Understanding these relational modes will especially allow for a better understanding of infectious pathology in the clinical context.

I. Positive Interactions: Symbiosis and Commensalism

Symbiosis: is a mode of relationship in which both bacteria and host benefit from their association. For example, bacteria living in the digestive tract (e.g., Escherichia coli) play a role in protecting against digestive tract infections and in vitamin synthesis. Among these symbiotic interactions, another deserves mention here, that which is responsible for the origin of "eukaryotes." Eukaryotic cells are characterized by the presence of a nucleus and mitochondria (as well as chloroplasts for plants). Both mitochondria and chloroplasts (organelles) are in fact former bacteria that have merged with another cell following a particularly successful symbiosis. Although the majority of studies concerning the relationships between bacteria and their hosts have focused, for obvious reasons, on their pathogenic power, the known cases of symbiosis involving bacteria are already relatively numerous at the present time. However, by prioritizing an approach to the bacterial world based on interactions with the environment, it is likely that even more will be discovered. The tremendous evolutionary success of bacteria is undoubtedly due more to their ability to establish mutually beneficial interactions with their environment than to their ability to cause harm.

Mutualism: is a symbiotic association between two living species with reciprocal benefits; in the case of an obligatory association, the relationship is called symbiosis. Commensalism: Etymology: cum = with, mensa = the table; eating at the same table. These are bacteria that colonize, live, feed, and multiply within the host, e.g., enterobacteria in the intestines. The set of species is called the commensal flora. When the host benefits from this cohabitation, the relationship is then called symbiosis. This state results from a dynamic balance between the multiplication of these bacteria and their control by the immune system. It is a physiological balance that is established from birth and can persist until the end of life. The fetus is sterile, at birth it initially acquires maternal vaginal flora, then during breastfeeding...etc. Many microbial species live on the skin and mucous membranes (mouth, nasal cavities, digestive system, vagina...) without causing any harm to the host: these species are part of the commensal flora.

Environmental conditions (temperature, pH, oxygen availability...) significantly influence the distribution of commensal microorganisms. In the digestive system, for example, the number and proportion of strict anaerobic bacteria increase from the stomach to the colon. A resident flora consists of microorganisms permanently established (on the skin, in the intestinal tract...). It plays an important role in resistance to colonization by other potentially more pathogenic microorganisms. A transient flora includes contaminating microorganisms usually absent from a given normal flora. On the skin, for example, microorganisms from the digestive tract, colonized or infected individuals (carriers), the environment, or contaminated material can be found. They stay briefly on the skin because these microorganisms cannot multiply there and cannot survive for long due to the protective effect of the resident flora and an unfavorable environment (cold, dryness...). In general, antiseptics have a limited action on the resident flora but are rapid and effective against transient flora. An asymptomatic carrier is a person hosting pathogenic microorganisms (bacteria, viruses, etc.) but showing no signs of disease and being in good health. However, these individuals can transmit the microorganism to others. It is more accurate to call them asymptomatic carriers (i.e., individuals showing no symptoms) than "healthy" carriers. However, the boundary between pathogenic and commensal microorganisms is not precise: the same bacterium can be both commensal and pathogenic. The location of the microorganism as well as the health status of the host ("terrain") influence this "unstable equilibrium." From birth, a bacterial flora settles on the skin and mucous membranes, and this constant association of bacteria with surfaces in contact with the external environment will last throughout life. During evolution, a complex defense system is established to prevent the invasion of the individual by bacteria. An equilibrium is established between the individual and the various commensal floras of the skin and mucous membranes. The flora varies over time depending on age, diet, health status, antibiotic therapy, etc. This flora is a source of certain nutrients and vitamins necessary for the host and constitutes an ecological barrier against the establishment of virulent germs. This flora is diversified and these bacteria are not pathogenic except in the case of invasive procedures. They are found: -In the oro-pharyngeal sphere (Streptococcus sp.), -On the skin surface (Staphylococcus sp., Propionibacterium sp., Corynebacteria), -In the digestive tract: -In the digestive tract: enterobacteria (Anaerobes), -In the genital flora: Lactobacillus sp. (Anaerobes) I.1- Human Commensal Flora: The normal flora refers to all microorganisms naturally present on the body's sites, mainly on external surfaces. It should be noted that the skin and mucous membranes of the respiratory and genitourinary tracts are considered external surfaces. The composition of this flora varies with age. An individual has 10^{14} eukaryotic cells for 10^{15} microbial cells. Living with this commensal flora

is an obligation and a necessity. The commensal flora is mainly located in the digestive tract (10^9 to 10^{12} bacteria per gram of stool). Most bacteria lead, in nature, a completely independent life from another living organism. They live on waste, which they destroy by deriving their energy from it and performing their syntheses. They are called saprophytes. Medically relevant bacteria find favorable conditions for their growth on the surface or inside a living organism. Depending on the various biological relationships that can be established between these bacteria and their host, various groups of microorganisms are distinguished (Fig 1):

I.1.1. Cutaneous flora: The established germs on the skin live on the most superficial layers of the epidermis and on the upper part of the hair follicles and sebaceous gland ducts. This cutaneous flora varies in quality and quantity (10^2 to 10^6/cm^2) depending on the topography.

-Resident flora consists of Gram+ potentially non-pathogenic germs such as Coagulase-negative staphylococci, Corynebacteria

-Transient flora is more polymorphic and may include potentially pathogenic germs from the digestive tract or nasopharynx: Enterobacteria, *Staphylococcus aureus*. The hands often carry abundant transient flora (role in cross-transmission).

I.1.2. Digestive flora:

-Oral flora: There are schematically two ecosystems in the mouth: the flora of the oral mucosa and that of dental plaque.

-Oral mucosa: this flora consists mainly of certain species of streptococci that adhere to the cells of the jugal and lingual epithelium (Streptococcus salivarius, *Streptococcus mitor, Streptococcus milleri*...). Other anaerobic species are associated with them. Saliva reflects this flora of the oral mucosa and contains a high number of bacteria (10^5 to 10^6 bacteria/ml) with a clear predominance of *S. salivarius.*

-Dental plaque: it is a bacterial film adhering to the enamel of the teeth. Under the electron microscope, it appears to be composed of numerous bacteria inserted into an organic matrix composed of glycoproteins from saliva and locally secreted bacterial polymers. This plaque forms in a few hours and can calcify, forming tartar, or complicate into dental caries or periodontitis.

I. 1.3. Gastric flora: Helicobacter pylori (responsible for gastric and duodenal ulcers) is one of the few bacteria found in the stomach. Thus, apart from transit bacteria brought by food, there are no bacteria in the stomach (acidic pH).

I. 1.4. Small intestinal flora: It has a poor flora due to peristalsis and abundant secretions. The germs present are mainly streptococci, staphylococci, and lactobacilli.

I. 1.5. Colonic flora: It is extremely varied and abundant. It contains 10^{11} to 10^{12} bac/g with a clear predominance of strict anaerobes (99.9%), especially Bacteroides, Bifidobacterium, and Clostridium. Aerobic germs are mainly Enterobacteria (*E. coli*), Enterococci, and Staphylococci. This flora is usually stable and limits the implantation of pathogenic species and the development of potentially dangerous commensal bacteria. It may vary with the type of food, age, environment, and antibiotic therapy.

I. 1.6. Respiratory Tract Flora (RT):

-Upper RT: The flora is variable and abundant in the nasopharynx (10^8/ml of pharyngeal secretion). It contains many major opportunistic pathogens: *Staphylococcus aureus* (nostrils), Streptococci (groupable or non-groupable)

-**Lower RT:** At the level of the trachea, the flora is minimal and actively combated by mucus, cilia, macrophages, etc. The lower respiratory tree is normally sterile.

I. 1.7. Genital tract flora:

-Urethral flora: It is found at the end of the urethral canal in men and women, over a small area. It consists of Staphylococci, Micrococci, Enterobacteria, Corynebacteria, and non-groupable Streptococci.

-V**aginal flora:** It plays an essential protective role in women. Lactobacillus acidophilus or Doderlein's bacilli, by their secretion of lactic acid, maintain a low pH that limits the commensal flora. This commensal flora is reduced to: Streptococci (mainly Group B Streptococcus), Corynebacteria, Bifidobacterium. After menopause, anaerobes and Enterobacteria are more abundant.

I. 2. Role of the commensal flora:

-Infection resistance: One of the major roles of the commensal flora is to create a state of resistance against the implantation of pathogenic bacteria on the skin and mucous membranes. The presence of a huge quantity of bacteria in contact with the mucous membranes permanently stimulates the immune system distributed along these membranes, especially the digestive and respiratory ones. Furthermore, this flora, by its barrier effect, prevents the implantation of exogenous bacteria.

-Nutritional contribution: The flora of the digestive tract partly contributes to digestion by destroying waste or hydrolyzing certain substances that have resisted digestion by intestinal juices. Moreover, this flora is capable of synthesizing vitamins (K, B12, folic acid) that will be used by the host as a supplement to dietary intake.

• Hydrolysis and fermentation of polysaccharides (for which enzymes are not available)

• Production of short-chain fatty acids (e.g., butyrate of microbial origin represents 50% of the carbohydrate sources of colonic epithelial cells)

-Barrier effect against pathogenic microorganisms: Competition (receptors and nutrients) and production of bactericidal molecules (lactic acid, bacteriocins)

-Homeostasis of the intestinal epithelial barrier.

I.3. Disadvantages of the presence of commensal flora:

-Responsible for opportunistic infection in case of immunosuppression.

-Reservoir of genes encoding multi-resistance to antibiotics that can be transmitted to pathogenic bacteria.

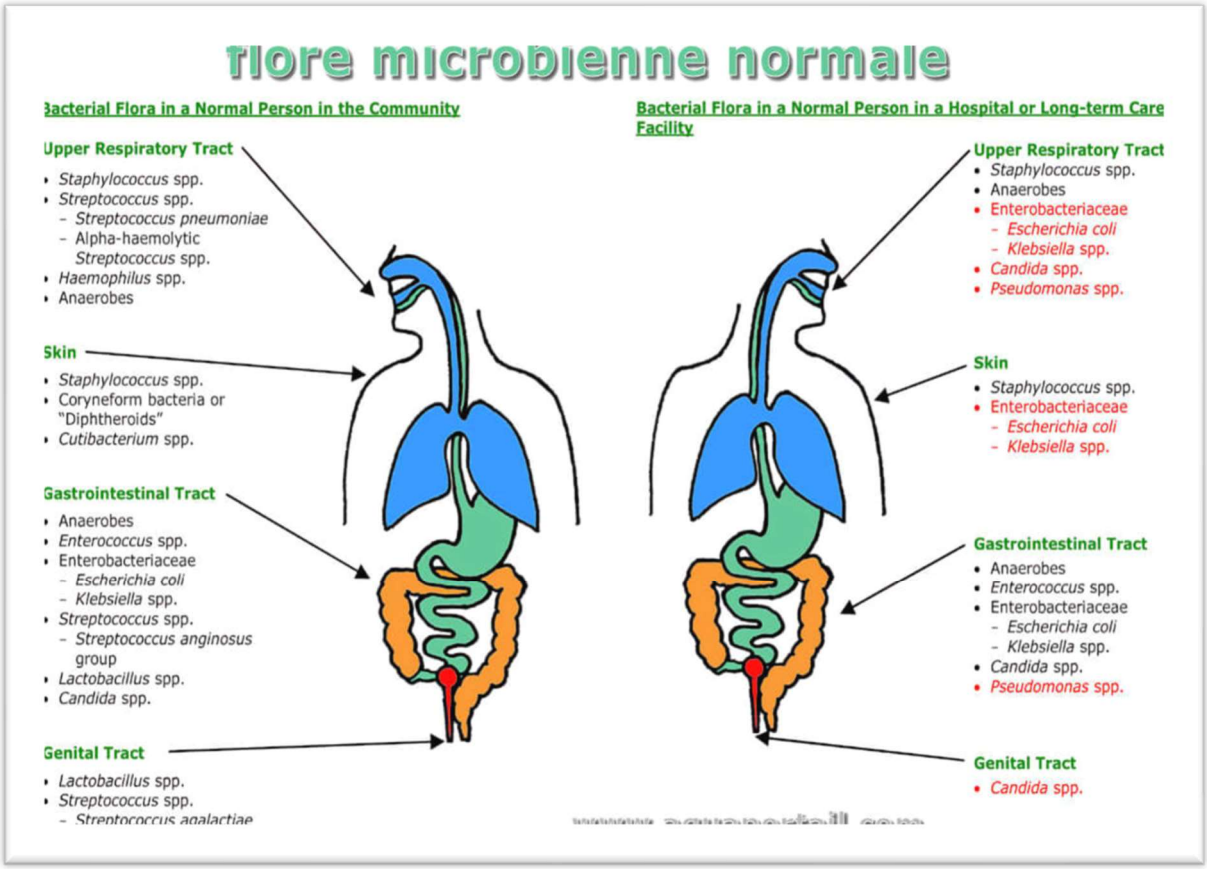


Figure 1. Bacterial floral in normal person in the community and normal person in hospital.

I.4. Favorable Conditions for Bacterial Growth:

I.4.1. Energy Sources:

Bacteria must find in their environment the substances necessary for their energy and cellular synthesis. Phototrophic bacteria use light energy for photosynthesis (ATP synthesis from ADP and inorganic phosphate). Chemotrophic bacteria derive their energy from mineral or organic compounds. They use electron donors and acceptors (mineral element: chemoautotrophic bacteria; organic element: chemoheterotrophic bacteria). The vast majority of medically relevant bacteria are chemoheterotrophs.

I.4.2. Carbon Sources:

Carbon is one of the most abundant elements in bacteria. The simplest compound is carbon dioxide or CO2. This can be used by bacteria for the synthesis of certain essential metabolites involving a carboxylation reaction. CO2 is the only carbon source for autotrophic bacteria. Heterotrophic bacteria facultatively use CO2. Heterotrophic bacteria degrade a large amount of hydrocarbon substances (alcohol, acetic acid, lactic acid, polysaccharides, various sugars).

I.4.3. Nitrogen Sources and Sulfur Requirements: Bacteria require nitrogenous substances to synthesize their proteins. This nitrogen can come from the direct fixation of atmospheric nitrogen or the incorporation of nitrogen compounds (deamination, transamination reactions). Sulfur is incorporated by bacteria in the form of sulfate or organic sulfur compounds.

I.4.4. Inorganic Requirements: Phosphorus is present in nucleic acids and is used in many enzymatic reactions. It allows the recovery, accumulation, and distribution of energy in bacteria. It is incorporated in the form of inorganic phosphate.

I.4.5. Other Elements: Other elements play a role in bacterial metabolism (sodium, potassium, magnesium, chlorine) and in enzymatic reactions (calcium, iron, manganese, nickel, selenium, copper, cobalt, vitamins).

I.4.6. Quorum Sensing: Bacteria communicate with each other and exhibit cooperative behavior. Bacteria control their own population density by sensing the level of signal molecules or autoinducers. For example, through quorum sensing, bacteria reach a high population density before releasing their enzymes. Examples include the synthesis and release of virulence factors in *P. aeruginosa*, stimulation of sporulation in Bacillus, production of toxins and virulence factors in *S. aureus,* and maturation of biofilm in *P. aeruginosa.*

I.5. Physico-Chemical Conditions for Growth:

I.5.1. Oxygen Effect: There are several classes of bacteria depending on their relationship with oxygen. Strict aerobic bacteria only grow in the presence of air. Their main source of energy is respiration. Molecular oxygen, the ultimate electron acceptor, is reduced to water (Pseudomonas, Acinetobacter, Neisseria). Microaerophilic bacteria grow better or exclusively when the partial pressure of oxygen is lower than that of the air (Campylobacter, Mycobacteriaceae). Facultative aerobe bacteria grow with or without air. This is the case for the majority of bacteria encountered in medical pathology: enterobacteria (Escherichia, Salmonella), streptococci, staphylococci. Energy comes from substrate oxidation and the fermentation pathway. Strict anaerobic bacteria only grow in the absence or near absence of oxygen, which is often toxic. These bacteria must be cultured under a reducing atmosphere. All energy is produced by fermentation. This is the case for intestinal bacteria (Bacteroides, Fusobacterium, Clostridium) and many bacteria present in the normal flora of the body.

I.5.2. Temperature Effect:

Bacteria can be classified according to their optimal growth temperature.

- Mesophilic bacteria (e.g., Escherichia coli): growth temperature close to that of the human body (37°C)
- Thermophilic bacteria (e.g., Thermus aquaticus): growth temperatures between 45°C and 70°C
- Hyperthermophilic bacteria (e.g., Archaea): growth temperatures above 80°C
- Psychrophilic bacteria (e.g., Pseudomonas): temperatures close to 0°C (optimum at 10-15°C)
- Psychrotrophic bacteria (e.g., Pseudomonas): growth temperatures close to 0°C with optimum growth close to mesophilic bacteria. I.5.3. pH Effect: The pH (hydrogen ion concentration [H+]) of the environment varies between 0.5 (acidic soils) and 10.5 (alkaline waters of lakes). Pathogenic or human ecosystem-related bacteria mostly grow in neutral or slightly alkaline environments. There are :
- Neutrophilic bacteria grow at pH between 5.5 and 8.5 with an optimum close to 7.
- Alkalophilic bacteria prefer alkaline pH: examples include Pseudomonas and Vibrio.
- Acidophilic bacteria grow better in acidic environments: examples include Lactobacillus. To maintain a neutral internal pH, bacteria have various resistance mechanisms:

- Cytoplasmic membrane becomes impermeable to protons,
- Neutrophilic bacteria: exchange potassium for protons,
- Alkalophilic bacteria: exchange sodium ions for protons,
- Production of acidic or alkaline metabolic wastes.

I.5.4. Osmotic Pressure Effect:

Bacteria are quite tolerant of changes in ion concentrations. Some species are osmotolerant (staphylococci, Vibrio cholerae).

I.5.5. Free Water Effect:

The availability of water present in the atmosphere or in a substance affects bacterial growth. Water activity (Aw) is inversely proportional to the osmotic pressure of a compound. Thus, it is affected by the presence of more or less significant amounts of salts or sugars dissolved in water. The presence of salts: Halophilic bacteria require salt (NaCl) for their growth. This concentration can vary from 1-6% for weakly halophilic bacteria up to 15-30% for extreme halophilic bacteria (Halobacterium). In this case, the bacterium accumulates significant amounts of potassium to remain hypertonic relative to its environment.

- Halotolerant bacteria accept moderate concentrations of salts but do not require them for growth (e.g., Staphylococcus aureus). The presence of sugars :
- Osmophilic bacteria require sugars for their growth.
- Osmotolerant bacteria accept moderate concentrations of sugars but do not require them for growth.
- Xerophilic bacteria can multiply in the absence of water in their environment.

I.5.6. Energy Metabolism:

Bacteria can be categorized as having fermentative metabolism or respiratory metabolism. For bacteria with fermentative metabolism, glucose degradation is incomplete and leads to the formation of various organic compounds (organic acids). For bacteria with respiratory metabolism, degradation occurs through the Krebs cycle. The final electron acceptor is oxygen. In bacteria, the electron transport system is located in the cytoplasmic membrane.

I.5.7. Iron Capture:

Necessity to use iron for cytochromes and enzymes. Difficult capture due to the insolubility of ferric ions. Synthesis of siderophores of hydroxamate nature (aerobactin, pyoverdine, mycobactin) or phenolate nature (enterochelin, pyochelin, vibriobactin). Their molecular weights range from 300 to 1000 Da. High affinity for trivalent iron: 10-38 M. Transport of the iron-siderophore complex by an ABC-Transporter system. Other pathways for bacterial iron uptake exist: heme, haptoglobin-hemoglobin, hemopexin, transferrin, lactoferrin.

I.6. Adaptation of Bacteria to Deficiencies and Environmental Stresses:

Like all unicellular organisms, bacteria must constantly adapt their physiology to fluctuations in physico-chemical factors in the surrounding environment. The adaptive mechanisms implemented include regulation of gene transcription whose products contribute to bacterial resistance and/or survival under stress conditions. We study the regulations implemented following various stresses (osmotic, acid, stationary phase), mainly on two global regulatory systems: the sigma factor of the generalized stress response σS (RpoS), and the Rcs CDB phosphorelay system. The adaptation of bacteria to deficiencies and environmental stresses is a crucial process for their survival and growth under difficult conditions. In situations of deficiency or stress, bacteria can adopt two types of survival strategies:

Strategy 1: The bacterium differentiates into a metabolically inactive resistance form. This is the case with Bacillus, which produces a spore. However, many of them have the ability to live in very different conditions, such as the bacterium Escherichia coli, the usual host of our intestines, and a model bacterium that has been used in most scientific studies concerning the functioning of the bacterial genome. In the presence of oxygen, which it breathes, it is capable of using a wide variety of sugars (including lactose, which has made it famous), or amino acids as a source of carbon and energy... But it can also live in anaerobiosis (in the absence of O2), either by "breathing" nitrate or fumarate, or by fermenting various sugars.

Strategy 2: *E. Coli* bacteria develop regulatory systems to control. In this type of situation, the bacterium presents the following adaptations:

-Degradation of total cellular RNA, releasing nucleotides used for the synthesis of new RNA or as a source of energy. Protein degradation: release of amino acids reused or degraded for energy production. Implementation of transport and assimilation systems as substitutes for missing elements, which are mainly nitrogenous, phosphorus, carbon compounds, and iron. Synthesis of stress proteins that protect the bacterium from nutrient deprivation and other stresses (presence of genes involved in deficiency or stress phenomena).

I.6.1. Biofilm Formation:

Biofilms are communities of bacteria enveloped in an extracellular matrix. Bacteria can form biofilms to protect themselves from unfavorable environmental conditions, such as nutrient deficiencies or pH changes. Bacterial genetic circuits exhibit fluctuations in the level of regulatory proteins. This fluctuation allows a subpopulation of cells to enter a transient state of antibiotic resistance, thereby improving their survival. This mechanism, called "bet-hedging" or risk minimization, ensures that bacterial cells do not all exist in the same transcriptional state at the same time. This process may ensure the survival of the bacterial colony in the face of future environmental changes. The study of risk minimization is therefore of primary importance in terms of public health. The spread of infectious diseases sometimes depends on the activation of these alternative genetic programs in terms of bacterial competence, general stress response, or antibiotic persistence in the bacterial cell. The BET-HEDGING BACTERIA project, funded by the EU, has therefore studied the mechanisms by which bacterial cells generate alternative transcriptional states.

I.6.2. Example of Adaptation in Enterobacteria:

To cope with the challenges, they encounter in their environment, enterobacteria develop adaptive responses that allow them to maintain their impermeable and functional protective envelope. Enterobacteria, Gram-negative bacilli mainly present in the intestinal flora of mammals, have an impermeable envelope that allows them to better resist toxic agents such as antibiotics or detergents. To ensure their survival in these hostile environments, bacteria must constantly preserve the integrity of this envelope, especially the outer membrane (OM) composed of fatty acids and proteins playing a crucial role. Thus, integral envelope proteins, called Outer Membrane Proteins (OMPs), perform various critical functions for bacterial growth, defense, or pathogenicity. In case of defects in these OMPs, the permeability barrier of bacteria is altered, and a transcriptional pathway of stress response, noted sE, is triggered. One of the responses of this pathway is the overproduction of the BAM machinery (b-barrel assembly machinery) responsible for assembling integral envelope proteins into the outer membrane, allowing their function to be restored. Scientists have discovered that an accumulation of BAM in Escherichia coli could be toxic to the cell, suggesting a finely regulated mechanism. In order to decipher the molecular basis of the balance between the "Yin and Yang" of stress response, they highlighted the crucial role of DolP, an ME lipoprotein induced by the sE response, in this process by showing that DolP comes into contact with the BAM complex at the ME level. By interacting directly with BamA, the central subunit of BAM,

DolP enables the correct folding of this membrane protein, which is altered during its overproduction. Thus, DolP ensures the full functionality of BAM required for maintaining envelope integrity. Furthermore, researchers show that under stress conditions, DolP loses its localization at the cell division zone (septum) where it is supposed to regulate peptidoglycan remodeling, a component of bacterial cell walls. This suggests a link between envelope stress caused by alterations in OMP biogenesis and the regulation of a late stage of cell division. By providing new insights into the mechanisms of biogenesis and maintenance of the envelope of Gram-negative bacteria, this work could help identify new targets of interest for the development of innovative antibiotics (Fig 2).

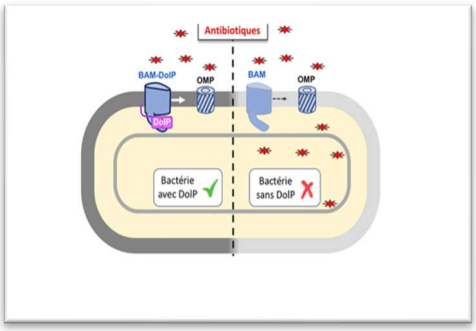


Figure 2: The BAM complex, by enabling the assembly of integral outer membrane proteins (OMPs), is essential for outer membrane biogenesis. DolP promotes the folding and function of the BAM complex, ensuring optimal integrity of the outer membrane to withstand

Chapter II: Negative Interactions: (Pathogenicity)

Introduction

Interactions between bacteria and their host are extremely diverse and complex, ranging from beneficial cooperation to harmful pathogenicity. A better understanding of these interactions is important for the development of therapies and strategies for preventing bacteria-associated diseases. The host-bacteria conflict pits two adversaries against each other: the bacterium characterized by its pathogenic power and the host characterized by its receptivity or defense capabilities. Infectious disease results from the disruption of the balance in favor of the bacterium. It is linked to the penetration and multiplication of a virulent microbial agent capable of causing disturbances in the infected organism. These are bacteria endowed with aggressive power in the host, leading to infectious diseases. They can be categorized as follows:

- Strictly pathogenic microorganisms or those with a high potential for pathogenicity. They are called parasitic bacteria: typically, always pathogenic for a given host, e.g., Mycobacterium tuberculosis.

- Occasional or opportunistic pathogenic microorganisms:

- These are commensal bacteria (resident flora of humans) or saprophytes (environmental bacteria) that can become pathogenic when the host's defenses are weakened (immunosuppression), e.g., bacteria from skin flora. These microorganisms cause diseases when specific conditions are met (immunosuppressed individuals, prolonged broad-spectrum antibiotic therapy, extreme ages of life, etc.), e.g., Pseudomonas aeruginosa, numerous Enterobacteria, Enterococcus...

- Saprophytism: These are environmental bacteria that live on organic waste and do not colonize the host; they are transient bacteria, e.g., Pseudomonas aeruginosa.

- Opportunism: Opportunistic bacteria do not cause disease in healthy individuals but can become pathogenic in immunocompromised individuals. They are environmental bacteria (saprophytes) or commensals of human skin and mucous membranes that, following immunosuppression or antibiotic therapy, are selected and proliferate. Any commensal or environmental bacterium can become opportunistic, e.g., Pseudomonas sp., Acinetobacter sp., Legionella pneumophila.

- Parasitism: Some bacteria are parasites that cause diseases in their host. They can cause acute or chronic infections, resulting in various symptoms and consequences for the host's health.

- Pathogenic interactions: Pathogenic bacteria are capable of colonizing their host, thwarting the host's immune defenses, and causing diseases. These interactions may include adhesion to host cells, release of toxins, and manipulation of the host's immune responses.

- Coevolution: Interactions between bacteria and their host can lead to coevolutionary processes, where both parties adapt and evolve in response to selective pressures exerted by each other.

II. Virulence and Pathogenicity:

II.1. Virulence: The virulence of a microorganism reflects the severity of the disorders it causes in the host, in other words, the degree of pathogenic power. Virulence can be estimated by measuring the LD_{50} or lethal dose at 50%. The LD_{50} is the quantity of a substance that, when administered in a single dose, causes the death of half (50%) of the animals tested. Virulence is a quantitative notion, whereas pathogenic power is a qualitative notion; thus, for the same pathogenic power, there can be more or less virulent strains. For example, Shigella dysenteriae and Shigella flexneri are both responsible for bacillary dysentery, but not with the same doses:

- A few bacteria are sufficient to develop an infection with S. dysenteriae.
- Several thousand are necessary with S. flexneri; therefore, this species is less virulent than S. dysenteriae.

II.1.1. Factors of virulence:

Microbiota-related factors:

- Surface structures: for attachment and adhesion to host cells: fimbriae, adhesins, flagella. Others allow them to escape resistance: capsule.
- Secretion of enzymes or toxins: (collagenases, hemolysins, etc.) promote the diffusion of bacteria into tissues and cause lesions in them.
- Bacterial multiplication: *In vivo* multiplication is much lower than in vitro.

II.1.2. Host-related factors:

- According to animal species: Some species are more susceptible to certain germs. E.g., Anthrax and sheep.
- According to age: the most exposed are the young and the elderly.
- According to nutrition :
- Protein deficiency.
- Corticosteroid treatment, immunosuppressants, antibiotics. (Selection of resistant mutants) Increases the pathogenicity of a bacterial strain.
- According to local factors: E.g., Streptococcus and pre-existing valve lesions.
- Defects (diabetic, cancerous)
- Overwork or fatigue.

II.1.3. Variation in Virulence: The virulence of a bacterial strain for a parasitic organism varies.

- **Attenuation of virulence:** Preservation, heat, slow desiccation, and frequent subculturing on culture medium lead to attenuation or even extinction of virulence. E.g., BCG vaccine (230 passages on glycerinated potato).
- **Enhancement of virulence :**

✓ By repeated passages on laboratory animals. E.g., Inoculation of pneumococcus in mice.

✓ This phenomenon is observed naturally with influenza viruses, measles, and meningococcal meningitis (the beginning of the epidemic is benign, the end is malignant with death).

✓ Some microbial associations enhance virulence. E.g., Streptococcus gives the TOX gene to diphtheria bacillus, which becomes virulent.

- **Conservation of virulence :**

✓ Normally it is the phenomenon of sporulation.

✓ In the laboratory: - Lyophilization: It is vacuum desiccation at very low temperature. - Freezing: at -70°C.

II.2. Pathogenic Power:

Pathogenic power or pathogenicity is a qualitative notion that translates into a set of mechanisms conditioning the type of disease dependent on a bacterium. Pathogenic bacteria are capable of causing a disease or morbid conditions in the parasitized organism, whose defense mechanisms are normal (e.g., Cholera, Typhoid...).

II.2.1. Reservoir and Transmission of Bacteria: It consists of humans, animals, or the environment. The source of infection is related to the status of pathogenic or opportunistic bacteria and to the ecology of the bacterium; this is the notion of a bacterial reservoir. A disease can be strictly human (meningococcal infection, pneumococcal infection, whooping cough) or animal; humans are accidentally infected: this is called Anthropozoonosis (brucellosis, plague). There are different modes of transmission :

- Direct transmission: contamination by contact with the reservoir, which can be an infected individual or animal.
- Indirect transmission: contamination through infected objects, contaminated food, water, etc. Bacterial survival in the environment is possible for a certain time.
- Vertical transmission: it occurs in utero, from mother to fetus through various contamination routes: Digestive route: ingestion of contaminated water or food (e.g., cholera, typhoid) Respiratory route: contamination by inhalation of contaminated aerosols (e.g., Legionnaires' disease, whooping cough). Cutaneous route: inoculation by contact (contaminated wound) (e.g., tetanus, wound infection). Transcutaneous route: iatrogenic inoculation (injection, catheter) or through insect bites carrying bacteria (e.g., plague, Lyme disease). Sexual route: sexually transmitted infections (e.g., syphilis, gonococcal urethritis).

II.2.2. Types of Host-Bacteria Interactions:

- Transit: Absence of bacterial implantation on the host for physiological reasons: e.g., growth temperature or nutritional requirements.
- Colonization: The first step in the infectious process corresponds to the implantation of bacteria on the skin or mucous membrane without causing damage to the host. E.g., Interaction of commensal flora.
- Healthy carriers (Carriage): Colonization by pathogenic bacteria found more or less transiently in the commensal flora.

- Infectious disease: Infectious diseases include diseases caused by the penetration into the body of an infectious agent: bacteria, viruses, parasites, pathogenic prions, or fungi. When they are contagious, these diseases can be transmitted directly or indirectly from one person to another, according to variable transmission modes. Some are benign, like the common cold or tonsillitis. Others are much more serious and can trigger public health disasters on a global scale, with epidemics or pandemics such as AIDS, tuberculosis, or more recently COVID-19.

II.2.3. Attachment of Bacteria to Host Cells and Biofilm Formation: The attachment of bacteria to the host cell is a fundamental step in biofilm formation because it allows bacteria to anchor to the surface and form a three-dimensional structure protected by an extracellular matrix, thus promoting biofilm formation and stability.

II.2.3.1. Mechanism of Adhesion to Host Cell:

Adhesion factors to cells are necessary for a bacterium to attach to the epithelium and colonize it. The presence of proteins called adhesins, which recognize specific receptors on the cell surface, thus promotes anchoring to the host tissue. It is a step dependent on the pathogen's ability to compete with the host's normal microflora for nutrients. There are two groups of adhesins:

1. Pili or fimbriae: filamentous protein adhesins (pilin subunit). These adhesins recognize glycoprotein or glycolipid receptors on cell surfaces. Pili are found on the surface of many Gram-negative bacteria (e.g., Neisseria gonorrhoeae, E. coli).

- In certain *Escherichia coli,* for example, enteropathogens (ETEC; responsible for diarrhea in infants) have surface proteins allowing adhesion to the digestive epithelium. This preliminary step is essential to trigger pathogenicity.

- In some strains of Escherichia coli in the genitourinary tract, causing cystitis. After adherence to the tissue, the bacterium can cross the epithelial barrier to invade and reach normally sterile tissues.

2. Non-fimbrial adhesins: Surface proteins or lipopolysaccharides allowing close contact between the bacterium and the cell. They are found in Gram-negative (proteins or LPS) and Gram-positive bacteria (teichoic acids, and lipoteichoic acids). The fixation of the adhesin is either directly to the cellular receptor (teichoic and lipoteichoic acids of Gram-positive bacteria) or sometimes to proteins anchored in the wall: Fibronectin-binding proteins (FnbA, FnbB) and fibrinogen-binding proteins (clumping factor, ClfA,

ClfB). Collagen, elastin, and fibronectin act as bridges between the bacterium and the host cell.

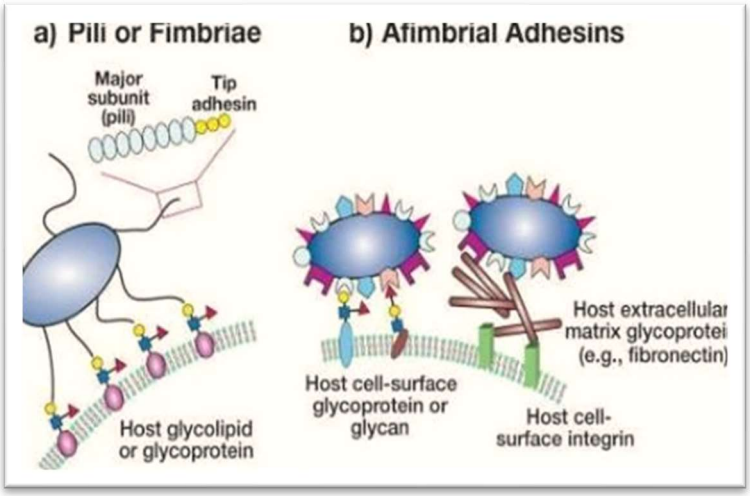


Figure 3: Schematic representation of fimbrial adhesins involved in specific substrate recognition

II.2.4. Biofilm Formation: Certain bacteria secrete polysaccharides into the external environment, which are involved in bacterial adhesion to each other and to the cell surface, resulting in the formation of a biofilm that shields bacteria from phagocytic cells and antibiotics. There is a physiological biofilm at the mucosal level, vaginal, oral, or digestive, secreted by the commensal flora. The formation of a biofilm is a characteristic of pathogenic power for some bacteria; Streptococcus mutans (part of the commensal flora of the oral cavity, is most often involved in dental caries) is involved in dental plaque formation; Staphylococcus epidermidis has the ability to colonize biomaterials such as catheters, probes, prostheses, etc., leading to iatrogenic infections on materials. The formation of a bacterial biofilm can be divided into three steps (Figure 04). Initiation corresponds to cell adhesion to the surface, maturation involves the development of spatially organized complex structures, and detachment involves either individual cells or entire portions of the community.

1-Initiation of a Biofilm: The initiation stage of the biofilm requires the transition of the cell from a planktonic to a sessile state and occurs in response to environmental factors. Regulation of mobility plays a major role in this transition. Adhesion to a support involves the structures and properties of the cell surface. Once in contact with the surface, bacteria will interact with the support via electrostatic, Van der Waals forces, or according to the hydrophobicity of the bacterial species. This step constitutes reversible and nonspecific initial adhesion. It is a very rapid phase that can occur between any bacterium and any support via the low-energy forces mentioned above. When the substrate and the microorganism are compatible, the latter will

regulate its gene expression and express a certain number of adhesion factors, including adhesins. These molecular motifs will allow the recognition of a specific receptor on the bacterium's support. These motifs may or may not be carried by pili (Figure 7). This recognition will result in irreversible adhesion, which constitutes a definitive anchoring of the bacterium to its support. Since this step requires the expression of adhesion factors, it is necessary for the bacterium to be viable to be able to establish itself permanently on its support. This is the major life form of the microbial world, accounting for over 95% of microbial life according to Costerton. This lifestyle indeed has many advantages, such as trapping and circulating nutrients in the matrix, optimizing resources by the diversity present in multispecies biofilms, or acting as a barrier against external aggressions (such as antibiotics).

2-Formation and Maturation of Structures: After their irreversible attachment to a surface, cells form areas of high cell density. Three mechanisms for the establishment of these cell aggregates have been proposed. The first, which involves no form of mobility, is the clonal replication of cells that have irreversibly adhered to the surface. The second mechanism is based on the recruitment of planktonic cells as the biofilm develops. The relative involvement of these two mechanisms depends on the nature of the organism, the surface to be colonized, and the physicochemical conditions.

3-Dispersion of the Biofilm: When the biofilm reaches a certain cell density, the accumulation of toxic metabolic wastes and the depletion of nutrients in the environment make biofilm dispersion beneficial. This phenomenon allows cells to migrate to more favorable environments. Biofilm dispersion has been observed in response to deficiencies in carbon, nitrogen, or oxygen, as well as nutrient depletion in the surrounding environment. In strains of the Pseudomonas genus, it has been shown that even slight variations in nutrient concentration induce biofilm dispersion. Low oxygen concentration in the deeper layers of a biofilm has also been associated with a higher frequency of dispersion events in Shewanella oneidensis.

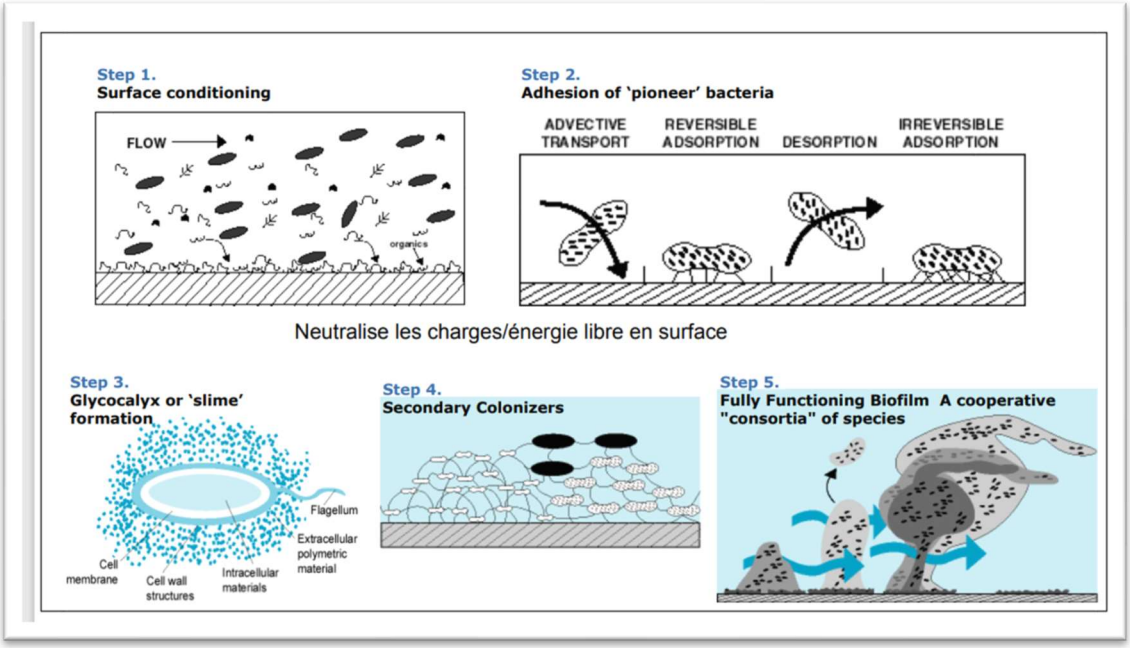


Figure 4 : Steps of biofilm formation

II.2.5. Stages of Bacterial Infection:

The latter enters the body through an entry point: The entry point can be the mucous membranes (respiratory tract), the urinary tract, the digestive tract, a skin lesion, or invasive devices such as catheters or cannulas; this is colonization. It depends on an essential mechanism of bacterial pathogenicity, bacterial adhesion. Once inside, bacteria multiply and invade the tissues of our body, associated with the development of nonspecific inflammation at the entry point, which is secondary to bacterial multiplication at this level, thus realizing a localized infection (pneumonia, urinary tract infection, etc.): this is the invasion stage (invasive bacteria). The third stage is dissemination, which may be followed by the invasion stage, starting from the entry point via the bloodstream (bacteremia) or lymphatic system, leading to secondary metastatic localizations at several levels (e.g., endocarditis, osteitis, meningitis, etc.). Among these invasive bacteria, there are bacteria with extracellular multiplication and bacteria with intracellular multiplication.

The pathogenicity of bacteria is conditioned by several factors:

- The ability of bacteria to multiply in the host: invasive power,
- The ability of bacteria to release toxins: toxic power.

II.2.5.1. Invasive Power:

An invasive bacterium is capable of multiplying in the host's tissues, thus causing an infection. Initially, it must penetrate the organism, meaning it must cross the host's mechanical barriers: skin and mucous membranes, for example. Regarding the skin, few bacteria are capable of crossing it when it is healthy. However, a skin lesion (cut, burn, etc.) constitutes an "entry point" for microorganisms. Mucous membranes are more fragile, and some bacteria are capable of locally destroying them to penetrate the host's tissues. Invasive bacteria generally possess adhesion structures for mucous membranes. These adhesins (or adhesion factors) are molecules specifically recognized by receptors on host cells. The crossing of the host's mechanical barriers can occur through:

-Endocytosis: transport into the host cell by budding of the plasma membrane after recognition between bacterial adhesion molecules and cell receptors, zipper-type entry is a mechanism used, for example, by Listeria monocytogenes. Conversely, other bacteria such as Salmonella or Shigella penetrate host cells after injection of proteins capable of modulating their structure. Direct interaction between a bacterial surface protein and a host cell receptor. Example: Listeria: interaction between bacterial internalin A with E-cadherin of epithelial cells or between bacterial internalin B and the Met receptor of hepatocytes. Formation of a significant number of contact points before invagination (zipper closure) responsible for a cascade of signals activating cytoskeletal components, trigger-type entry. Injection occurs via a secretion system (protein structure similar to a needle), and the injected proteins divert cellular machinery in favor of the bacterium (Type III secretion in Salmonella and Shigella). After its internalization, the bacterium can either follow inside the endocytosis vacuole (Salmonella, Legionella) or escape into the cytoplasm (Shigella, Listeria).

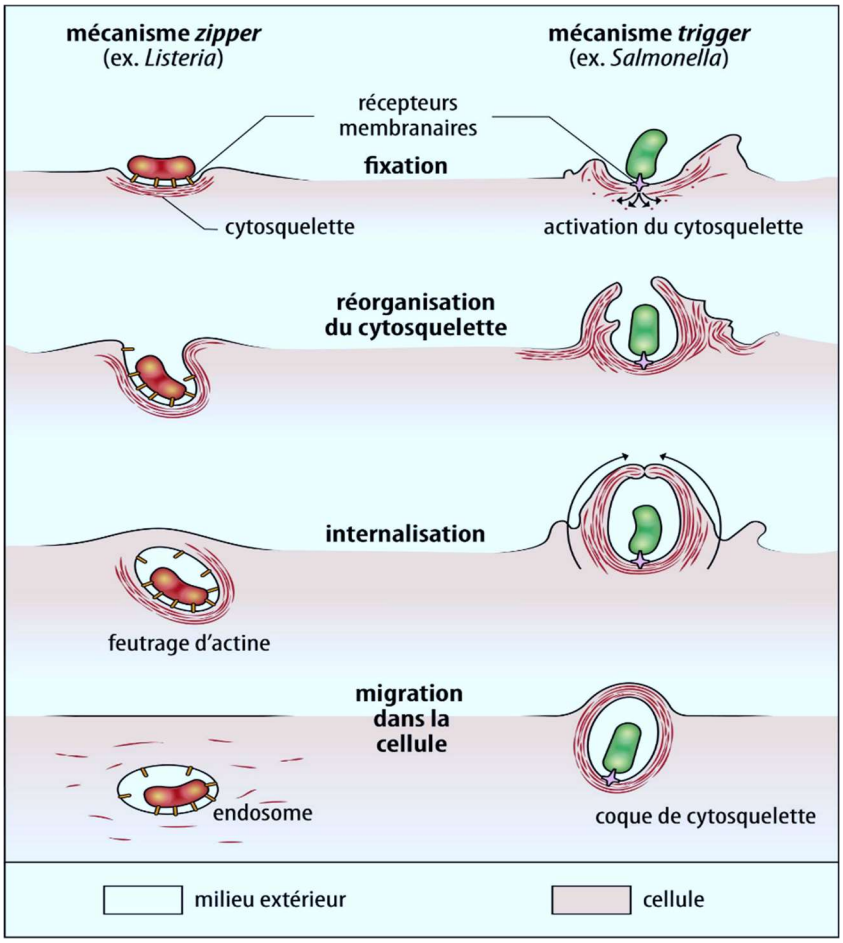


Figure 5: Mechanism of bacterial penetration into host cells

II.2.5.1.1. Intracellular Survival Mechanisms: Intracellular bacteria share the ability to grow inside eukaryotic cells. They originate from very different genetic backgrounds but have all adapted to the cellular environment in the same way. This is called convergent evolution. Strict intracellular bacteria are those that cannot be cultured outside of a cellular support. They are incapable of autonomous replication, e.g., Chlamydia Rickettsia, Coxiella burnetii. Facultative intracellular bacteria can multiply in environments with or without cells: e.g., Bartonella-Brucella melitensis, Legionella pneumophila. Often, the compartment in which multiplication takes place is within macrophages for Brucella (brucellosis) and Legionella (Legionnaires' disease). For some bacteria, the cellular type in which multiplication occurs is endothelial cells: Rickettsia (spotted fever). Internalization is a means of dissemination for pathogenic bacteria but also a means of escaping environmental constraints such as host cell defenses and often the acidic environment that hosts it.

II.2.5.1.2. Adaptation Mechanisms: It is suggested that ancestral amoebae served as meeting places facilitating the exchange of genetic material between different bacteria and accelerating the adaptation of these bacteria to the intracellular environment of eukaryotic cells. This process may have conferred upon these bacteria the ability to infect cells of higher animals, especially phagocytes, which share many physiological similarities with amoebae.

II.2.5.1.3. Classification :

1. **Obligate Intracellular Pathogens:**
    - Bacteria incapable of surviving outside of cells.
    - Complete adaptation to the host.
    - Example: Rickettsia
2. **Facultative Intracellular Pathogens:**
    - Bacteria adapted to environmental predators like amoebae (e.g., Legionella pneumophila).
    - Bacteria with a stage of the cycle involving intracellular passage (e.g., mucosal traversal like Salmonella).
    - Bacteria seeking a shelter from host defenses (e.g., Brucella, BK).

II.2.5.1.4. Factors of Intracellular Parasitism:

Entry into cells ($\not\subset$ epithelial or phagocytes): Specific interaction between bacterial surface molecules and cell membrane receptors results in cytoskeleton rearrangements leading to bacterial ingestion $\Rightarrow$ phagocytosis induced by the bacterium.

II.2.5.1.5. Fate of Bacteria in Invaded Cells: Pathogenic bacteria can invade non-phagocytic cells like epithelial cells by two mechanisms: "zipper" and "trigger." After internalization, the bacterium can survive inside the endocytic vacuole (Salmonella, Legionella) or escape into the cellular cytoplasm (Shigella, Listeria).

-The bacterium is found in a phagocytic vacuole or phagosome.

Intracellular Survival and Multiplication: Several mechanisms are possible for the fate of the bacterium in the cell, either survival in the phagosome: inhibition of phagosome-lysosome fusion (e.g., Legionella, Brucella). Intravacuolar bacterial multiplication

-Intracytoplasmic bacterial multiplication.

Survival in the cytoplasm: escape from the phagosome after lysing the vacuole membrane ⇒ release of the bacterium into the cytoplasm. Intracytoplasmic bacterial mobility and propulsion through the cell to adjacent cells without external passage (Rickettsia). Intracytoplasmic bacterial multiplication.

-Escape from host defenses: Intracellular multiplication allows the bacterium to remain sheltered from host immune defenses. Induction of a cellular immunity directed against infected cells.

-Survival and multiplication in the vacuole: (e.g., Legionella Legionnaires' disease). The Legionellaceae family includes the single genus Legionella, comprising 50 species and over 70 serogroups. Legionella pneumophila presents 15 antigenic types. L. pneumophila serogroup 1 is responsible for 70 to 90% of Legionnaires' disease cases worldwide. This bacterium is capable of multiplying inside cells, particularly in free-living amoebae and human macrophages. The key factor in the pathogenicity of Legionella pneumophila comes from its ability to survive and multiply within human macrophages and monocytes. Two loci are involved in this process: the dot locus (defective organelle trafficking) and the icm locus (intracellular multiplication), necessary for bacterial multiplication inside the phagosome and also involved in macrophage lysis. The combination of these genes enables the survival and multiplication of L. pneumophila in the phagosome according to the following sequence: Role of dot locus products for the invasive power of the bacterium:

- Inhibition of phagosome-lysosome fusion
- Recruitment and fusion of cellular organelles with the phagosome
- Intracellular multiplication of *L. pneumophila*

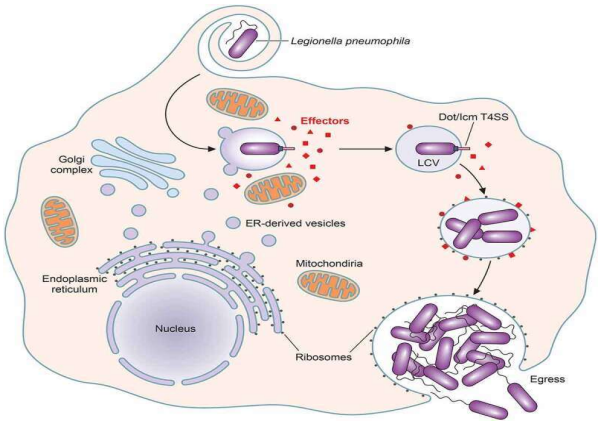


Figure 6: Survival and multiplication within the Legionella vacuole

II.2.5.2. Toxinogenesis (or "toxic power")

Toxic power is assessed by two parameters: the lethal dose 50 (LD50) tested on a laboratory animal (rats or mice) and the minimum lethal dose (MLD). Toxigenic bacteria produce toxins, i.e., toxic biological molecules (antibiotics, hormones, etc.) capable of harming the host, even in the absence of the producing microorganism. Nearly 300 toxic substances had been identified by 1880. Recently, new toxins are discovered in bacteria identified as human pathogens, such as saprophytic opportunistic bacteria, responsible for infections in immunocompromised patients (e.g., Aeromonas hydrophila). Some pathogenic bacteria produce several toxins, to which high-pathogenicity extracellular enzymes can be added: example of Staphylococcus aureus, methicillin resistance, and which produces several types of toxins.

-All strains produce hemolysins, hyaluronidases, DNAses, coagulases.

-In some strains: superantigens, exfoliatins, leukocidins.

Two types of toxins are distinguished:

• **Exotoxins** are proteins produced during bacterial growth, totally or partially released during the growth of the microorganism.

• **Endotoxins** are complex molecules (LPS), part of the bacterial wall, and released only during the destruction of the microorganism.

II.2.5.2.1. Exotoxins (Protein Toxins):

Exotoxins are proteins sometimes associated with a carbohydrate or lipid whose action can be local (at the site where they are synthesized) or distant (away from the site of production). Protein toxins have a very high toxic power: tetanus and botulinum toxins have a lethal minimal dose (LMD) in mice on the order of 10-11 grams (0.00000000001 g)! They are much more "active" substances than the most toxic chemical poisons. Protein toxins act specifically and therefore cause specific symptoms. A toxin binding to the membrane of intestinal cells can therefore be called an "enterotoxin" (Vibrio cholerae; cholera toxin and Shigatoxin of E. coli). A toxin acting on nerve cells is a neurotoxin (Clostridium botulinum; botulinum toxin and Clostridum tetani; tetanus toxin). Many toxins secreted by phylogenetically distant bacteria have a common mode of action.

1- ADP-ribosylation Toxins of proteins essential for cellular metabolism: Target proteins are:

• Elongation factor EF2,

• GTP-dependent proteins (G proteins), regulators of adenylate cyclase: cholera toxin, heat-labile enterotoxin of E. coli.

Types of Action:

• Inhibition of protein synthesis

• Alteration of transmembrane signal transduction

• Disorganization of actin in the cytoskeleton

• Exoenzyme

Toxins have 2 components: A subunit A: enzymatic activity, active after dissociation and a subunit B: specific binding with the membrane of the host cell, transfer of the enzyme through the membrane, the subunit B is non-toxic alone and acts on specific cellular receptors: - Cholera toxin: gangliosides GM1 - Tetanus toxin: GT1 or GD1, botulinum toxin: GD1

2- Membranolytic toxins: Membranolytic toxins will totally destabilize the membrane of the eukaryotic cell (Cytolysin), which destroys the integrity of the cell membrane. Cytolysins forming pores in the cytoplasmic membrane belong to the RTX (Repeat in ToXIn) hemolysin family because of the duplication of a motif of 9 amino acids: example of hemolysin HlyA produced by pathogenic strains of E. coli (UPEC). Perfringolysin toxin: toxin that forms pores, cholesterol dependence: perfringolysin O, streptolysin, Listeriolysin

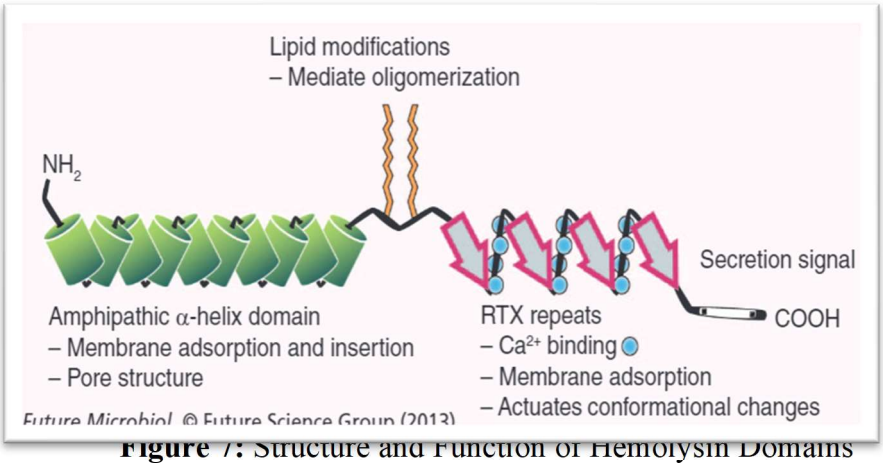


Figure 7: Structure and Function of Hemolysin Domains

3- Superantigens:

Superantigens are bifunctional molecules that associate with at least two types of receptors: TL-cell receptors and the major histocompatibility complex. Binding of superantigens to these receptors leads to differentiation and polyclonal proliferation of T cells, resulting in the release

of inflammatory cytokines from activated T cells and antigen-presenting cells, inducing fever, chills, and vomiting.

4- **Exotoxins of Staphylococcus aureus and Streptococcus pyogenes target three main sites**: T-cell receptors, antigen-presenting cells, and hepatocytes. Actions of toxins on T cells: release of cytokines inducing fever and immunosuppression.

Figure 8: Superantigen Toxins from *Staphylococcus aureus* (Enterotoxins)

II.2.5.2.2. Endotoxins.

Endotoxins correspond to the lipopolysaccharides (LPS) found in the outer membrane of Gram-negative bacteria. Among the three components of the molecule (lipid A, central polysaccharide or "core," side chain or O antigen), only lipid A carries toxicity. At low concentrations, endotoxin triggers alarm reactions, leading to three major events:

- Production of cytokines by monocytes, macrophages, and endothelial cells
- Activation of the complement system
- Activation of the coagulation system

LPS can lead to disseminated intravascular coagulation, followed by systemic infection, which can complicate into septic shock and often proves fatal. At high concentrations, LPS can lead to disseminated intravascular coagulation followed by systemic infection (sepsis), causing endotoxic shock, which is often fatal.

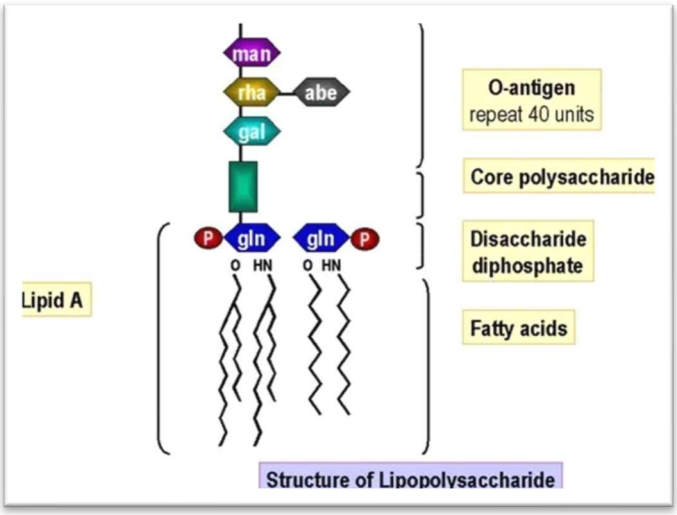


Figure: Pathogenic Potential of Escherichia coli Strains: (LPS structure)

II.3. Bacterial Secretion Systems and Cellular Effectors:

Pathogenic bacteria use a multitude of methods to invade mammalian hosts, damage tissue sites, and prevent the immune system from responding. An essential element of these strategies for many bacterial pathogens is the secretion of virulence proteins across the lipid membranes. These "virulence factor" proteins are exported from the bacterium's cytosol into host cells or the environmental host. Secretion systems allow the transport of macromolecules (proteins or nucleoproteins) between the bacterial cytoplasm and the external environment, across the internal and external hydrophobic bacterial membranes, which are impermeable to hydrophilic compounds. For this, bacteria need secretion systems. Secretion systems are complex structures composed of several proteins. The assembly of these proteins is a highly regulated process essential for the function of the secretion system. Five types of secretion systems are commonly described in bacteria: type I (T1SS), type II (T2SS), type III (T3SS), type IV (T4SS), and type V (T5SS). They are classified according to their protein composition, amino acid sequence, and overall structure. These secretion systems are used to transport different types of molecules, including proteins, DNA, and protein-DNA complexes. They can transport virulence factors (proteins involved in the pathogenicity of the bacteria), toxins, enzymes, and other proteins involved in various cellular processes.

-The cell wall of Gram-negative bacteria is organized into three main parts:

1. **The inner membrane (IM)**

2. **The periplasmic space (periplasm):** contains enzymes involved in the degradation and transport of nutrients.
3. **The outer membrane (OM):** is in direct contact with the external environment, containing LPS and transport proteins called porins.

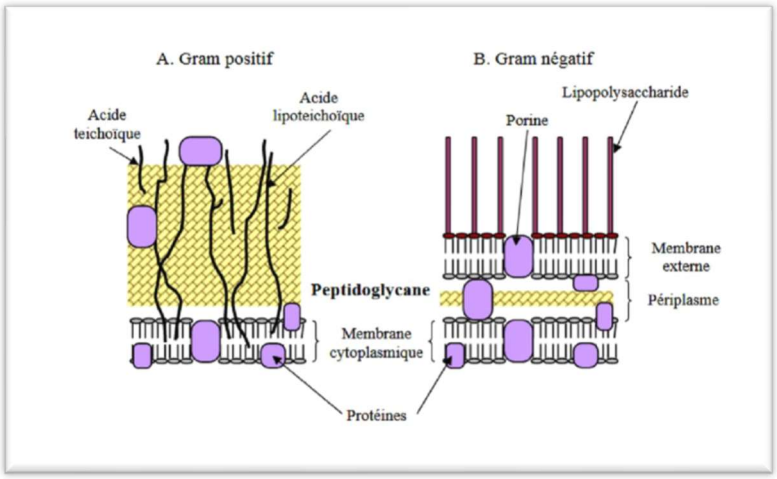


Figure 10: Organization of the Gram-negative and Gram-positive cell wall

II.3.1. Bacterial Protein Secretion: Protein secretion poses different challenges depending on whether the bacterium is Gram-positive or Gram-negative:

-For Gram-positive bacteria to secrete proteins: they must transport them across the cytoplasmic membrane. Once the cytoplasmic membrane is crossed, the protein either passes through the relatively porous peptidoglycan to the external environment or remains buried or attached.

-Gram-negative bacteria face more hurdles when secreting proteins: they also need to transport proteins across the cytoplasmic membrane, but to complete secretion, proteins must be able to escape proteolytic enzyme attack in the periplasmic space and be transported across the outer membrane.

-Secreted proteins can play various roles in promoting bacterial virulence:

• Enhancing adherence to eukaryotic cells

• Trapping resources in an environmental niche

• Directly targeting host cells and disrupting their functions, including inhibiting immune functions.

II.3.1.1. Passage through the inner membrane:

Proteins synthesized in the cytoplasm and destined for post-cytoplasmic compartments face passage through the inner membrane. The export pathways allowing the translocation of these proteins are the General Secretion (Sec) pathway and the Tat (*Twin-arginine translocation*) pathway.

-**The General Secretion (Sec) pathway:** is the most important protein export pathway. Its presence is essential as its absence is lethal for the bacterium. It allows the passage of unfolded and inactive proteins to the periplasm or their insertion into the inner membrane.

- Proteins that need to be transported across the cytoplasmic membrane by this pathway are synthesized as pre-secretory proteins called preproteins:
- The preprotein has a signal peptide at its N-terminal end recognized by the Sec machinery.
- Special proteins called chaperones (e.g., SecB) bind to the signal peptide as soon as it is synthesized; this delays protein folding and allows the preprotein to maintain a conformation required for transport by the Sec machinery.
- Some Sec proteins (SecY, SecE, and SecG) are thought to form a pore in the membrane through which the preprotein passes.
- Another protein (SecA) binds to SecYEG proteins and the SecB-preprotein complex:
- SecA acts as a motor to transport the preprotein (but not the chaperone protein) across the cytoplasmic membrane using ATP.
- Upon emerging from the cytoplasmic membrane, the protein folds to its proper conformation, and disulfide bridges are formed when needed.

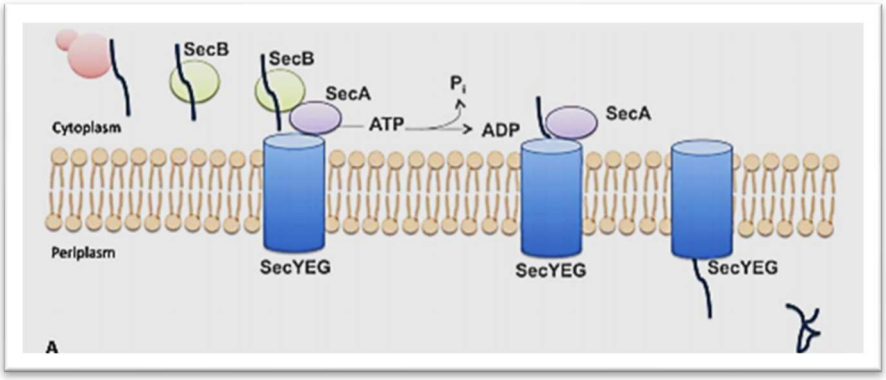


Figure 11: The General Secretion (Sec) pathway

-**The Tat (Twin Arginine Translocation) pathway:** Unlike the Sec pathway, which transports unfolded proteins, the Tat pathway can translocate folded proteins across the lipid bilayer membrane. It is an alternative system identified in *E. coli* allowing the translocation of folded and activated proteins within the cytoplasm. (The need for certain enzymes to bind cytoplasmic cofactors to become active, such as the metalloenzymes of the respiratory chains).

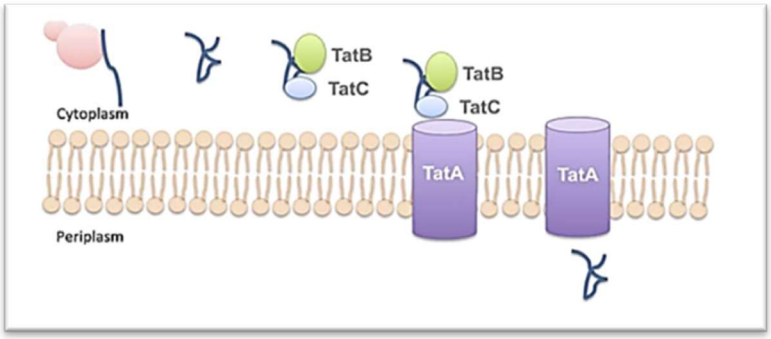


Figure 12: The Tat (Twin Arginine Translocation) pathway

II.3.1.2. The Different Secretion Systems in Gram-Negative Bacteria.

In general, bacterial protein secretion systems can be divided into classes based on their structures, functions, and specificity. Some systems are conserved across all bacteria and secrete a wide range of substrates, while others are found only in a small number of bacterial species and/or are specific to one or a few proteins only. Six secretion systems are known in Gram-negative bacteria. These systems differ in their macromolecular organization and the type of transported substrate (protein or DNA). These systems can be categorized into two categories based on the number of steps required for the transportation of the secreted protein:

- Secretion can occur in a single step (from the cytoplasm to the extracellular milieu): these are called Sec-independent. This category includes two secretion systems:
    - Type I (T1SS)
    - Type III (T3SS)
- Secretion can occur in two steps (periplasmic step): they can be Sec-dependent or Tat-dependent. This category includes:
    - Type II (T2SS)
    - Type IV (T4SS)
    - Type V (T5SS)

- Type VI (T6SS)

II.3.1.3. Different Types of Secretion Systems

II.3.1.3.1. Type I Secretion System:

The type I secretion system has a relatively simple structure and is composed of only three protein subunits (Baron and Coombes, 2007; Fig. 1). It consists of an outer membrane protein (OMP) and a membrane fusion protein (MFP). The MFP protein forms a complex in the periplasmic space with an ATP-binding cassette protein (ABC; Angkawidjaja and Kanaya, 2006). Type I systems are responsible for the secretion of extracellular enzymes by Gram-negative bacteria, such as the secretion of α-hemolysin in Escherichia coli, adenylate cyclase in Bordetella pertussis, and proteases in Pseudomonas aeruginosa.

II.3.1.3.2. Type II Secretion System.

It participates in bacterial virulence and allows secretion in two steps. It begins with the export of exoproteins into the periplasm by the Sec system for the majority or by the Tat system. Once in the periplasmic space, proteins acquire their three-dimensional conformation. Finally, these exoproteins are secreted outside the bacterium. This system is complex and requires assembly of 15 different proteins called Gsp for General Secretion Pathway.

II.3.1.3.3. Type III Secretion System.

This system is found only in pathogenic animal or phytopathogenic Gram-negative bacteria. It consists of about twenty proteins forming a syringe allowing the injection of bacterial cytoplasmic proteins directly into the host cell (needles and syringe), where they can modulate a wide variety of functions such as immune responses. T3SS substrates are generically called effector proteins. Pathogenic bacteria can secrete only a few effector proteins, as in the cases of Pseudomonas and Yersinia, or several dozens, as in the cases of Shigella and EHEC.

II.3.1.3.4. Type IV Secretion System.

It is involved in various processes, such as toxin secretion, DNA transformation, cell adhesion, and bacterial mobility. The system consists of an extracellular pilus formed by an assembly of pilins.

II.3.1.3.5. Type V Secretion System:

It corresponds to the autotransporter family and the chaperone-usher system.

- **Autotransporters:** Proteins secreted by this pathway contain all the necessary information for export. Autotransporters pass through the general Sec secretion pathway to reach the outer membrane.
- **Chaperone-usher pathway:** It consists of a periplasmic chaperone protein and an integral outer membrane protein called usher. This system is dedicated to the secretion and assembly of surface appendages such as pili and fimbriae, but may also be involved in the formation of the bacterial capsule.

II.3.1.3.6. Type VI Secretion System.

Recently discovered in Vibrio cholerae, the structure of this system remains unknown, but it is known to allow secretion of a virulence factor called Hcp1 (Hemolysin coregulated protein).

II.4. Secretion in Gram-Positive Bacteria. The main pathway for transporting proteins across the cytoplasmic membrane of Gram-positive bacteria is the Sec-dependent pathway. These bacteria use a modified version of the type I system to transport proteins across the cytoplasmic membrane. Genome analysis of Bacillus subtilis has identified 77 ABC transporters. This suggests that ABC transporters carry, in addition to proteins, a wide range of solutes, such as carbohydrates and amino acids, and export inhibitors out of cells.

II.5. The ABC System: ATP-Binding Cassette. This system involves a substrate-binding protein, a membrane transporter, and an ATP-hydrolyzing protein. It depends on substrate-binding proteins attached to the membrane near the outer part of the transporter. In Gram-positive bacteria, molecules to be transported must diffuse through the wall to reach the surface of the membrane where they will be captured by binding proteins.

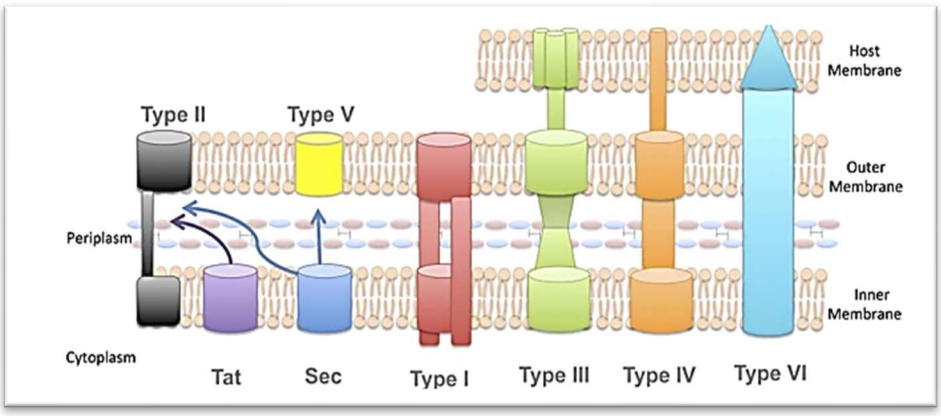


Figure 13: Different Types of Secretion Systems in Gram-negative bacteria

Chapter III: Bacterial Vectorization

Introduction

Vectorization is a technique that allows for the controlled distribution of an active ingredient to a target by associating it with a vector. In the case of bacteria, vectorization can be used to deliver antibiotics or other therapeutic agents directly to bacteria, thereby improving their effectiveness and reducing side effects. Bacterial vectorization refers to the process by which foreign genes, often in the form of vectors such as plasmids or viruses, are introduced into a bacterium so that it expresses the characteristics or products of these foreign genes. This technique is widely used in biotechnology and genetic engineering to modify bacteria so that they produce specific proteins, acquire new functions, or serve as delivery vectors for therapeutic or industrial applications. Bacterial vectorization can involve different methods, such as transformation by electroporation, transformation by heat shock, transduction by bacteriophage viruses, or bacterial conjugation. Once the foreign genes are integrated into the bacterial genome or maintained in extrachromosomal genetic elements such as plasmids, bacteria can express them and pass them on to their offspring. Vectorization is a powerful tool that enables the production of drugs, industrial enzymes, biofuels, and other products of interest, as well as the development of genetically modified bacteria for applications in medical research, agriculture, and the environment. Active Principles Involved (API) Make the distribution of drugs in the body as independent as possible from the inherent properties of the active molecule; to subject it to the physicochemical properties of a chosen vector based on the target aimed at.

- Short plasma half-life
- Poorly distributed active principle
- Active principle toxic to certain tissues
- Low intracellular penetration
- Poor subcellular distribution

III.1. Potential Application Potentials of Vectors:

- Protect the active molecule from the administration site to the site of action,
- Improve the transport of active principles to certain difficult-to-reach sites and their penetration into targeted cells,

- Increase the specificity of action, effectiveness, and regularity of active principles at the target level.
- Decrease toxicity to certain organs by modifying the tissue distribution of active principles

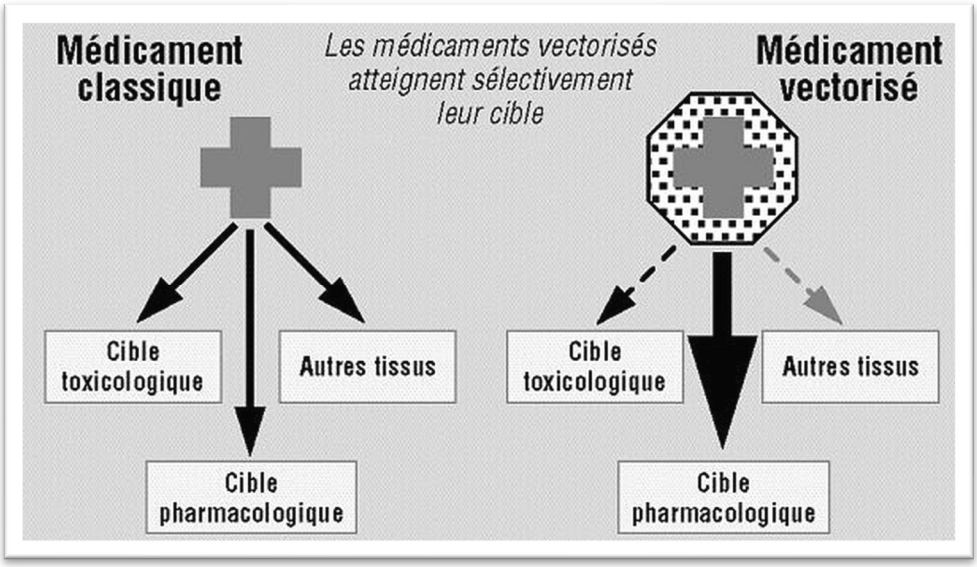


Figure 13: Drug vectorization**III.2. Characteristics of a good vector:**

- Non-toxic
- Biodegradable
- Appropriate size allowing for:

✓ Incorporation of a range of active ingredients.

✓ Internalization of the molecule into the target cell.

✓ Easy administration.

- Possessing positive tropism for a specific organ or tissue type.
- Stable but reversible binding of drug to vector.
- Protection of the active molecule from the administration site to the targeted destination.
- Easy manufacturing.

III.3. Classification of vectors:

-Classification according to size:

✓ Microparticle vectors

- ✓ Nanoparticle vectors

-Classification according to nature:

- ✓ Organic Nanoparticles
- ✓ Lipid particles
- ✓ Polymeric particles

- **Inorganic Nanoparticles**
  - ✓ Mineral and metallic particles

- **Classification by generation (based on their potentialities):**
  - ✓ 1st generation vector
  - ✓ 2nd generation vector
  - ✓ 3rd generation vector

III.4. Different types of vectors:

III.4.1. Microparticles:

Microparticles (MPs) are spherical particulate systems, made from a biodegradable polymer base, used to transport active ingredients. They range in size from 1 to 1000 µm. Microparticles can be either microspheres or microcapsules. The systems used are either solid (microspheres) or hollow (microcapsules). Hollow systems consist of a reservoir delimited by a polymeric wall in which the active ingredient is in solid or liquid form. Solid systems consist of a polymeric matrix in which the active ingredient is dispersed or dissolved.

III.4.2. Organic Nanoparticles: Lipid vectors and polymeric vectors. Among lipid vectors, liposomes, niosomes, solid lipid nanoparticles, and nanostructured lipid nanoparticles are included.

III.4.2.1. Liposomes: These are small spherical vesicles consisting of one or more bilayers of phospholipids, with an aqueous cavity in the center. Depending on its nature, the active ingredient can be located either in the aqueous cavity or in the lipid bilayer. The morphology of liposomes, particularly lamellarity, as well as their size distribution, depends on the manufacturing processes. Based on size and number of lamellae, liposomes are categorized into: LUV: large unilamellar vesicule, MLV: multi lamellar vesicule, SUV: small unilamellar vesicule.

Preparation Methods: Numerous processes have been developed for the preparation of liposomes, distinguishing between...

II.4.2.2. Bangham Method (1965): The simplest method for preparing liposomes involves evaporating the organic solvent in which the lipids are dissolved, and then re-suspending them in an aqueous solvent.

Principle and steps:

- ✓ Dissolution of phospholipids and other components of the liposome membrane in a volatile solvent such as chloroform.
- ✓ Evaporation of the solvent under reduced pressure in a rotary evaporator: a translucent lipid film forms on the surface of the flask.
- ✓ Addition under agitation of an aqueous solution to hydrate the phospholipids, which is done at a temperature higher than the phase transition temperature of the phospholipids.
- ✓ Addition of the active ingredient:
    - If it is lipophilic: it will be added to the organic solution of phospholipids at the beginning of the operation.
    - If it is hydrophilic: it will be added to the aqueous solution.

The Bangham method produces multilamellar liposomes (MLVs) with high diameter and highly heterogeneous size. In order to homogenize, reduce the size of MLV vesicles, or transform them into small unilamellar vesicles (SUVs), several techniques can be used, including ultrasound. The main lipid components (and possibly lipophilic drugs/macromolecules) are dissolved in an organic solvent (A). After solvent evaporation, a dry lipid film (thin) is formed (B). The lipid film is then rehydrated in a saline buffer (containing potentially hydrophilic drugs to be trapped), causing swelling of the lipid bilayer stacks (C). Subsequent agitation of the sample promotes the formation of multilamellar vesicles (polydispersed) (D). The final steps of the production process include reducing the size of the liposomes (E), their purification (F), and their characterization (G).

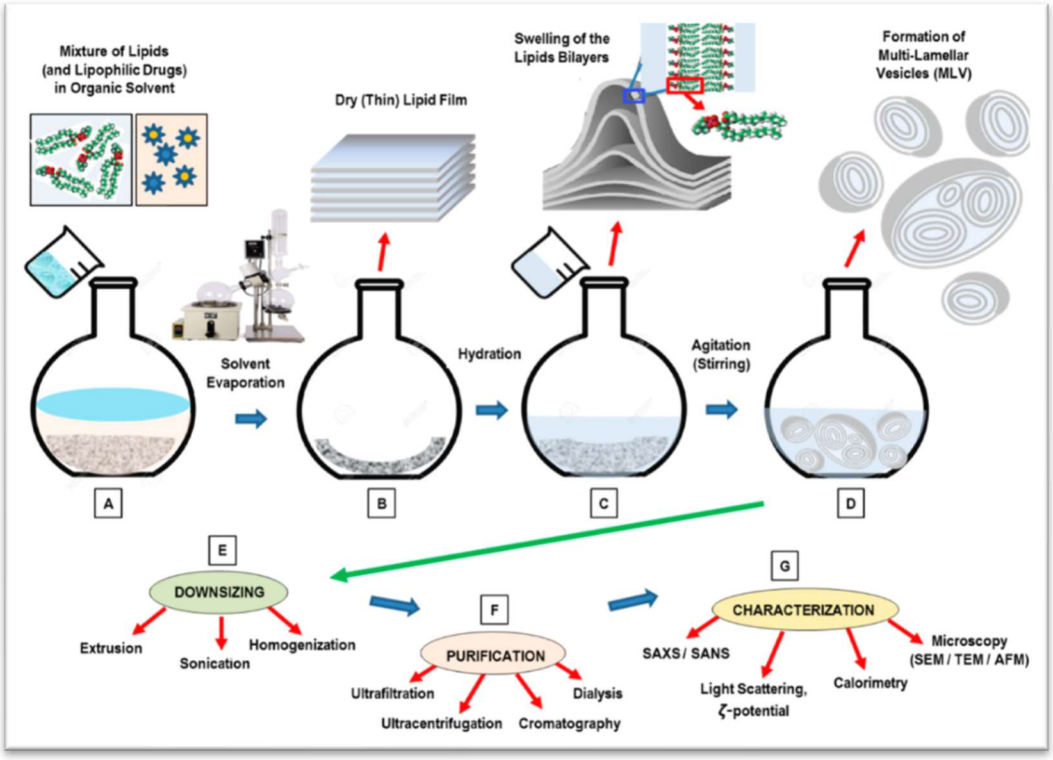


Figure: Schematic representation of the main steps of the thin-layer hydration method for liposome preparation

III.4.2.3. The inverse phase evaporation method:

Phospholipids are dissolved in an organic solvent such as ethyl ether. The aqueous phase is added to an excess of emulsified organic phase with lipids placed at the interface. Solvent removal by evaporation under reduced pressure leads to the approximation of reverse micelles and then to gel formation. The pressure is further reduced to promote total evaporation of ether. This causes the rupture of the gel phase and the approximation of monolayers to form liposomes.

III.4.2.4. The method of injecting an organic solution of phospholipids:

Several techniques exist, including ethanol injection, ether injection, and isopropanol injection. This technique involves the spontaneous formation of SUV liposomes following injection of an organic lipid solution into an aqueous solution and removal of the organic solvent by evaporation under reduced pressure, dialysis, or tangential filtration. Lipids are dissolved in an organic solvent (A), and the formation of reverse micelles is observed (B). Addition of an aqueous medium (buffer), followed by emulsification of the solution, promotes the formation

of a homogeneous dispersion of an E/H microemulsion (C). With the final removal of the organic solvent (by rotary evaporation, under vacuum), a viscous gel forms in the solution, which eventually collapses to form liposomes (D) (LUVs)

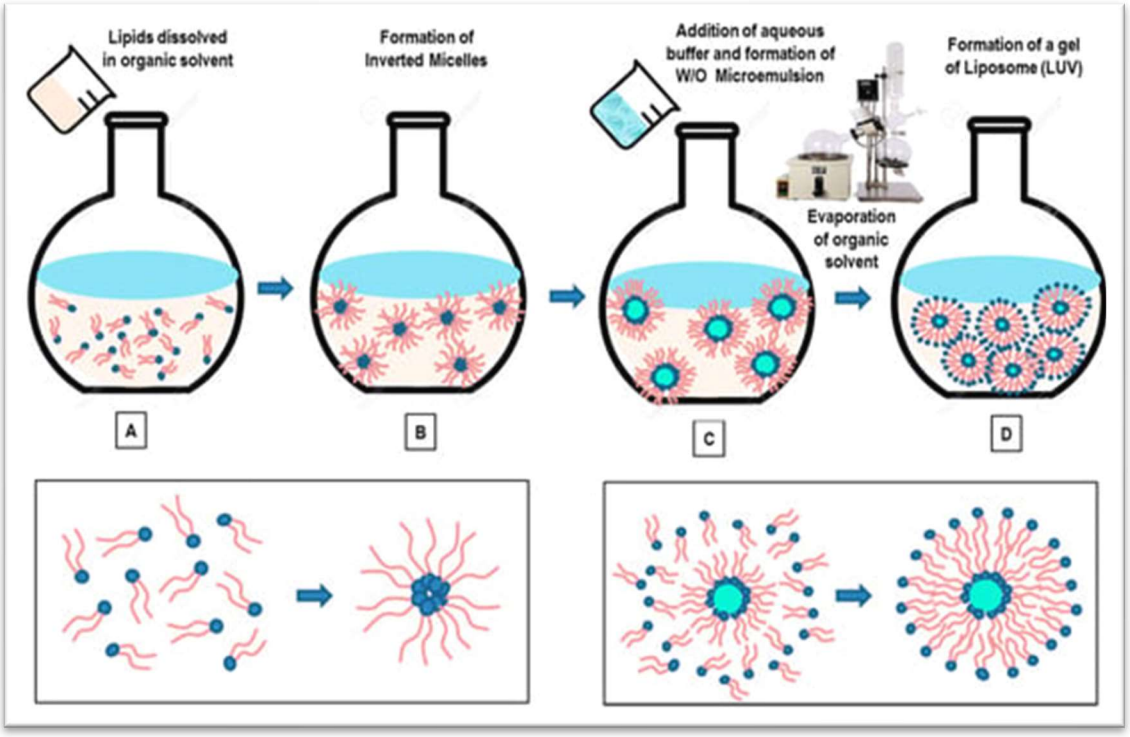


Figure 15: Schematic representation of the main steps of the inverse phase evaporation method.

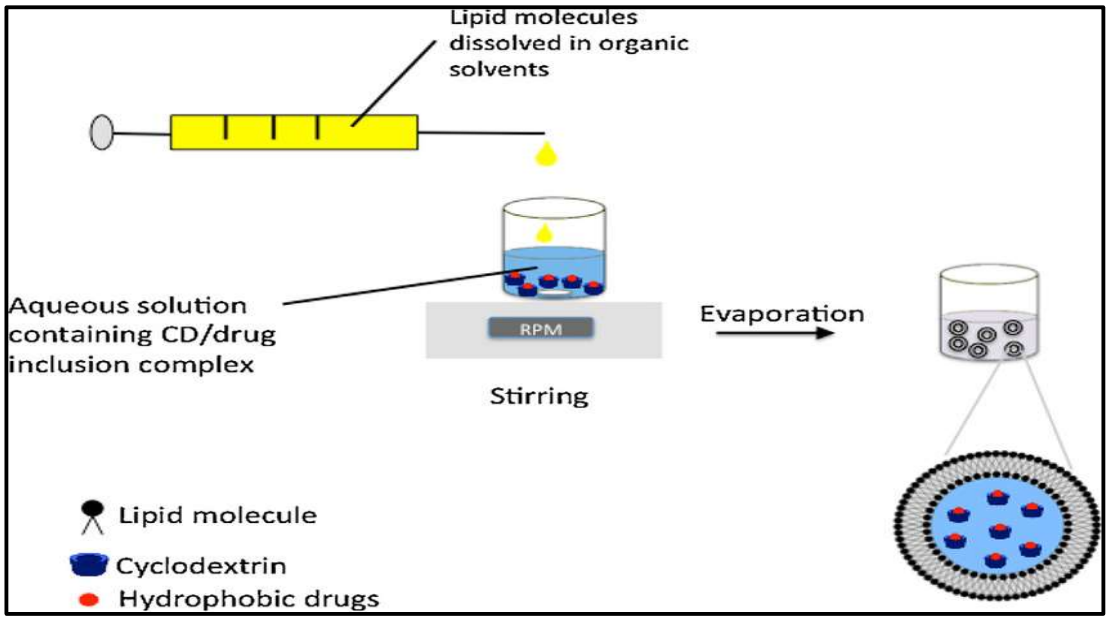


Figure 16. Schematic representation of the main steps of the method of Injection of an organic solution of phospholipids.

III.4.3. Vecteurs polymerics:

III.4.3.1. Polymer Nanoparticles:

These are colloidal systems (10 to 1000 nm) whose structure is generally composed of biodegradable polymers (poly lactic acid PLA, albumin) or non-biodegradable ones (methacrylate, alpha-cyanoacrylate). Nanoparticles can be of matrix type (nanospheres); in this case, the active ingredient can be dispersed or dissolved in the polymer matrix and be released by simple diffusion or as a result of polymer biodegradation in the body. Nanoparticles can also be of reservoir type (nanocapsules); in this case, they consist of a central core generally liquid surrounded by a thin polymer wall whose thickness does not exceed a few nanometers. Several methods for preparing polymer nanoparticles exist, such as:

- Preparation of nanocapsules by polymerization
- Preparation by the emulsification-solvent extraction method

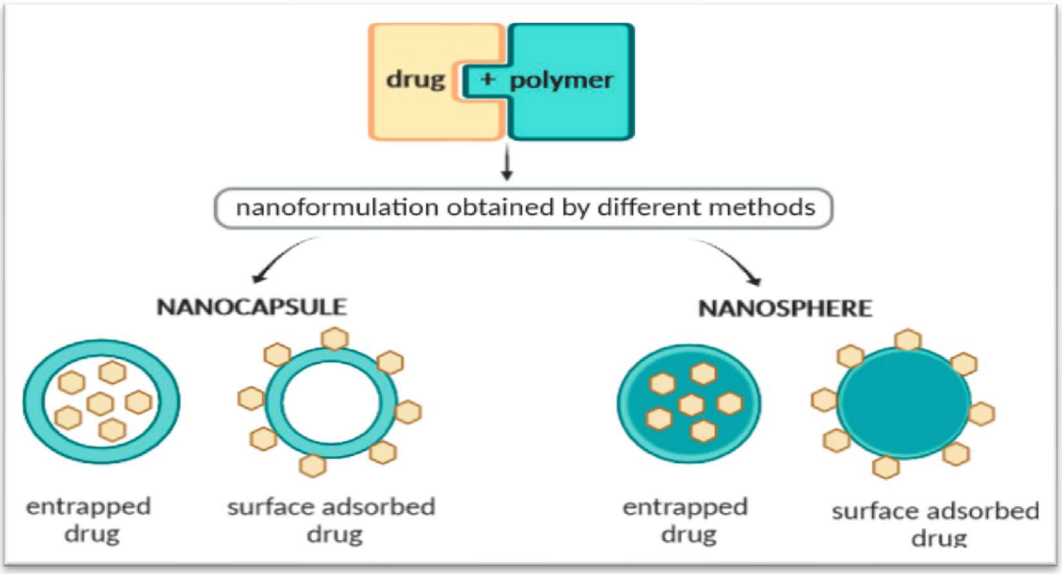


Figure17. Nanoformulation obtained by different methods

III.4.3.2. Polymer Micelles: Micelles are ideal nanocarriers for insoluble agents. They are made up of copolymers consisting of hydrophilic and hydrophobic monomeric units. The formation of polymer micelles results from the reduction of free energy obtained by the association of hydrophobic units with each other to reduce contact with the surrounding

aqueous environment. The self-assembly of amphiphilic copolymers in an aqueous medium lead to the formation of spherical micelles in such a way that the hydrophilic end of the copolymer is oriented towards the surface of the micelle while the hydrophobic end is oriented towards the core of the micelle. Generally, when the hydrophobic segment of the amphiphilic block is longer than the hydrophilic segment, the micelle will have a spherical shape. Otherwise, the copolymer blocks form non-micellar structures, namely rods and lamellae. There are at least three main categories of micelles based on linear block copolymers: Common block copolymer micelles, Copolymer-drug conjugate micelles, and Ionomer complex micelles. Figure 42 illustrates the different categories of micelles (Figure 18)

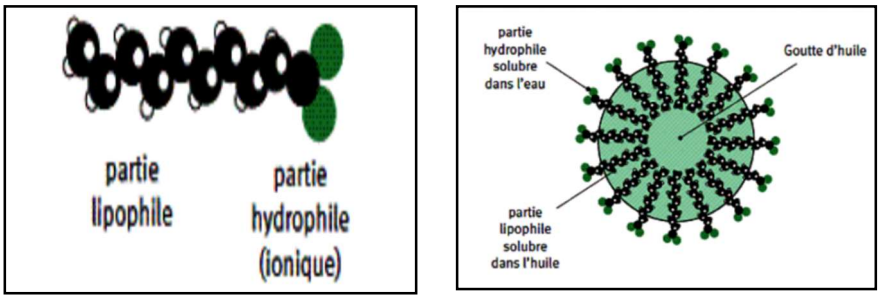


Figure 18: Polymer Micelles:

III.4.4. Drug Vector Generations: Three generations of vectors are distinguished.

III.4.4.1. First-generation vectors: All vectors described previously that have undergone no chemical modification of their surface. Upon intravenous administration, the surface of these vectors becomes covered with opsonin, antibodies recognized by specific receptors on macrophages of the mononuclear phagocyte system (MPS), liver, spleen, and bone marrow. Colloidal vectors decorated with opsonins circulating in the blood are therefore captured by Kupffer cells (liver macrophages) and marginal zone macrophages in the spleen whose receptors specifically recognize opsonins. The hepatic-splenic accumulation of these vectors can thus be advantageous for improving the treatment of certain conditions localized to these tissues. (Fig 19)

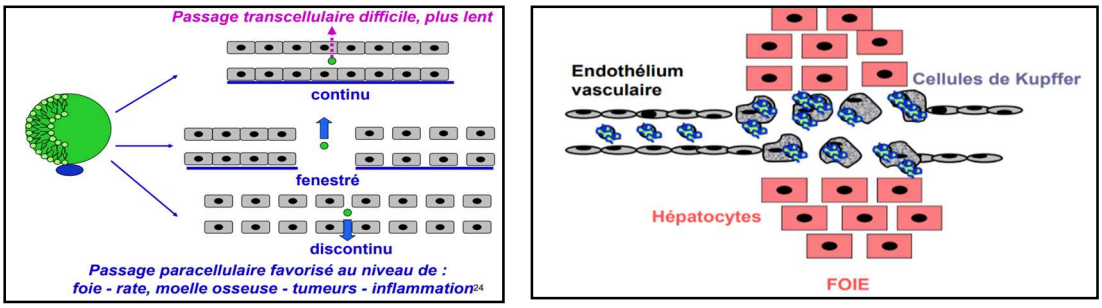


Figure 19: Hepatic Capture of First-generation Vectors**III.4.4.2. Vecteurs de deuxième génération :** "Concept de résidence vasculaire prolongée.The coating of vectors with hydrophilic and flexible polymers, among which PEG (polyethylene glycol) is the best representative, prevents opsonins from binding to their surface (Concept of steric repulsion). After intravenous administration, these "pegylated" vectors are characterized by an extended plasma half-life and reduced hepato-splenic uptake (Fig 20).

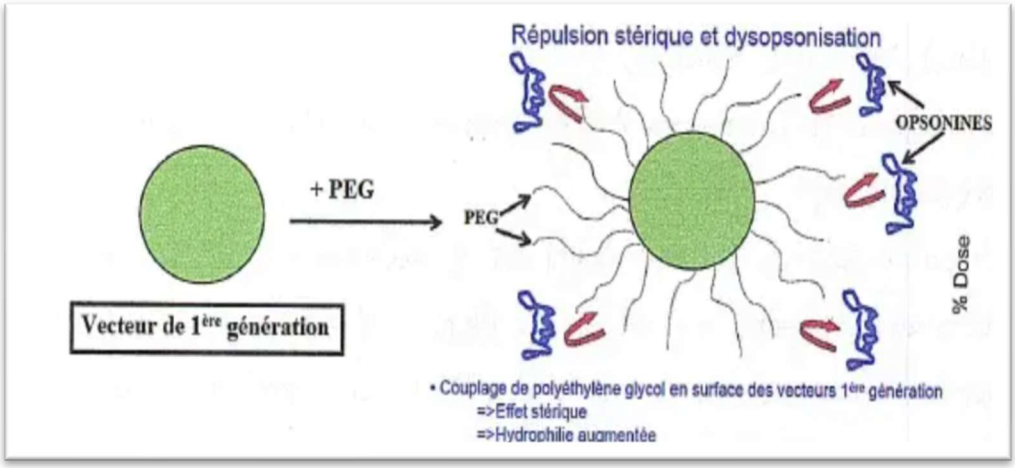


Figure 20: Schematic representation of the concept of steric repulsion that prevents opsonization and recognition by macrophages

The "stealth" nature (lack of recognition by liver, spleen, and bone marrow macrophages) is even more pronounced when the vectors are small in size. These "stealth" vectors, with prolonged vascular residence, have a significant probability of crossing vascular endothelia with increased permeability, such as those located in tumors or infectious foci (Fig 21).

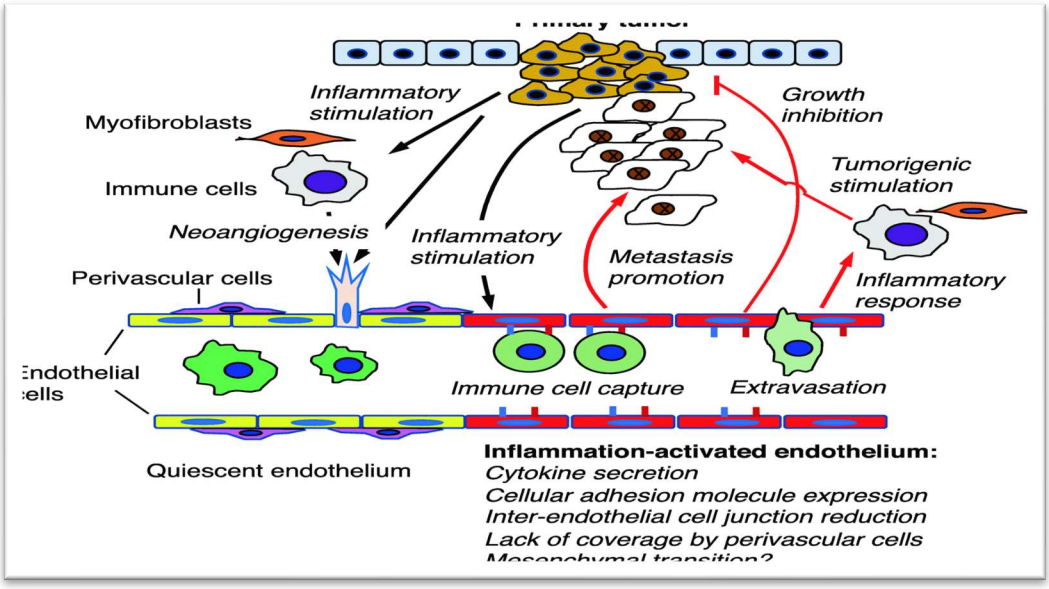


Figure 21: Schematic representation of selective diffusion through tumor vascular endothelium

Applications:

Nanoparticle passage through tumor-permeabilized endothelium: passive targeting. Example: Doxil® or Caelix® (Doxorubicin Lipo-PEG) Doxorubicin • Anemia, alteration of veins and tissues at the injection site • Decrease in platelet count • Cardiac toxicity Formulated in PEGylated liposomes Indication: Breast cancer, ovarian cancer, and Kaposi's sarcoma (HIV patients).

III.4.4.3. Third-generation vectors:

When second-generation vectors are decorated with ligands (antibodies, peptides, sugars, folic acid), they are then capable of selectively recognizing antigens or receptors that are overexpressed on the surface of target cells (cancer cells, infected cells, etc.). The design of these third-generation vectors requires the construction of supramolecular structures composed of:

- A biodegradable core (phospholipids or polymers),
- A layer of hydrophilic and flexible polymers (PEG) to avoid hepato-splenic recognition,
- A membrane recognition ligand at the end of certain PEG chains.

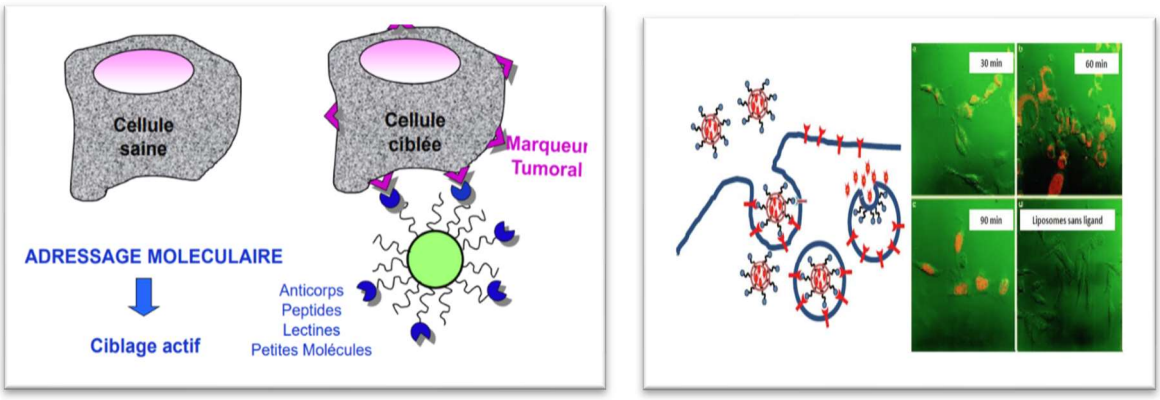


Figure 22: Schematic representation of molecular targeting of a vector to a target cell using a recognition ligand

Cellular internalization generally occurs via the endocytic pathway, with the vector then localized in lysosomes or the cellular cytoplasm depending on the intracellular trafficking followed by the ligand. However, these vectors still face certain obstacles including (Fig 22):

- Heterogeneity of tumor antigens
- Possible antigenicity: vector-antibody binding decreases affinity for the target. This disadvantage is corrected by including a SPACER between the vector and antibody.

Chapter IV: Experimental Models for the Identification of Virulence Factors.

Introduction

One of the current challenges in microbiology is to characterize the virulence mechanisms of pathogenic bacteria, particularly from a molecular perspective. Thus, some 100 years after Robert Koch, microbiologists like Stanley Falkow have adopted a molecular version of Koch's postulates to define a virulence gene, which can be summarized in three points: a) the gene confers a certain virulence phenotype to the studied bacterium; b) inactivation of this gene abolishes the phenotype in question; c) reintroduction of the gene restores the wild-type phenotype in the mutant. This strategy, essentially based on mutation and easily applicable to experimental models, allows the identification of genes that, by meeting these criteria, can be designated as virulence genes. It can be advantageously complemented by studies of differential expression, either at the transcriptional level (transcriptome) or at the protein level (proteome). All these techniques, now supported by complete sequencing of bacterial genomes, provide information that defines what could be called the "virulome": the set of genes contributing to the establishment of the pathogenic power of a bacterium, the knowledge of which will allow for new vaccine approaches or the development of new anti-infective strategies. The identification of virulence factors in bacterial pathogens can be achieved using various experimental models. Studies of bacterial pathogenicity allow for the analysis, either finely or globally, of the evolution of infection in a host or an aspect of the infectious process. Knowledge gained from studies on animals or cells is valuable for better understanding pathogenesis and can lead to strategies for combating these infections, including vaccination. Many ongoing studies focus on intracellular parasitism (e.g., Salmonella), highlighting numerous genes involved in this mechanism. Analysis of host-pathogen interactions generally focuses on a particular aspect of pathogenicity. However, the pathogenesis of these infections appears complex, involving many genes and functions in virulence. The identification of these genes/functions can rely on several strategies, with validation of their role in developed *in vivo* and *in vitro* study models. Here are some of the most commonly used models:

IV.1. Experimental models:

Animal Models:

-Murine Models (mice, rats, hamsters): Animals are infected with the studied bacterium to assess virulence and pathological effects.

-Non-Murine Models (rodents, rabbits, guinea pigs): These models can be used to study specific aspects of virulence or host-pathogen interactions.

-Avian, Fish, or Invertebrate Models: Used to study virulence in specific contexts, such as avian diseases or infections in fish.

-In Vitro Models: Various in vitro tests have been developed as alternative methods for studying virulence factors of bacterial strains. These methods are more flexible and allow for relative standardization.

-Cell Cultures: Human or animal cells are infected with the bacterium to study host-pathogen interactions.

-Organ-on-a-Chip Models: Microfluidic devices are used to simulate the *in vivo* cellular environment and study virulence and pathogenicity.

-Artificial Biofilms: Bacterial biofilms are cultured *in vitro* to study biofilm formation and associated virulence factors.

-Alternative Models:

Ex Vivo Models: Tissues or organs isolated from animals are infected with the bacterium to study virulence in an environment closer to that *in vivo.*

-Mathematical and Simulation Models: Used to predict and model the spread of infection and the impact of virulence factors.

IV.2. Identification of Genes Involved in Virulence:

These experimental models can be used individually or in combination to gain a comprehensive understanding of bacterial virulence mechanisms and to identify specific factors involved in pathogenicity. They can also be used to test the effectiveness of drugs, vaccines, and other strategies to combat bacterial infections. Several strategies have been developed to identify genes involved in virulence, applying a molecular version of Koch's postulate.

IV.3. Steps of infected mouse model.

1-Pathogen selection: Choose a pathogen known to cause disease in mice.

2-Gene mutation or deletion: Genetically modify the pathogen to delete or specifically alter genes involved in the virulence factors you wish to study.

3-Infection of mice: Inject the modified or unmodified pathogen into a group of mice

4-Monitoring of infection: Monitor the progression of infection in mice by measuring parameters such as survival, bacterial load in different organs, disease symptoms, and immune response

5-Comparison of results: Compare the results between mice infected with the unmodified pathogen and those infected with the modified pathogen to determine how altered virulence factors affect disease progression and host response.

6-Molecular analysis: Conduct molecular analyses to study the underlying mechanisms of the observed differences in virulence between modified and unmodified pathogen strains.

IV.4. Identification of Virulence Factors in *E. coli*

Defining virulence factors and understanding the mechanisms involved in the pathogenicity of Escherichia coli strains are essential prerequisites for assessing the public health risk associated with these pathogens. Thus, the combination of virulence factors involved in the pathogenicity of the strains remains to be determined. The study of the pathogenicity factors of Escherichia coli has shown that within the species, there are numerous variants of factors:

IV.4.1. Potential Virulence Factors:

- A capsule that prevents phagocytosis.
- Outer membrane proteins and LPS that give bacteria the ability to evade the bactericidal activity of the host serum by preventing complement fixation.
- Iron uptake systems:
  - ✓ **Siderophores:** notably encoded by the pathogenicity island providing bacteria with the iron necessary for their multiplication, at the expense of transferrin.
- Toxins:
  - ✓ The endotoxin common to enterobacteria.
  - ✓ ST (Stable Toxin) and LT (Labile Toxin) enterotoxins. These are cytotoxic toxins that act on the enterocyte control of hydro-electrolytic secretion. LT toxin is similar to cholera toxin.
  - ✓ SLT1 and SLT2 (Shiga-like toxin). These toxins alter the integrity of enterocytes. They are also called vero-toxins (VT) because of their toxic effect on vero cells in culture (originating from vervet, an African monkey).

- ✓ Sat and Vat: Sat (Secreted Autotransporter Toxin) and Vat (Vacuolating Autotransporter Toxin) are type V toxins, from the autotransporter family. They induce vacuolization and cellular engorgement. Vat: a toxin involved in the pathogenesis of an extraintestinal E. coli infection has not yet been well identified. Sat: the toxin causes severe damage to the kidneys, loss of the glomerular membrane, loss of tubule epithelium, and tissue vacuolization, but its role in bacterial colonization has not been determined.

- Proteases, such as serine protease, catalase peroxidase, metalloprotease.
- Gastric acidity resistance systems and ureases.

The list of these potential virulence factors continues to grow, but to date, their respective roles in Escherichia coli pathogenicity have not been demonstrated. Virulence factors can be encoded by genes found in transposons (Tn), pathogenicity islands (PAIs), bacteriophages (Phages), and/or plasmids. Thus, strains causing various diseases and symptoms such as dysentery, meningitis in newborns, diarrhea, urinary tract infection (UTI), or hemolytic uremic syndrome (HUS) carry different genetic elements, which confer specific virulence factors and therefore colonization and survival advantages in a specific environment.

IV.4.2. Shiga Toxins (Stx) of *Escherichia coli*:

IV.4.2.1. Shiga Toxin Production: Although the production of toxins (Stxs) is necessary, it is not sufficient for an STEC strain to induce disease in humans. Indeed, Escherichia coli strains associated with disease in humans are capable of adhering to enterocytes, often causing attachment and effacement (A/E) lesions. Some Escherichia coli strains produce a cytotoxin that is lethal to cells. This toxin, similar to Shiga toxin from Shigella dysenteriae, has been designated Shiga-like toxin (Slt) and Shiga toxin (Stx). Several studies have distinguished two antigenic types of toxins.

IV.4.2.2. Genetics and Structure of Shiga Toxins: These toxins are 70 kDa holoproteins consisting of an A subunit with enzymatic activity and 5 B subunits for binding to the receptor. The A subunit is encoded by the stxA gene and the B subunit by the stxB gene. These two genes form an operon carried by specific lambda-type lysogenic phages, except for the production of Stx 2 toxin, the stx1 operon codes for Stx1 toxin, and the stx2 operon codes for Stx2 toxin.

IV.4.2.3. Adhesion Factors:

Adhesins give *Escherichia coli* strains the property of attaching to epithelial cells. Most often proteinaceous, they are most often carried by common pili. Adhesion is an essential step in the pathogenesis of enteric bacteria. The main ones are factors involved in the development of attachment and effacement (A/E) lesions of enterocytes, which are responsible for diarrhea and are characterized by effacement of the microvilli of intestinal epithelial cells in the contact zone between the bacterium and the target cell. Adhesins give the bacterium the ability to attach to host-specific receptors. For example, for fimbriae production using galactose-α - 1-4-galactose receptors of uroepithelial cells. The genes (pa or prs) that code for this adhesin are often genetically linked to the hly and cnf clusters, encoding CNF toxin. Another adhesin, fimbriae S, encoded by the sfa genes, is used to attach to sialic acid receptors. Type 1 fimbriae (fim), and pap (pyelonephritis-associated pilus) or P fimbriae, are the best-characterized adhesins; they give bacteria the ability to attach to host cells, a key step in infection pathogenesis. Their expression is regulated by over 80 antigenic capsule types (K antigens). Capsules consist of layers of polysaccharides assembled on the surface of the bacterium.

IV.4.2.4. Types of *Escherichia coli Plasmids:* A large number of virulence factors are encoded on plasmids. In addition to the adhesins and toxins mentioned earlier, factors coded on the pINV plasmid can be added, as well as other genes conferring selective advantages for the carrier strains (antibiotic resistance genes, colistin genes, siderophore-coding genes). Thus, the major categories of plasmids are exchanged within the population of Escherichia coli.

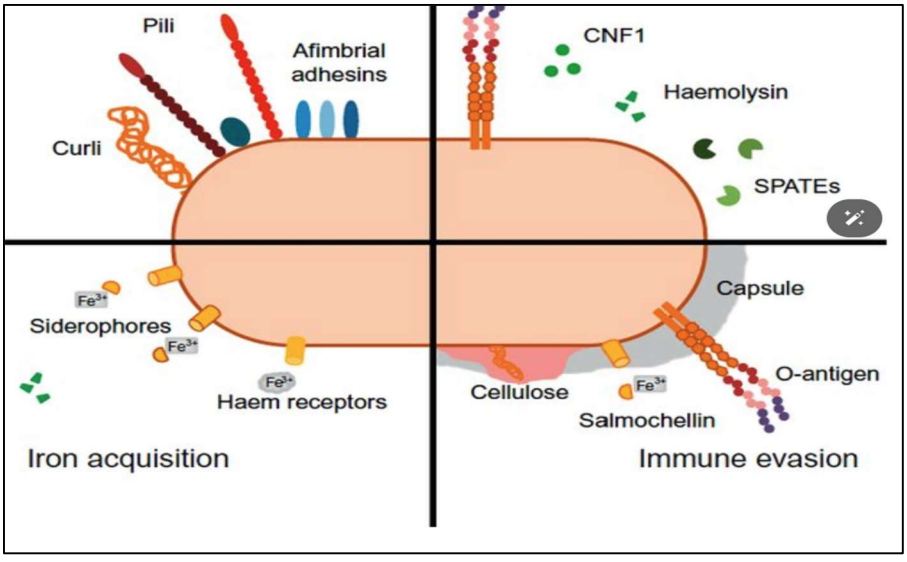
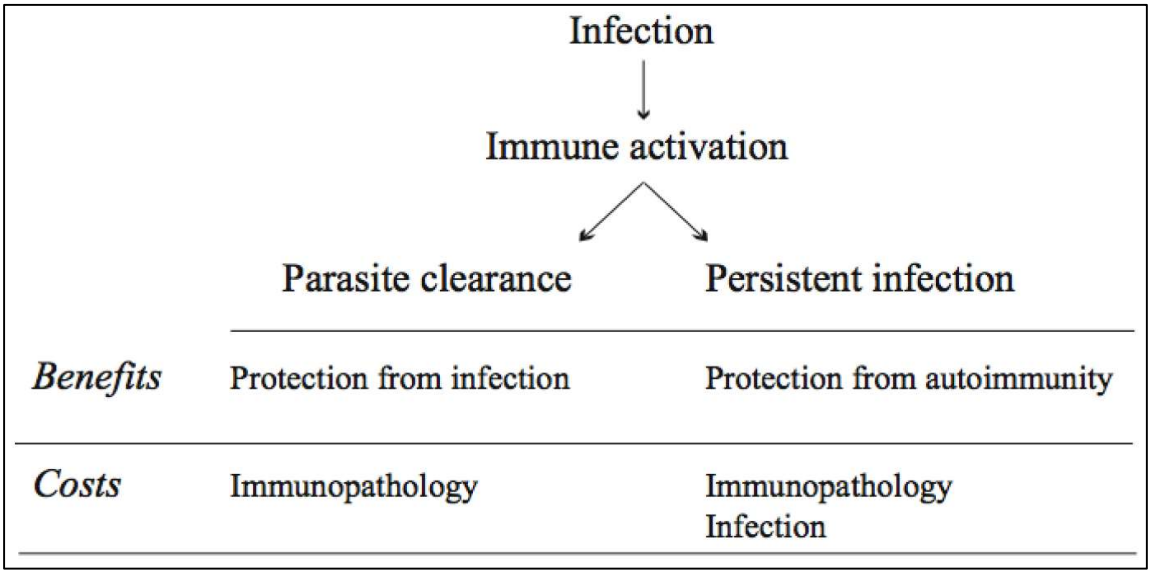


Figure 23: Fitness and virulence factors of uropathogenic *E. coli*

Chapter V: Strategies of Infectious Agents Evasion:

Introduction

Pathogens (bacteria, viruses, protozoa, parasites, etc.) have developed strategies to evade the immune response. Depending on the pathogens, the thwarted steps vary widely and can be multiple for the same pathogen. The establishment of an infection usually occurs following the bypassing of various defense mechanisms deployed by the infected organism. This evasion first requires an adhesion step, tissue colonization at the entry point, then a phase of invasion and evasion of defense mechanisms deployed by the organism for the elimination of these pathogens, and finally toxins capable of destroying cells and tissues. Each of these steps involves several mechanisms and factors.



V.1. Antibiotic Resistance Mechanisms:

Antibiotic resistance is a phenomenon as old as the emergence of antibiotics. Today, often of synthetic origin and produced by humans, antibiotics are originally natural substances generated by fungi but also by certain bacteria to "defend" themselves against other bacteria. Since bacteria are not suicidal, the first ones that learned to synthesize antibiotics also developed means to protect themselves. This is natural antibiotic resistance. Natural or intrinsic resistance is a species characteristic that affects all bacteria of the considered species. It is stable, transmitted to offspring (it is genetically supported by the bacterial chromosome), but it is not or little transmissible horizontally (from one bacterium to another within the same species or between different species). Example of natural resistances in Klebsiella spp.: Naturally

produces beta-lactamases. This enzyme is then present in the periplasmic space of the bacterium and leads to the destruction of antibiotics such as penicillins A before they can reach their bacterial target; anaerobic bacteria are naturally resistant to aminoglycosides because the passage of aminoglycosides through the cytoplasmic membrane requires an active transport system absent in anaerobes. In addition to natural resistance, there are also acquired resistances; it is a characteristic that concerns only a few (or sometimes many) strains of a given species. Acquired resistance is less stable, but it often spreads extensively in the bacterial world. Acquired resistance results from a modification of the bacterium's genetic capital, allowing it to tolerate a higher antibiotic concentration than that inhibiting sensitive strains of the same species.

V.1.1. Genetic Mechanisms of Acquired Resistance:

A bacterium's genetic potential consists of the chromosome and one or more facultative and extra-chromosomal genophores, the plasmids. Genes are also carried by transposable genetic elements and integrons. A bacterium can acquire antibiotic resistance through two major genetic mechanisms. One is supported by the chromosome and defines chromosomal resistance; the other is supported by plasmids or transposable elements or integrons and defines extra-chromosomal resistance.

V.1.1.1. Chromosomal Resistance:

It results from a mutation. It is a rare phenomenon, due to chance. It is not caused by the presence of the antibiotic. However, the antibiotic reveals the resistance mutation by selecting resistant mutant bacteria (or more precisely, by destroying the other bacteria of the species, those that remain sensitive to the antibiotic). It is an independent phenomenon: the appearance of a mutation does not promote the appearance of other resistance mutations to other antibiotics. The probability of two simultaneous mutations is therefore very low. This independence of mutations is one of the best arguments for justifying antibiotic combinations. It is transmissible; it is permanent and therefore hereditary (vertical transmission from mother bacterium to daughter bacteria). All mutations result in the loss or modification of a structural or enzymatic protein, and a mutated bacterium is often counter-selected in the absence of an antibiotic.

V.1.1.2. Extra-chromosomal Resistance (Plasmids): Two facts explain the importance of plasmid resistance:

-Plasmid resistance is related to the synthesis of additional proteins and not to a modification of the bacterium's normal constituents. Bacteria carrying plasmids are normal, while bacteria

resistant by mutation are often weakened. Also, bacteria carrying plasmids are not or little counter-selected in the absence of an antibiotic.

-Many resistance plasmids are conjugative or mobilizable, allowing horizontal transfer; these transfers are responsible for a very significant dissemination of resistance within bacterial populations, which qualifies plasmid resistance as "contagious or infectious." Resistance plasmids are likely to evolve through successive acquisitions or losses of resistance determinants carried by transposable genetic elements. Transposable genetic elements allow: --**Gene dissemination** between phylogenetically distant bacteria by allowing the implantation of a gene where plasmid implantation fails. As for chromosomal resistance, genes for extra-chromosomal resistance are not induced by antibiotic use, which simply selects bacteria carrying such genes. It is important to note that extra-chromosomal resistance often involves multi-resistance, so the use of a single antibiotic will select multi-resistant bacteria (MRB) that are not counter-selected in the absence of an antibiotic.

-**The most worrying MRB** are multi-resistant enterobacteria. Enterobacteria such as Escherichia coli and Klebsiella pneumoniae are gastrointestinal bacteria responsible for a large number of infections; methicillin-resistant Staphylococcus aureus, multi-resistant tuberculosis bacilli, or Pseudomonas aeruginosa and Acinetobacter baumannii, which are bacteria infecting the lungs of people with cystic fibrosis and are responsible for nosocomial infections (acquired in healthcare settings, especially hospitals and clinics).

V.1.2. Biochemical Mechanisms of Acquired Resistance: They can be grouped into three main types of mechanisms:

1-Decreased permeability (mutation affecting the structure of porins or decreasing the synthesis of porins through which the antibiotic can enter the bacterium) and active efflux: Efflux relies on a pump inserted into the membrane capable of ejecting the antibiotic out of the bacterium through a channel. This efflux leads to a decrease in the intracellular concentration of the antibiotic.

2-Modification of antibiotic targets: Example: modification of PBPs (Penicillin-Binding Proteins): PBPs are enzymes that catalyze the final step of peptidoglycan biosynthesis (bacterial cell wall) and are the target of beta-lactams (by binding to PBPs, beta-lactams prevent them from performing their role; peptidoglycan synthesis is therefore hindered). *Three mechanisms can be involved:

- ✓ Decreased affinity of PBPs for beta-lactams (e.g., *Streptococcus pneumoniae*); beta-lactams have difficulty binding to PBPs, which remain available for peptidoglycan synthesis.
- ✓ Increased synthesis of existing PBPs with overexpression of PBPs naturally possessing low affinity for beta-lactams (e.g., Enterococcus spp.; similar to the previous case, with an increase in the number of PBPs available for peptidoglycan synthesis, making it impossible for the same dose of beta-lactams to block them all).
- ✓ Synthesis of one or more new PBPs insensitive to beta-lactams (e.g., Staphylococcus aureus: the acquisition and integration into the chromosome of a gene (mecA), of unknown origin, induces the synthesis of a new PBP, PBP 2a, which alone can assemble peptidoglycan and confers resistance to all beta-lactams.

3-Production of enzymes inactivating antibiotics: Example: production of beta-lactamases encoded by plasmids or transposable genetic elements. The number of plasmidic beta-lactamases is very high, and they are classified according to their hydrolysis rates. Their affinity constants for beta-lactams, their ability to be inhibited by inhibitors such as clavulanic acid, etc. On a practical level, beta-lactamases can be grouped into 4 categories:

a*) Strictly penicillinases;* in Staphylococcus aureus, they inactivate penicillin G, penicillins A... However, they have no action on penicillin M (oxacillin or methicillin) or cephalosporins. These penicillinases are inducible and encoded by plasmids or transposons.

b) Extended-spectrum beta-lactamases (ESBLs); these beta-lactamases, encoded by plasmids, confer resistance (or reduced activity) against penicillins G, penicillins M, carboxypenicillins, ureidopenicillins, first- and second-generation cephalosporins (except cephamycins). Extended-spectrum beta-lactamases are well inhibited by clavulanic acid, sulbactam, or tazobactam.

c) Extended-spectrum beta-lactamases (ESBLs); these beta-lactamases derive from the previous enzymes through mutation of genes coding for extended-spectrum beta-lactamases. The resistance profile conferred is similar to that conferred by extended-spectrum beta-lactamases but extends to third-generation cephalosporins and aztreonam. Extended-spectrum beta-lactamases remain sensitive to inhibitors.

d) Inhibitor-resistant beta-lactamases; inhibitor-resistant beta-lactamases derive from certain extended-spectrum beta-lactamases through point mutations. The resistance profile conferred

is similar to that of extended-spectrum beta-lactamases, but these enzymes are not inhibited by clavulanic acid, sulbactam, or tazobactam.

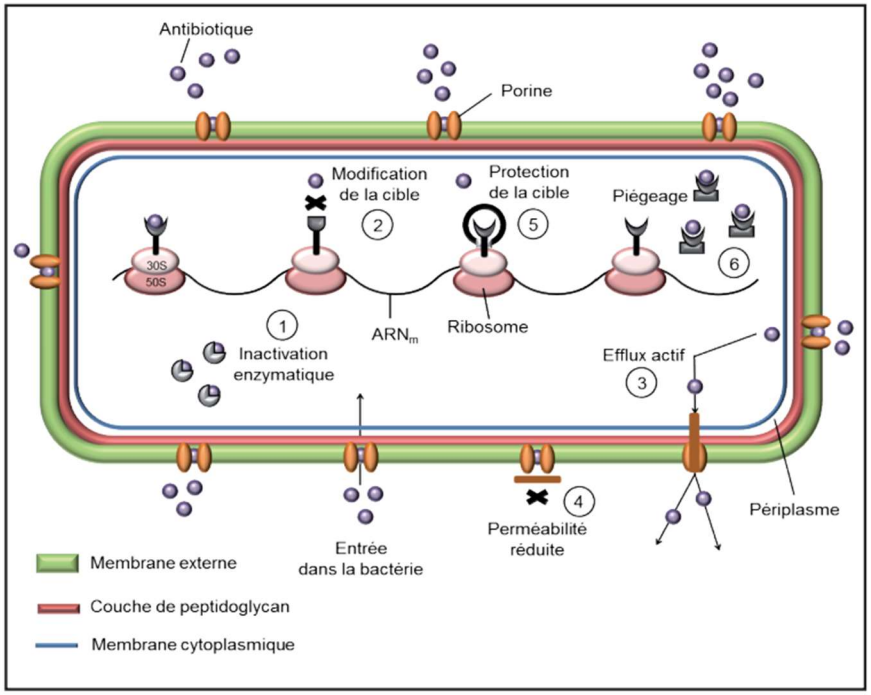


Figure 24. Biochemical Mechanisms of Acquired Resistance

V.2. Evasion Strategies of the Immune System:

The establishment of an infection usually occurs following the circumvention of the various defense mechanisms deployed by the infected organism. This evasion first requires an adhesion step, tissue colonization at the entry point, then a phase of invasion and evasion of defense mechanisms deployed by the organism for the elimination of these pathogens, and finally toxins capable of destroying cells and tissues. Each of these steps involves several mechanisms and factors, which can be grouped into:

- **Resistance to phagocytosis:** Some bacterial components protect them against phagocytosis, such as the capsule. These bacteria are called extracellular pathogens.
- **Resistance to ingestion:** mainly concerns bacteria with an extracellular development that are capsulated or have a slime layer, achieved through electrostatic repulsion and/or preventing opsonization. For example, Pneumococci.
- **Escape from the phagosome:** e.g., Listeria.
- **Inhibition of fusion with lysosomes:** e.g., Chlamydia.

- **Resistance to digestion by the phagolysosome**: e.g., Mycobacteriaceae, Salmonella.
- **Resistance within the phagocyte:** Other bacteria do not escape phagocytosis but have the property of persisting and multiplying inside phagocytes, these are intracellular bacteria (either facultative like Listeria or obligate like Mycobacteria).
- **Escape from antibody response:** Through antigenic variations, mimicking host surface compounds, action of proteases (complement, C5a peptidase, immunoglobulin, IgA peptidase), and fixation of the Fc fragment by Staphylococcus aureus protein A (inhibition of opsonization).

V.2.1. Colonization and Adhesion Factors:

- Penetration through intact skin, by insect vector bite as in the case of Lyme disease (Borrelia burgdorferi) or by iatrogenic cutaneous infection by bacteria from the skin flora (surgical wounds).
- Penetration at mucous membranes, by bacterial motility (flagella), secretion of IgA protease, which cleaves mucosal IgA (Haemophilus influenzae, pneumococcus, meningococci).
- Entry through "M" cells [mucous membranes of the digestive tube, in Peyer's patches] where the mucus layer is thin, facilitating access to underlying tissues and blood (Yersinia, Shigella, Salmonella).
- Bacterial adhesion, involving specific surface constituents of the bacterium (adhesins) and host cell receptors. This interaction is specific. Like the Pili (Fimbriae) adhesin in Gram-negative bacteria Neisseria gonorrhoeae, E. coli enterotoxigenic, and uropathogenic. Non-fimbrial adhesins are surface proteins allowing tight contact, binding either to host fibronectins, which in turn bind to integrin-type membrane receptors, or by direct binding to the cell receptor (Streptococcus pyogenes and S. aureus).
- Mechanisms of iron acquisition, through the synthesis of high-affinity iron chelators, allowing competition with host lactoferrins and transferrins. This mechanism is encountered, for example, in Mycobacterium tuberculosis.

V.2.2. Host Defense Evasion Factors:

1. **Bacterial capsule:** It is the outer envelope of the bacterium, usually of polysaccharide nature, and has a protective role against complement activation. This capsule is generally

immunogenic (of interest for Haemophilus influenzae type b vaccine or pneumococcal vaccine), but sometimes the nature of this capsule resembles host polysaccharides (e.g., hyaluronic acid in Streptococcus pyogenes, sialic acid in Neisseria meningitidis type B). This latter is non-immunogenic, leading to a lack of humoral response and thus protection against phagocytosis. Pathogenic bacteria that escape phagocytosis are called extracellular pathogenic bacteria.

2. **Modification of LPS (O antigen):** It is another factor of evasion or resistance to complement, preventing the formation of MAC (e.g., serum-resistant GNB). Note: the wall structure differs depending on whether it is Gram-negative or positive, Peptidoglycan is the common constituent to both, being a macromolecule consisting of polysaccharide chain polymers. Gram-positive wall: the peptidoglycan layer is thick, with teichoic acids, polysaccharides, and enzymes attached. Gram-negative wall: the peptidoglycan layer is thin, covered by an outer lipopolysaccharide membrane called the outer membrane, which contains the somatic O antigen, rich in lipids. The main interest of LPS is its toxic effect, explaining its name as endotoxin of Gram-negative bacteria. The richness in lipids explains the Gram-negative nature of these bacteria.

3. **Antigenic variations:** Some bacteria are capable of varying their surface antigens and evading the immune response, such as phase variations involving Salmonella flagella (Salmonella can produce 2 very different types of flagellar antigens, but only one of them is expressed at any given time, genetic bases are responsible for the expression of one or the other).

V.2.3. Host-Damaging Factors:

1. **Hydrolytic Enzymes:** The secretion of these enzymes by pathogenic bacteria leads to tissue destruction, dissemination, and pus production (e.g., Staphylococcus aureus).

2. **Bacterial Protein Toxins (Exotoxins):** After release, toxins diffuse into the body and act on target cells, sometimes expressing the pathogenicity of a bacterium (e.g., Streptococcus pyogenes) primarily through these toxins, playing a major role in the clinical expression of the disease (e.g., Tetanus, Diphtheria).

- ✓ **A-B Toxins:** Most protein toxins are in this category, consisting of two portions, B, which allows binding to the receptor of the target cell, and A, which possesses the enzymatic activity responsible for toxicity. The latter becomes active only once inside the cell cytoplasm and then exerts its toxic activity. These toxins often account for all

clinical symptoms without bacterial multiplication in the body, termed toxemia. They are responsible for the synthesis of neutralizing antibodies, providing effective protection against the disease. This immunogenic property is used in vaccine production (anatoxins). (e.g., Tetanus vaccine, Diphtheria vaccine). An anatoxin is a substance that has lost all toxic power through formalin detoxification but retains its immunogenic power. Use of antitoxin: Human-specific Ig, which provides immediate, transient protection, with limited effectiveness on toxin already bound to cellular receptors.

- ✓ **Cytolysins or Hemolysins:** Toxins causing membrane rupture of the target cell. They are either proteins that integrate into the cell membrane causing pore formation and cell lysis (e.g., Streptolysin O from Streptococcus pyogenes) or enzymes destabilizing the plasma membrane through action on membrane phospholipids: phospholipases or lecithinases (e.g., Clostridium perfringens toxin).
- ✓ c. Superantigens: Substances causing hyperstimulation of CD4+ helper cells, inducing significant inflammatory reaction, sometimes leading to shock (e.g., toxin of Staphylococcus aureus Toxic Shock Syndrome and the pyrogenic toxin of *Streptococcus pyogenes).*
- ✓ Components of the bacterial wall causing inflammatory reaction: Microbial molecular structures highly conserved, originating the inflammatory reaction.

***In Gram-negative Bacteria*:** LPS or endotoxin is a constituent of the outer membrane of the wall. It consists of lipid A, the lipid portion with toxic properties common to all bacteria, responsible for septic shock, and an antigenic polysaccharide portion composed of a central core and polysaccharide chains supporting antigenicity, known as the O antigen or somatic antigen.

In Gram-positive Bacteria: Teichoic (lipo) acids and peptidoglycan have an equivalent role to LPS in Gram-negative bacteria in the genesis of septic shock. Note: Excessive inflammatory response can have detrimental consequences either by impairing the function of an organ (lungs, CNS) or by causing systemic circulatory disorder (septic shock)(Fig 25).

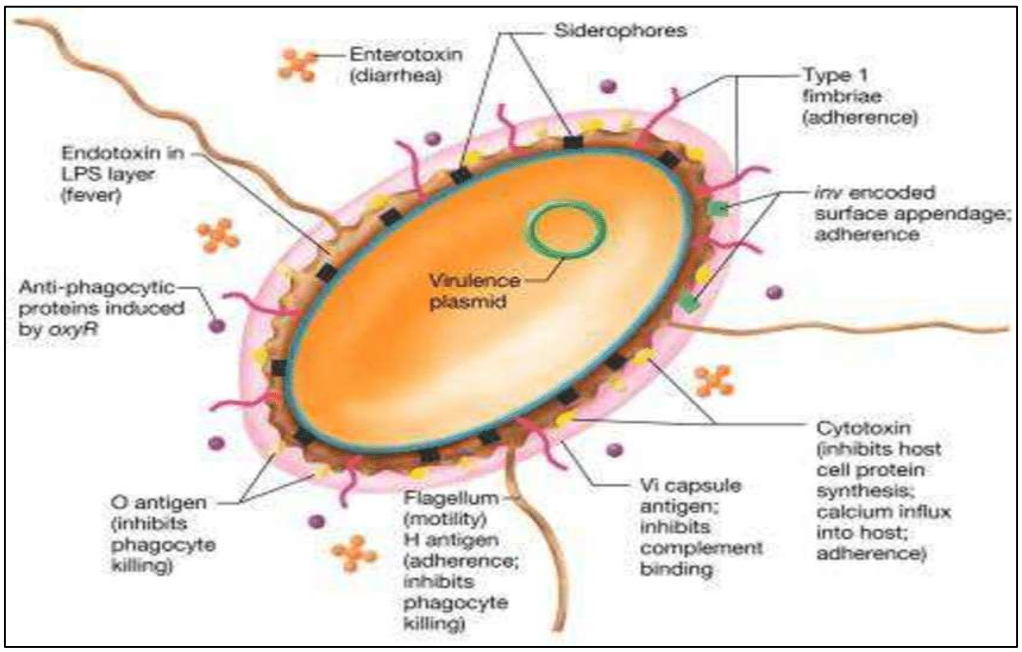


Figure 25. Host-Damaging Factors

V.2.4. Examples of Viruses Evading the Immune Response:

Some viruses employ much more sophisticated approaches: their existence, multiplication, and ability to thrive are the result of complex interactions with the host's specific immune system, leading to chronic infection. Studying this delicate balance allows for understanding viral pathogenesis and is essential for the development of various therapeutic strategies aimed at protecting the individual.

1-Blocking the Innate Response:

- Inhibiting the Effect of Innate Response Cytokines: Under normal circumstances, cells infected by a virus secrete IFN-alpha and beta, which have strong antiviral activity (by blocking virus replication). Evasion: The hepatitis C virus blocks the action of IFNs by inhibiting IFN-IFN-R signal transduction in infected cells.

- Inhibiting Complement Effect: Herpes simplex virus (HSV) and certain gram-negative bacteria produce a protein neutralizing C3b, a key fragment in complement activation.

2-Blocking Antigen Presentation: The pathogen becomes immune-invisible: Presentation on MHC I: Adenoviruses produce a protein inhibiting TAP => Viral antigen-derived peptides cannot be loaded onto class I molecules => no CTL response. Presentation on MHC II: HIV reduces the expression level of class II molecules on the cell membrane => Unstimulated TH.

3-Inducing Generalized Immunosuppression: Infecting a host cell that is a key factor in the immune system (lymphocyte or macrophage): HIV, measles virus, CMV. Modifying Cytokine Balance: EBV produces a protein mimicking IL-10 => TH2 pathway blockade => Inhibition of antiviral cytotoxic cellular response.

4-Antigenic Variation: A cat-and-mouse game: Exploiting two weaknesses of the adaptive immune response:

- Its specificity => If the antigen is modified, even very modestly, specific BCRs and TCRs can no longer recognize the neo-Ag.
- Its slow implementation => cat-and-mouse game: In the 7 days post-antigenic variation required for establishing a new specific immune response, the pathogen proliferates freely. Highly prevalent strategy: Viruses (HIV, Influenza Virus), bacteria (Neisseria gonorrhoeae), protozoa (Trypanosome),... Makes vaccine development challenging.

Chapter VI: Control of Bacterial Infections

Introduction:

In some cases, the balance between microorganisms and the host is not established, and the patient develops an infection. An infection involves the presence of an infectious agent and is always accompanied by an inflammatory response from the host: the appearance of local signs (inflammatory signs such as redness, heat, pain, and swelling) and/or general signs (fever, chills, drop in blood pressure). The risk of infection depends on several factors:

- ✓ type of microorganism
- ✓ number of microorganisms (inoculum)
- ✓ virulence of the microorganism
- ✓ patient's defense mechanisms
- ✓ presence of medical devices or foreign bodies.

VI. Pathophysiology of the infection

VI.1. Modes of Transmission:

- ✓ Direct (from a reservoir)
- ✓ Indirect (from contaminated object)

- ✓ Horizontal (human-to-human)
- ✓ Vertical (in utero)

VI.2. Routes of Contamination: Entry Port

- ✓ Digestive (cholera, typhoid)
- ✓ Respiratory (Legionnaires' disease, whooping cough)
- ✓ Cutaneous (tetanus, wound infection)
- ✓ Transcutaneous (injection, catheter)
- ✓ Sexual (syphilis, AIDS)

VI.3. Clinical Manifestations of Infection

1-Localized: The germ multiplies at the entry portal on the skin or mucous membranes (e.g., furuncle, localized abscess). The bacterium may remain localized at the entry portal but produces a diffusible toxin responsible for the disease (diphtheria, tetanus) or may directly or secondarily reach a deep tissue, creating a focus of infection (deep abscess).

2-Generalized: Occurs when the body poorly defends itself against a particularly virulent germ; the bacterium then enters the bloodstream. It may involve a transient passage without particular gravity, called bacteremia, or repeated permanent passages accompanied by severe infectious signs, known as septicemia. The presence of germs in the infected organism leads to the appearance of clinical signs of infection but also stimulates the defense mechanisms (production of antibodies).

VI.4. Acute Apparent Generalized Infection: It evolves in four stages:

1. **Incubation period:** Clinically silent, the number of germs present in the organism is too low to induce visible clinical manifestations. The duration of this period depends on the virulence of the germs and the multiplication time of the germs.
2. **Invasion period:** Marked by the appearance of initial clinical signs.
3. **State period:** Clinical signs are at their maximum; at this phase, the infection encounters the organism's defense mechanisms that prevent its progression.
4. **Convalescence period:** Corresponds to the terminal phase of the disease, which can evolve either towards complete spontaneous healing or after treatment with germ disappearance, or towards apparent healing with silent bacterial persistence, leading to chronicity.

VI.5. Latent Infection: These infections correspond to unrecognized foci of infection. The reactivation of these infections may occur after months or years due to a decrease in the body's defense mechanisms. c. Inapparent Infections: No clinical signs are perceptible. In this type of infection, it is possible to isolate the infectious agent, specific antibodies appear, and the infection is followed by lasting immunity (rubella, measles, hepatitis A).

VI.6. Mode of Infection:

- **Simple Toxi-infection:** Bacteria are outside or in transit (no colonization) in the digestive tract. Only toxins produced in the intestinal lumen or ingested are responsible for the pathogenic power. Example: foodborne intoxications by Staphylococcus aureus and Clostridium botulinum.
- **Colonization followed by Toxi-infection:** In this case, there is first adhesion of the pathogenic agent followed by colonization without penetration beyond the cutaneous-mucous lining, followed by secretion of toxins responsible for the pathogenic power. Example: Clostridium tetani and Corynebacterium diphtheriae.
- **Colonization followed by Bacterial Invasion:** Occurs after bacterial adhesion, colonization of the skin or mucous membranes, and then invasion of subepithelial tissues. Most pathogenic bacteria are invasive.

VI.7. Defense mechanisms of the organism:

VI.7.1. Escape factors from host defenses: The higher organism has implemented a multitude of defense mechanisms against aggressions by external agents that invade it. These invaders include viruses, bacteria, protozoa, or even parasites. Moreover, it is also capable of developing immune responses against its own proteins (autoimmunity) and aberrant cells. The first line of defense against foreign organisms consists of barrier tissues, such as the skin and mucous membranes, which prevent the entry of the organism into the body. However, if these barrier layers are penetrated, the body contains cells that respond quickly to eliminate the invader. These cells include macrophages and neutrophils that engulf foreign organisms. An immediate reaction of the organism also comes from soluble molecules that deprive the invading organism of essential nutrients (such as iron) and certain molecules found on epithelial surfaces, in secretions (such as tears and saliva), and complement proteins. This form of defense is known as innate or nonspecific immunity, which is always ready to respond to invasion. A second line of defense is constituted by specific or adaptive immunity, which may take several days to respond to an invasion. In specific immunity, there is a response by antibody production

(humoral immunity) and cell-mediated response, in which specific cells recognize foreign pathogens and destroy them. This response is also indispensable for the recognition and destruction of cells infected by viruses and tumor cells. The response to a second infection is often faster than that of the primary infection, due to the activation of memory immune cells (B and T lymphocytes) established during the first contact with the pathogen. Cells of the immune system interact with each other through a variety of signaling molecules to establish coordinated action. These signals can be proteins such as lymphokines, produced by lymphoid system cells, cytokines, and chemokines produced by other cells depriving bacteria of iron through iron-chelating proteins such as lactoferrin, transferrins, and ferritins.

VI.7.2. Nonspecific (innate) immune defense: The immune response is triggered by danger signals or the specific recognition of molecules identified as foreign (antigens). When bacteria breach the epithelial lining, the organism has means to destroy them:

a- Phagocytosis: Ingestion and intracellular destruction of microorganisms. Phagocytes are of two types: polymorphonuclear leukocytes and mononuclear phagocytes (monocytes, macrophages of the spleen and lymphoid organs, alveolar macrophages of the lungs). (Figure)

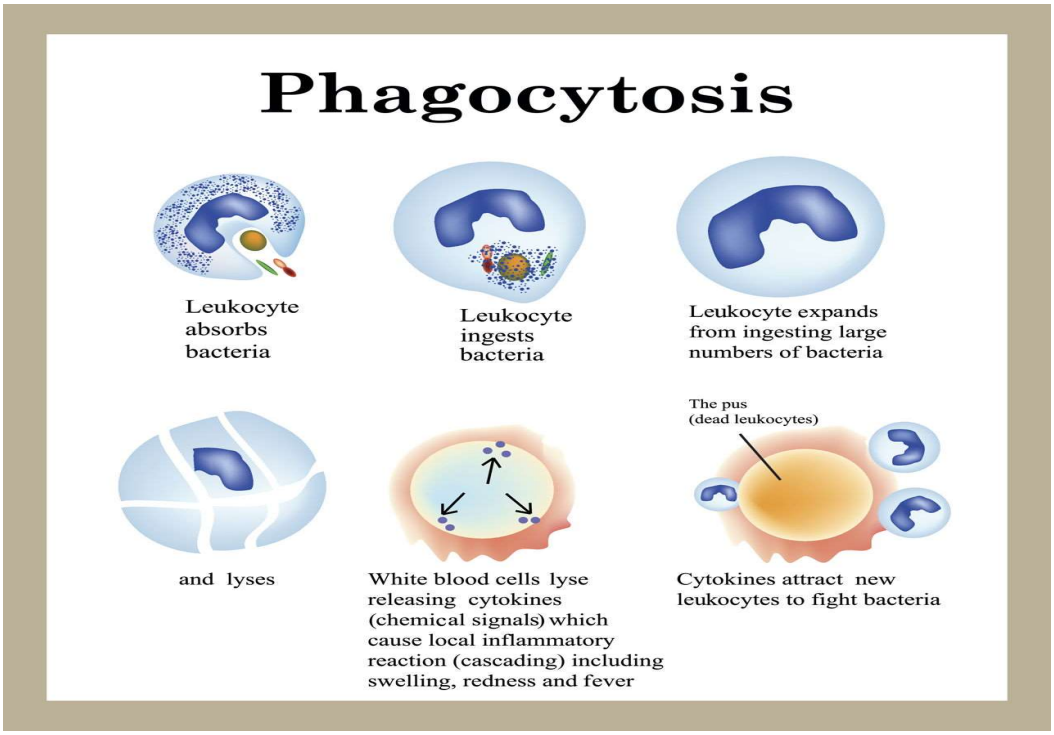


Figure 26. Steps of phagocytosis

b- Complement activation: Bacteria often present polymers containing sugars (LPS of Gram-negative bacteria) that activate the complement system, resulting in:

- **Inflammatory reaction:** This occurs through mast cells, leading to an influx of leukocytes.

- **Deposition of complement degradation** products on bacteria allows their ingestion by phagocytes possessing complement receptors, a process known as opsonization. For example, the activating microbial substance-opsonin-receptor complex for opsonin (e.g., bacterial wall-C3b complement-RC3b on neutrophils). Additionally, a membrane attack complex (MAC) forms on the outer membrane of Gram-negative bacteria, leading to bacterial lysis. Complement deficiencies can render humans more susceptible to certain infections. Opsonization is a process whereby a molecule called opsonin covers the membrane of a target cell (bacteria) to facilitate its phagocytosis by a cell with an opsonin receptor. c- Inflammatory response: Its goal is the rapid elimination of pathogens present in normally sterile tissue, representing an immediate host response based on the recognition of highly conserved bacterial antigens. Recruitment of phagocytic cells and the action of inflammatory proteins lead to a rapid inflammatory reaction at the infected site, with an increase in neutrophil leukocytes and inflammatory proteins in the blood (such as CRP or C-reactive protein). Sometimes, excessive inflammatory response can lead to septic shock (Fig 27).

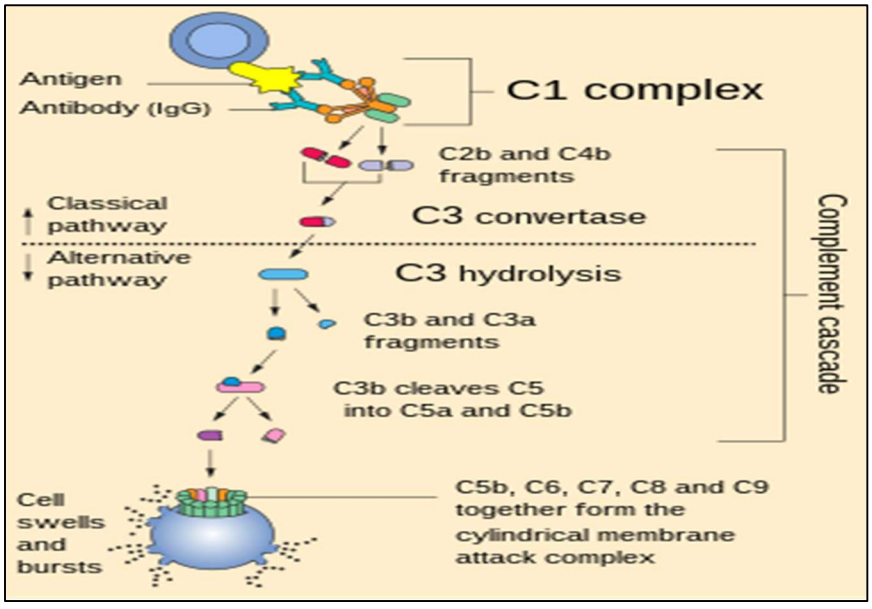


Figure 27. Complement system

VI.7.3. Specific (acquired) immune defense: It is delayed and characterized by the production of antibodies (mostly active against extracellular pathogens) and a cellular response (active

against intracellular pathogens). It is important to note that acquired immunity includes memory, which is the basis of vaccination. a- Immunoglobulins (Ig):

- Active mainly against extracellular bacteria.
- Sequential appearance: IgM followed by IgG.
- Direct neutralization: especially for toxins (benefit of serotherapy and vaccination with toxoids).
- Opsonization.
- Activation of complement.
- Special role of IgA in mucosal protection by preventing bacterial adhesion. b- T lymphocytes:
- CD8 cytotoxic T effector cells, which have a direct cytolytic action on infected cells.
- Activation and enhancement of macrophage action.
- Immune memory.
- Support for delayed hypersensitivity.
- Associated with the inflammatory response, this immune response leads to the formation of a inflammatory granuloma, which is a histo-immunological structure that is extremely bactericidal...(Fig 28).

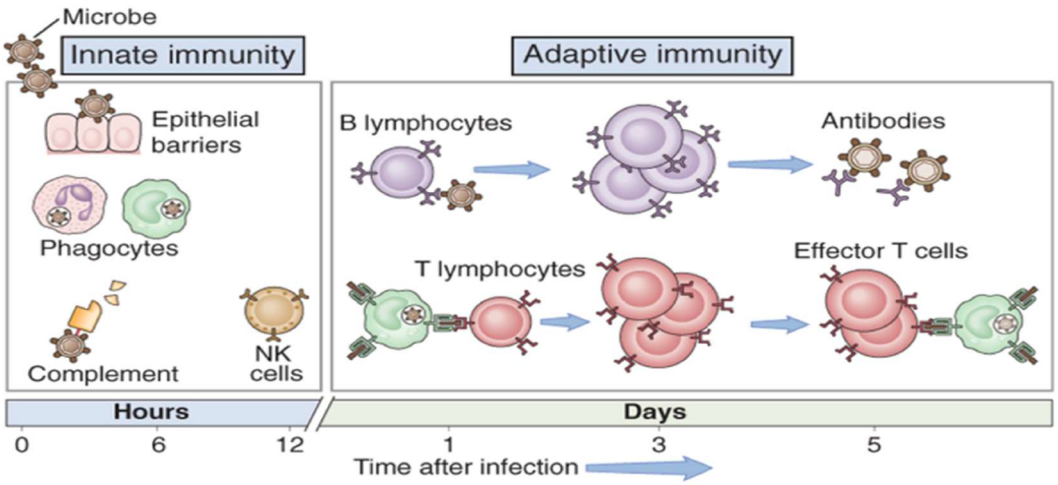


Figure 28. Specific (acquired) immune defense

VI.7.4. Protecting oneself from pathogenic microorganisms: The first step in fighting against pathogens is to prevent contamination. There are different protective measures for this purpose. In case of a breach in the natural barriers, such as a wound, the first step is to disinfect the wounds to destroy the microorganisms before they have time to multiply. This is called antisepsis. When the body needs to be opened during surgery, it is done in a completely sterile environment, meaning a place where all microorganisms have been destroyed. This is known as asepsis. Aseptic practices date back only to the 19th century with the work of Louis Pasteur. Before that, patients were operated on without concern for possible infections. Instruments, bandages, and premises were not necessarily sterilized, and as a result, patients developed infections after operations, some of which led to death. Regarding sexually transmitted infections (STIs), the use of a condom prevents contamination by many microorganisms, such as the HIV virus responsible for AIDS. There are STIs caused by viruses or bacteria where, following contamination, symptoms are not necessarily visible or felt, making it difficult to treat the patient. After contamination by a bacterium, it is possible to fight against it by taking antibiotics. They destroy bacteria or prevent their multiplication; however, they are ineffective against viruses. An antibiotic act against one or more bacteria, making it specific, and therefore ineffective (or less effective) against other bacteria. Before starting antibiotic treatment, it is necessary to identify the bacterium causing the infection to target the effectiveness of the medication. This can be achieved by performing an antibiogram, which involves culturing bacteria and testing the resistance of this bacterium to several antibiotics.

VI.7. 5. Prevention and control of infections:

Strengthening prevention measures against infections through improved general hygiene conditions and vaccination is essential to limit the spread of resistant microorganisms and reduce the misuse and overuse of antimicrobials.

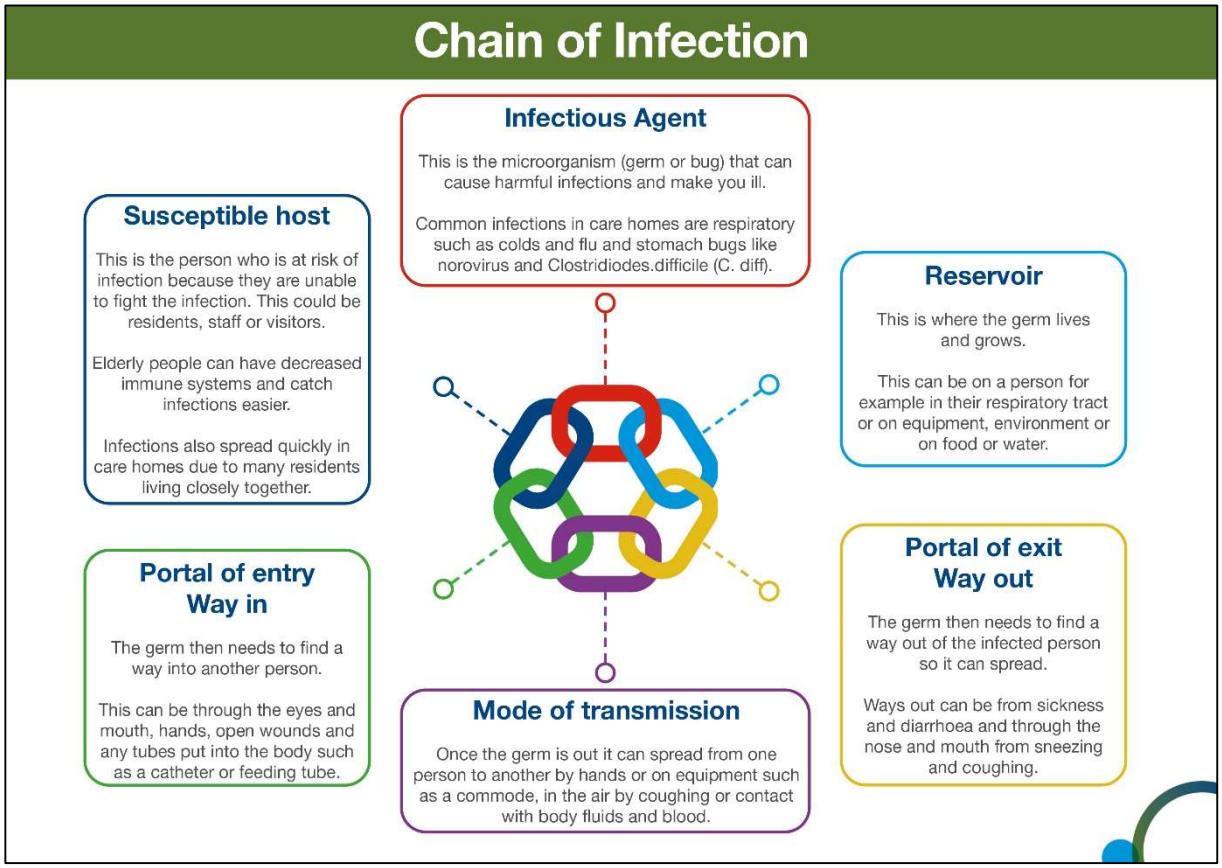


Figure 29. Chain of infection

References

Adak, A., and Khan, M.R. (2019). An insight into gut microbiota and its functionalities. Cellular and Molecular Life Sciences 76, 473–493.

Baron, C. and Coombes, B. (2007). Targeting bacterial secretion systems: benefits of disarmament in the microcosm. Infectious Disorders - Drug Targets, 7: 19-27

Bingle, L.E.H., Bailey, C.M. et Pallen, M.J. (2008). Type VI secretion: a beginner's guide. Current Opinion in Microbiology, 11: 3-8.

Costerton, J. (1999). Introduction to biofilm. International Journal of Antimicrobial Agents 11, 217–221.

Filloux, A., Hachani, A. and Bleves, S. (2008). The bacterial type VI secretion machine: yet another player for protein transport across membranes. Microbiology, 154: 1570-1583. Finck-Barbancon, V., Yahr, T.L. et Frank, D.W. (1998). Identification and characterization of SpcU, a chaperone required for efficient secretion of the ExoU cytotoxin. Journal of Bacteriology, 180: 6224-6231.

Foulongne, V., Michaux-Charachon, S., O'Callaghan, D. et Ramuz, M. (2002). Systèmes de sécrétion des protéines de type IV et virulence bactérienne. Medecine/Sciences, 18: 439-447.

Green, E.R., Mecsas, J., 2016. Bacterial Secretion Systems: An Overview. Microbiology Spectrum 4. https://doi.org/10.1128/microbiolspec.VMBF-0012-2015

Jean-Pierre Dedet. 2007. La microbiologie, de ses origines aux maladies émergentes. Dunod Edition. - Claude Dreux, Jeanne Brugere-Picoux, Jean-Philippe Brandel, Jean-Louis Laplanche, Jacques-Christian Darbord. 1998. Les maladies à Prions. Cahier de formation biologi

Jean-Yves Cesbron, Catherine Lemaire, Nadira Delhem, Tobias Schulze, Françoise Blanquet. 1998. Rôle du système immunitaire dans les maladies à prions. M/S novembre 1998 n° 11 volume 14

Jorand, F. (2018). Introduction." Biofilm et" adhésion des microorganismes : une stratégie du vivant ? Cours magistral de Master 2 Microbiologie (Nancy)

Kobayashi S.D. et De Leo F.R. 2009. An update on community-associated MRSA virulence. Curr. Opin. Pharmacol. 9:545Ŕ551

Lachmann PJ, Oldstone MBA.2006. Microbial Subversion of Immunity: Current Topics. Wymondham : British Library, Cataloguing-in-Publication Data. P 71-83.

Lavigne, J.-P., Botella, E., O'Callaghan, D., 2006. Les systèmes de sécrétions de type IV et leurs effecteurs. Pathologie Biologie 54, 296–303. https://doi.org/10.1016/j.patbio.2005.04.006

Le Faou A. 2012. Précis de virologie humaine. Rueil-Malmaison :

Navarro-Garcia, F., Ruiz-Perez, F., Cataldi, Á., Larzábal, M., 2019. Type VI Secretion System in Pathogenic Escherichia coli: Structure, Role in Virulence, and Acquisition. Front. Microbiol. 10, 1965. https://doi.org/10.3389/fmicb.2019.01965

Nessar Ahmed, Maureen Dawson, Chris Smith et Ed Wood.2007. Biology of disease. Taylor et Francis Group.

Pallen, M.J., Chaudhuri, R.R. et Henderson, I.R. (2003). Genomic analysis of secretion systems. Current Opinion in Microbiology, 6: 519-527.

Ranava D, Yang Y, Orenday-Tapia L, Rousset F, Turlan C, Morales V, Cui L, Moulin C, Froment C, Munoz G, Rech J, Marcoux J, Caumont-Sarcos A, Albenne C, Bikard D, Ieva R. Elife. 2021 Apr 13. doi: 10.7554/eLife.67817.

Ranava D, Yang Y, Orenday-Tapia L, Rousset F, Turlan C, Morales V, Cui L, Moulin C, Froment C, Munoz G, Rech J, Marcoux J, Caumont-Sarcos A, Albenne C, Bikard D, Ieva R.

Spitz, O., Erenburg, I.N., Beer, T., Kanonenberg, K., Holland, I.B., Schmitt, L., 2019. Type I Secretion Systems, One Mechanism for All, in: Sandkvist, Cascales, Christie (Eds.), Protein Secretion in Bacteria. American Society of Microbiology, pp. 215–225. https://doi.org/10.1128/microbiolspec.PSIB-0003-2018

Wolters Kluwer. Lever AML, Jeang KT, Berkhout B. 2010. Recent advances in human retroviruses: principles of replication and pathogenesis. New York : World Scientific Publishing Co. P 29

Webographie Morice,

http://www.chups.jussieu.fr/polys/bacterio/bacterio/POLY.Chp.1.2.5.html date de consultation : 9 mai 2019 NCBI,

https://www.ncbi.nlm.nih.gov/genome/1272 date de consultation : 9 mai 2019

https://www.researchgate.net/publication/233956110_The_RTX_pore-forming_toxin_alpha-hemolysin_of_uropathogenic_Escherichia_coli_progress_and_perspective