SECTION 3: BACTERIAL PATHOGENICITY AND GENETIC

Learning objectives:

- to describe various normal floras
- to explain host - bacteria relationships
- to describe mechanisms of bacteria pathogenicity
- To explain how a mutation occur and what’s a plasmid
- To study main mechanisms of genetic transfers: transformation, transduction and conjugation
1/Normal microbiota and the host

- Utero of human (and animals) are free of microbes protected by the placenta.

- At birth, however, normal and characteristic microbial populations begin to establish themselves.

- The newborn’s first contact with microorganism is usually with lactobacilli of the mother’s vagina flora.

- More microorganisms are introduced to the newborn’s body from the environment when breathing begins and feeding starts.

- After birth, *Escherichia coli* and other bacteria acquired from foods begin to inhabit the large intestine or colon. These microorganisms remain there, normally harmless, throughout live, may increase or decrease in number and contribute to disease.
Many other normally harmless microorganisms establish themselves inside other parts of the normal adult body and on its surface.

A typical human body contains $10^{13}$ body cells, yet harbors $10^{14}$ bacterial cells.

Members of the body’s normal microbiota or normal flora are those microorganisms that establish more or less permanent residence (colonize) but that do not produce disease under normal conditions. These bacteria are commensals.
Locations of normal microbiota on and in human body
In nose and the throat (upper respiratory system):
= in nose, are present: gram-positive cocci such as *Staphylococcus aureus*, *Staphylococcus epidermidis* and aerobic diphteroids which are gram-positive bacillus;
= in the throat, *S.epidermidis*, *S. aureus*, diphteroids, *Streptococcus pneumoniae*, *Hemophylus bacillus gram-negative*, and *Neisseria coccus gram-negative*

Mouth contains various species of *Streptococcus*, *Lactobacillus*, *Treponema*, *Corynebacterium* and anaerobies *Actinomyces*, *Bacteroides*, *Fusobacterium* and fungus such as *Candida albicans*

The large intestine contains the largest number of resident microbiota: dominated by anaerobes such as *Bacteroides*, *Fusobacterium*, *Bifidobacterium*, with enteric bacteria such as *Escherichia coli*, *Enterobacter*, *Proteus*, *Shigella*, *Klebsiella*, *Citrobacter* which are gram-negative bacilli and *Enterococcus* a gram-positive coccus
On skin are living: *Propionibacterium acnes* an anaerobe gram-positive coccus with *Staphylococcus epidermidis*, *Staphylococcus aureus* which are gram-positive cocci, *Corynebacterium xerosis* a gram-positive bacillus and fungi of *Candida* spp.

In the urogenital system:
- In urethra we meet: *Gram-positive cocci* such as *Staphylococcus epidermidis*, aerobic micrococci, *Enterococcus*, with *gram-positive bacilli* such as *Lactobacillus*, aerobic diphtheroids, and *gram-negative bacilli* *Pseudomonas*, *Klebsiella*, and *Proteus* which are present;
- In vagina, are present *Lactobacilli*, aerobic diphtheroids *gram-positive bacilli* with *Gram-positive cocci* *Streptococcus*, *Staphylococcus*, and anaerobe *Bacteroides and Clostridium*; we also meet fungus such as *Candida albican* and a protozoan *Trichomonas vaginalis*.
the vagina has its acid-tolerant population of lactoballli also called **bacilli of Döderlein**

=**male lower urethra** has skin resident bacterial population

- **On conjunctiva of eyes**
  =are present:

  *Staphylococcus epidermidis* and *Staphylococcus aureus* with diphteroids

- **We must keep in mind** that under certain conditions commensal relationship can change. For example, enteric *Escherichia coli*, when gains access to other body sites such urinary bladder, may cause urinary tract infectious; such as *Neisseria meningitis* generally harmless in the throat can become the causative agent of meningitis outbreaks
2/ Microbial Mechanism of Pathogenicity

- We will take a look at some of the specific properties of microorganisms that contribute to **pathogenicity**, the ability to cause disease by overcoming the defense of the host, and **virulence**, the degree of pathogenicity.

- We must keep in mind that many of the properties contributing to microbial pathogenicity and virulence are unclear or unknown. We do know, however, that if the microbial attack overpowers the host defense, disease results.

- Most pathogen must **gain access to the host, adhere to host tissues, penetrate or evade host defenses, and damage the host tissues to cause disease**.
Pathogenicity of *Staphylococcus aureus* which colonize, excrete enzymes and cause damages into tissues
Figure 15.8 Microbial mechanisms of pathogenicity. A summary of how microorganisms cause disease.
a/ The portals of entry

- The **portals of entry** for pathogens are **mucous membranes, skin, and parenteral route**

- Many bacteria and viruses gain access to the body by penetrating mucous membranes lining the **respiratory tract, gastrointestinal tract, genitourinary tract, and conjonctiva**.

- Most pathogens enter through the mucous membranes of **gastrointestinal and respiratory tracts**
Microbes are inhaled into nose or mouth in drop of moisture and dust particules. Diseases that are commonly contracted via respiratory tract include the common cold, pneumonia, tuberculosis, influenza, measles and smallpox.

Microorganism can gain access to the gastrointestinal tract in food and water and via contaminated fingers. Most microbes that enter the body in these ways are destroyed by hydrochloric acid (HCl) and enzymes in the stomach, or by bile and enzyme in the small intestine. Those that survive can cause disease.

Microbes in the gastrointestinal tract can cause poliomyelitis, hepatitis A or E, typhoid fever, bacillary dysentery (shigellosis) cholera and gastroenteritis. These pathogens are then eliminated with feces and can be transmitted to other hosts via contaminated water, food, or fingers.
The genitourinary tract is a portal of entry for pathogens that are contracted sexually. Some microbes that cause sexually transmitted diseases (STDs) may penetrate an unbroken mucous membrane. Others require a cut or abrasion of some type. Examples of STDs are HIV infections, AIDS, genital warts, chlamydia, herpes, syphilis and gonorrhea.

Unbroken skin is impenetrable by most microorganisms. Some microbes, such as *Staphylococcus* species, gain access to the body through openings in the skin, such as follicles and sweat gland ducts.

In addition, larvae of the hookworms *Necator americanus* and *Ancylostoma duodenalae* and larvae of *Schistosoma* various species, actually bore through intact skin. Some fungi such as dermatophytes grow on the keratin in the skin or infect the skin itself.
Although, the skin is one of the largest organs in terms of surface area and, when unbroken, an important defense against disease.

**Punctures, injections, bites, cuts, wounds, surgery** and **splitting** due to **swelling** or **drying** can all establish **parental** routes, which mean, gain access to body when microorganisms are deposited directly into the tissues beneath the skin or into mucous membranes when these barriers are penetrated or injured.

Many pathogen have a **preferred portal** of entry that is a prerequisite to their being able to cause disease. For example, the bacteria of typhoid fever, *Salmonella typhi*, produce all the signs and symptoms of the disease when **swallowed** (**preferred route**). Streptococci that are **inhaled** (**preferred route**) cause pneumonia.
b/ Adherence

- Once pathogens gain entry of the host, almost all of them have means of attaching themselves to host tissues.

- For most pathogens, this attachment, called **adherence**, is a necessary step of pathogenicity.

- The attachment between pathogen and host is accomplished by means of surface molecules on the pathogen called **adhesins** or **ligands** that band specifically to complementary surface **receptor** on the cells of certain host tissues.
Majority of adhesins on the microorganisms studied so far are glycoproteins or lipoproteins. Adhesins are incorporated into the fimbriae.

If adhesins, receptors, or both can be altered to interfere with adherence, infection can often be prevented (or at least controlled).
For examples:

=E. coli enteropathogenic strains that cause gastrointestinal disease, have adhesins in fimbriae that adhere on cells of the small intestine.

Electronic microscope picture of fimbriae surrounding E. coli cell
=Neisseria gonorrhoeae, the causative agent of gonorrhea, also has fimbriae containing adhesins, which adhere on their genitourinary tract cell’s receptors
c/ Penetration into host cells

- Once a pathogen is attached to host cells, it can pass through them to invade other tissues through **parenteral route**. During invasion, the pathogens metabolize and multiply to kill host cells.

- Some bacteria can also penetrate the host cells by secreting enzymes and by their own motility; such penetration can itself damage the host cells.
d/ How bacterial pathogens damage host cells

Although some pathogen can cause damage on the surface of tissues, most penetrate tissues to cause disease. Several factor contribute to the ability of bacteria to invade host.

When a microorganism invades a body tissue, it initially encounters phagocytes of the host. If phagocytes are successful in destroying the invader, no further damage is done to the host. Phagocytes are main effectors cells of natural immunity that acts as the primary barrier against invaders.

But pathogens can overcome the host defense, that produce enzymes, that have a capsule, or that secrete toxins.
1/ excreted enzymes

The virulence of some bacteria is thought to be aided by the production of extracellular enzymes:

- such as leucocidins which are produced by streptococci and staphylococci. Leucocidins can destroy leukocytes or white blood cells that are very active in phagocytosis. This type of damage to white blood cells decreases host resistance.

=Collagenases break down the protein collagen, which forms the connective tissue of muscles or other body organs and tissues. Produced by several species of Clostridium, collagenases facilitate the spread of gas gangrene which main causative agent is Clostridium perfinger.
Some bacteria make glycolax material that forms capsules around their cell walls. The capsule resists the host’s defense by impairing phagocytosis, the process by which certain effective cells of the natural immunity (phagocytes: macrophages, neutrophiles) engulf and destroy microbes.

Some strains of *Streptococcus pneumoniae*, the causative agent of lobar pneumonia, have capsules, others do not. *S. pneumoniae* strains with polysaccharide capsule are virulent, but strains without capsules are avirulent because they are susceptible to phagocytosis.
Other bacteria that produce capsules related to virulence are:

- *Klebsiella pneumonia*, a gram-negative bacilli causative agent of a bacterial pneumonia
- *Hemophilus influenza*, a gram-negative coccobacilli causative agent of pneumonia and meningitis in children
- *Bacillus anthracis*, etiologic agent of anthrax
- *Yersinia pestis*, the causative agent of bubonic and pneumonic plague

3/ toxins

Toxins are poisonous substance that are produced by certain microorganisms. They are often the primary factor contributing to the pathogenic properties of those microbes.

- Some toxins produce fever, cardiovascular disturbances, diarrhea, and shock. Toxins can also inhibit protein synthesis, destroy blood cells and blood vessels, and disrupt the nervous system by causing spasms.
They are two types of toxins, **exotoxins** and **endotoxins**

(a) **Exotoxins** are produced inside mostly gram-positive bacteria as part of their growth and metabolism. They are then released into the surrounding medium.

(b) **Endotoxins** are part of the outer portion of the cell wall (lipid A; see Figure 4.12c) of gram-negative bacteria. They are liberated when the bacteria die and the cell wall breaks apart.
Exotoxins are produced inside some bacterial and are released into the surrounded medium. Because exotoxins are soluble in body fluid, they can easily diffuse into the blood and are rapidly transported throughout the body.

Most bacteria that produce exotoxin are gram-positive.

Exotoxins are among the most lethal substances known. Only 1 mg of botulinum exotoxin is enough to kill 1 million guinea pigs.

Exotoxins may be grouped into three principal types, based on their mode of action:

1) cytotoxins, which kill host cells or affect their functions, such as
   = diphteria toxin of Corynebacterium diphteriae, that inhibits protein synthesis in eucaryotic cells.
   = and erythrogenic toxins A, B and C of Streptococcus pyogenes, which damage blood capillaries under the skin and produce the red skin rash of scarlet fever caused by Streptococcus pyogenes.
b) neurotoxins which interfere with normal nerve impulse transmission such as botulinum neurotoxin and tetanos neurotoxin also called tetanospasmin

- *Clostridium botulinum neurotoxin* inhibits the release of a neurotransmitter called acetylcholine and cause as result a flaccid paralysis in which muscle tone is lacking

- *Tetanospasmin of Clostridium tetani* binds to nerve cells that control the contraction of various skeletal muscles. This binding of tetanus neurotoxin produces uncontrollable muscle contraction such as the spasmic contractions of tetanus
c)enterotoxins which affect cells lining the gastrointestinal tract, such as *vibrio enterotoxin* and *staphylococcal enterotoxin*

- *Vibrio cholera* produces an enterotoxin called *vibrio toxin*, which induces the formation of a cyclic AMP from ATP in the cytoplasm of infected cells. As result, epithelial cells discharge large amount of fluid (20l/day) and electrolytes.

- *Heat-labile enterotoxin* produced by certain strains of *E. coli* has an action similarly but no so strong to that of cholera toxin
Exacerbation of adenylcyclase pathway by choleric toxin and *E. coli* enterotoxin action with loose of water and Cl-
- *Staphylococcus aureus* produces enterotoxin, staphylococcal enterotoxin that affects the intestines in the same way as cholera toxin.

- A strain of *Staphylococcus aureus* also produces enterotoxins that result in the symptoms associated with a toxic shock syndrome.
Pathogenicity of *Staphylococcus aureus* and their caused diseases
Endotoxins differ from exotoxins in several ways.

- Endotoxins are part of the outer portion of the cell wall of Gram-negative bacteria. This outer membrane consists of lipoproteins, phospholipides, and lipopolysaccharides (LPS).

- The lipid portion of the LPS, called lipid A, is an endotoxin.

- Endotoxins exert their effects when gram-negative bacterial die and their cell wall undergo lysis, thus liberating their endotoxins.

- Host responses, to those endotoxin effects, include chills, fever, weakness, generalized aches, and, in some cases, shock and even dead.
Figure 15.5 Endotoxins and the pyrogenic response. The proposed mechanism by which endotoxins cause fever.

All endotoxins produce the same signs and symptoms, regardless of the species of microbe.
e) Defenses of the host

- Pathogenic microorganisms are endowed with special properties that enable them to cause disease if given the right opportunity.

- If microorganisms never encountered resistance from the host, we would constantly be ill and would eventually die of various diseases. But in most cases, our body defenses prevent this from happening.

- Our ability to ward off disease is called resistance.

- Vulnerability or lack of resistance is called susceptibility.

- In discussing resistance, we will divide our body’s defenses into two general kinds:
  - non-specific
  - and specific.
- Non specific resistance refers to defenses against any pathogen, regardless of species.

- Specific resistance refers to defenses against a specific pathogen.
1/ the non-specific resistance

- It is also called **innate or natural immunity**
- That it act **immediately and non-specifically**
- **Innate immunity** forms the first line of defense using:

**a/ mechanical factors**

a) intact skin, waterproof protein keratin provide resistance to microbial invasion

b) Mucous membranes inhibit the entrance of many microorganisms

c) Viscous secretions of respiratory and gastrointestinal tracts which trap microorganisms

d) Cilia which cover epithelia of lower respiratory tract that propel microorganism trapped by the mucus upward toward the throat
immunité non spécifique : des dizaines de mécanismes tissulaires

biochemical
lysozyme in most secretions
mucus
cilia lining trachea
acid in stomach
commensal organisms in gut and vagina
spermine in sperm

biochemical and physical

immunité spécifique ➔ Système lymphoïde: organes, tissus et lymphocytes circulants

système myéloïde : cellules tissulaires
b) chemical factors

1) Sebum produced by sebaceous glands which forms a protective film over the skin.

2) Sweat gland produce perspiration which contains also **lysozyme**, an enzyme capable of breaking down cell wall of gram-positive bacteria. Lysozyme is also found in tears, saliva, nasal secretions, and tissues fluids where it exhibits its antimicrobial activity.

3) The acidity of gastric juice (pH 1.2-3) preserve the usually sterility of the stomach. This acidity destroy bacteria and most bacteria toxin, except those of *Clostridium botulinum* and *Staphylococcus aureus*

c) commensal flora

1) Commensal flora inhibit implantation of pathogen in many area of the body, such as the vagina, the gastrointestinal tract.
D) Complement system and their functions

1) **Complement** is a defensive system consisting of serum proteins that participate in *lysis* of foreign cells, *inflammation* and *phagocytosis*

2) The complement system consist of a group of at least 25 interacting proteins found in normal serum.

3) The complement system can be activated in either of two ways:
   
   a) by an immune reaction of antibodies to antigen in the classical *pathway*
   
   b) by the direct interaction of certain protein with polysaccharides in the alternative *pathway*

4) The protein of the classical and alternative pathways act in an ordered sequence, or *cascade*. In a series of steps, each protein activate the next one in the series, usually by cleaving it
Classical and alternative pathways of complement system
5) Both the classical or alternative pathway, lead to the cleavage of C3 into C3a and C3b.

6) C3b initiates a sequence of reactions involving C5-C9 which known collectively as the **membrane attack complex** that attack the invading cell’s membrane, produce circular lesions, called **transmembrane channels** that lead to the loss of ions and eventual cytolysis.
7) *In fine*, activation of complement induce three kinds of consequences destructive to microorganisms: *cytolysis, inflammation* and *opsonization*

= complement system has an intrinsic ability to lyse the cell wall of many bacteria by his membrane attack complex: *cytolysis*

= complement products released attract phagocytes to the site of infection: *inflammation*

= on the site of reaction, other complement components coating bacterial surface allow the phagocyte to recognize the bacteria and facilitate bacterial phagocytosis: *opsonization*

8) *These are functions of the innate immune system, although the reactions can be triggered by the adaptative immune system*
e) phagocytosis

1) **It is** the main effective mechanism of the innate or natural immunity. It means ingestion of microorganisms or any particulate matter by a cell.

2) The cells that perform this function are collectively called **phagocytes**.

3) All of which are types of white blood cells or derivatives of white blood cells such as:
   - Neutrophiles or polymorphonuclear leukocytes
   - Monocytes - macrophages

4) Some macrophages, called **fixed macrophages** are located in certain tissues and organ such as the dendritic Langerhans cells of the skin which play role of cells presenting antigen to the adaptative immunity.
5) All phagocytic cells have receptors for a variety of molecules
6) Most pertinent to non-specific immunity are receptors for IgG Fc, complement, interferon, TNF and certain bacterial components. Receptor interactions with these ligands promote phagocytosis and activation for efficient killing of pathogens.

7) Phagocytes may be activated by components of bacteria such as LPS of gram-negative bacteria and especially by all expressed bacterial antigens

8) Among the more activators of the phagocytes are small proteins hormones secreted by phagocytes and others cells involved in immunity called cytokines; especially by prolific T helper CD4 “the orchestra chief” of immune response
9) Process of inflammation refers on vasodilatation and increase permeability of blood vessels, migration of phagocytes on sites of infection to initiate phagocytosis
10) For the convenience of study, we will divide phagocytosis into four main phases: &text;chemotaxis, adherence, ingestion&text; and digestion.
Not all phagocytized microorganisms are killed by lysosomal enzymes.

Toxin-producing staphylococci kill phagocytes.

*Listeria monocytogenes* (causative agent of listeriosis) and *Shigella flexneri* (the causative agent of shigellosis or bacillary dysentery) have enzymes that lyse phagolysosomes.

*Mycobacterium tuberculosis* prevent fusion between lysosome and phagosome.

In addition to providing non-specific resistance for the host, macrophages play a role in specific immunity as *CPA or cells presenting antigen*.
To summarize:

Non specific or innate, natural immunity used:

- physical factors
- biochemical factors
- complement system
- phagocytosis

Non specific immunity recognize foreign antigens such as bacteria and react immediately and nonspecifically by the phagocytosis, main immune mechanism of innate immunity the first line of organism's defenses.

The most pathogenic bacteria evade this first line of our organism's defenses. So those pathogen must be manage by the specific immunity which is able to destroy them and to establish an immune memory.

Non specific immunity and specific immunity always interact and so act immune system of our organism.
2/ the specific resistance

- Specific resistance or **acquired immunity** is specific to an antigen, take a delay to response and has an immunological memory

- It’s sustained by the **duality of the specific immune system**:
  - humoral or antibody-mediated immunity
  - cell-mediated immunity

a) **Humoral immunity**

- **Humoral immunity** involves the production of **antibodies** that act against foreign organisms and substances which are generally **non-self antigens**. Cells called **B cells** or **B lymphocytes** are responsible for the production of antibodies. The humoral immune response defends primary against **extra cellular bacteria, bacterial toxins and virus that are circulating freely in the body’s fluids**.
Generally antibodies recognize and interact with specific regions on antigens called **epitopes** or **antigenic determinants**.
Antibodies are Y-shaped proteins that are made in response to an antigen and can recognize and bind to specific one antigen by is **Fab or fragment of antigen binding**.

Whereas **Fc or crystallizable fragment** has receptor on macrophages and is a receptor for the complement on the antibodies.
Antibodies are members of a group of soluble proteins collectively known as immunoglobulins (Igs) composed by two light and two heavy chains.

Immunoglobulins are classed, accordingly to chemically composition of the heavy chains into five classes or isotypes: IgG, IgM, IgA, IgE and IgD. Each class plays a different role in the immune response and has different structures and characters.

IgM antibodies are the first antibodies to appear in response to initial exposure to an antigen. In primo-infections we usually detect IgM which is relatively short lived.

A second exposure to antigen result mostly in increased production of IgG. The detection of IgG, which is relatively long-lived, may indicate only that immunity against a particular pathogen was acquired in the more distant past.
Primary response

Secondary response

IgG

IgM

Initial exposure to antigen

Second exposure to antigen

(arbitrary units)

Time (days)
Mother’s IgG (not IgM) can be transferred through placenta to the fetus. Those various immunoglobulins protect newborns during first 18 months of life.

Antibodies are screening in many infectious diseases, by serological assays which consist of indirect biological diagnostics or serodiagnostic assays.

For example, usually biological diagnosis of syphilis are serodiagnostic assays that screen antibodies against syphilis antigen:  
=VDRL an agglutination assay  
=TPHA a hemagglutination assay  
=Indirect Immunofluorescence assay  
=an immunoblotting assay which is the referred assay
For direct biological diagnostic of syphilis we use a darkfield microscope specimen examination from primary syphilis lesions.

Spiral aspect of *Treponema pallidum* with thiny spires observed on darkfield microscope.
b/ Cell-mediated immunity

Cell-mediated immunity involves specialized lymphocytes called T cells or T lymphocytes that act against foreign organisms or tissues.

Antigens that stimulate cell-mediate immunity are mostly intracellular in nature, such as Mycobacterium tuberculosis in alveolic macrophages, or virus inside his host cell.

Unlike humoral immunity, cell-mediated immunity is not transferred to fetus via placenta.

Cell-mediated immunity is based on the activity of certain specialized lymphocyte primary T cells.
Antigen recognition

CD8+ T cell

CD4+ helper T cell

TNF

IFN-γ

Phagocytes with ingested microbes; microbial antigens in vesicles or cytosol

Inflammation

Macrophage activation \( \Rightarrow \) killing of ingested microbes

Lysis of infected cell

Infected cell with microbes in cytoplasm

Effector functions of T cells

Section IV EFFECTOR MECHANISMS OF IMMUNE RESPONSES

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Like B cells, T cells develop from stem cells in the bone marrow. T cells are influenced by the thymus gland, where they differentiate into mature cells (the T in T cell is for thymus).

There seem to be two main functional type of T cells:

- helper T cells (Th) or CD4 cells
- cytotoxic T cells (Tc) or CD8 cells

a) Cytotoxic T cells destroy target cells on contact and especially virus and some bacteria, reproduce within host cells, they cannot be attack there by antibodies.
Figure 17.13 Cell-mediated cytotoxicity. A cytotoxic T (T<sub>C</sub>) cell binds to the MHC-antigen complex on the surface of an infected cell. The T<sub>C</sub> cell then discharges a protein called...
b) Ones activated by the antigen, **helper T cells (Th) CD4+ T lymphocytes** play central role in immune responses. **Th cells** are especially prolific producers of cytokines that **induce formation of Tc cells, and activation of macrophages.** **Th cells** with aids of cytokines influence the activity of all others immune system cells and stimulate B cells formation of many antibodies.

c) Mature B and T cells are located in peripheral lymphoid organs mainly in lymph nodes where antigens are trapped and react with immune effectors cells.

d) Each immune response **involves in effectors cell actions and establishing of immune memory**
Immune responses in lymph nodes
3) Types of acquired immunity

- Immunity can be acquired either *actively or passively*.

- Immunity is acquired *actively* when a person is exposed to microorganisms or foreign substances and the immune system responds.

- Immunity is acquired *passively* when antibodies are transferred from one person to another.

- Both actively acquired immunity and passively acquired immunity can be obtained by natural or artificial means.
Natural acquired passive immunity involves the natural transfer of antibodies from a mother to her infant; especially transplacental transfer to the fetus or in the breast milk of the first secretion called colostrum.

Artificial acquired active immunity result from vaccination. Vaccination, also called immunization, introduces special prepared antigens called vaccines into the body. Vaccines may be inactivated bacterial toxin or toxoids, killed microorganisms, living but attenuated microorganisms or part of microorganisms such as capsules. These substances can no longer cause disease, but they can stimulate an immune response much as naturally acquired pathogen do.
Artificially acquired passive immunity involves the introduction of antibodies into the body.

These antibodies come from an animal or a person who is already immune to the disease and protect against that disease.

We use such prevention against tetanos, hepatitis, herpes virus diseases and so on.
Figure 17.1 Types of acquired immunity.
f) Microbial genetics

1) DNA and chromosomes

- **Genetics** is the study of what gene are, how they carry information, how their information is expressed, and how they are replicated and passed to subsequent generations or other organisms.

- **Chromosomes** are cellular structures that physically carry hereditary information; the chromosomes contain the genes.

- Bacteria typically have a single circular chromosome consisting of a single circular molecule of DNA, attached at one or several points of the plasma membrane.

- **Genes** are segments of DNA that code for functional produces.
DNA is a macromolecule composed of repeating units called **nucleotides** which consist of a nitrogenous base ( adenine, thymine, cytosine or guanine), desoxyribose ( a pentose sugar) and a phosphate group.

DNA molecule consists of **two long strands** wrapped around each other to form a **double helix**, each strand is composed of many nucleotides.

The desoxyribose ( pentose sugar ) of one nucleotide is joined to the phosphate group of the next.
Figure 2.17 The structure of DNA. Nucleotides (top) are composed of a deoxyribose sugar, phosphate, and a nitrogenous base. In the DNA double helix, complementary base pairs are hydrogen bonded: thymine (T) pairs with adenine (A), and cytosine (C) pairs with guanine (G).
Every strand of DNA has a “backbone” consisting of alternating desoxyribose sugar and phosphate group and a nitrogenous base is attached to each sugar in the backbone.

The two strand are held together by hydrogen bonds between their nitrogenous bases

The base pairs always occur in a specific way: the purine base adenine (A) always pairs with the pyrimidine base thymine (T), and the purine base guanine (G) is always paired with the pyrimidine base cytosine (C)
Because of this specific base pairing, the base sequence of one DNA strand determines the sequence of the other strand. The two strands are thus complementary.

That means that if the sequence of bases of one strand is known, then the sequence of the other strand is also known.

The order in which the nitrogen base pairs occur along the backbone is extremely specific and in fact contains the genetic instruction for the organism.
Genetic information is encoded by the sequence of bases along the strand of DNA.

The information in the sequence of nucleotides specifies the complete collection of proteins to be found in the cell.

Which in turn determines the characteristics of the cell and transfers these characteristics to subsequent generations of cells.

Chargaff coefficient means A+T/G+C or GC% that indicates the specie’s DNA base composition. Two species that are closely related and hence has many identical or similar genes will have similar amounts of the various bases in their DNA. For each species GC % is constant. It is therefore a powerful criteria of taxonomy or classification.
DNA can change or **mutate**

Although many genetic mutations cause harm or kill the cell, others create new characteristics that give the offspring inheriting the mutation a better chance of survival in new environments.

Thus, mutation is, in the long run, an advantage that contributes to the successful evolution of the specie.
2) DNA replication

- The double helix of the parental DNA separates as weak hydrogen bonds between the nucleotides on opposite strands break in response to the action of replication enzymes.

- Next, hydrogen bonds form between new complementary nucleotides and each strand of the parental template to form new base pairs.

- Enzymes catalyze the formation of sugar-phosphate bonds between sequential nucleotides on each resulting daughter strand.

- DNA replication makes possible the flow of genetic information from one generation to the next.
DNA replication
Figure 8.3 A summary of events at the DNA replication fork. Enzymes at the replication fork unwind the parental double helix. The leading strand is synthesized continuously by DNA polymerase. The lagging strand is synthesized discontinuously. RNA polymerase synthesizes a short RNA primer, which is then extended by DNA polymerase. DNA ligase joins the discontinuous fragments of the lagging strand.
3) DNA and protein synthesis

- How is the information in DNA used to make the proteins that control cell activities?

- In the process of transcription, genetic information in DNA is copied or transcribed into a complementary base sequence of RNA which is called mRNA for messenger RNA.

- The cell then uses the information encoded in the mRNA to synthesize specific proteins through the process of translation.

- The overall goal of translation is to produce proteins using mRNAs as the source of biological information.

- The language of mRNA is in form of codons, group of three nucleotides as AUG, CGG or AAA.

- Each codon “codes” for a particular amino acid. This is the genetic code.
1. RNA polymerase binds to the promoter, and DNA unwinds at the beginning of a gene.

2. RNA is synthesized by complementary base pairing of free nucleotides with the nucleotide bases on the template strand of DNA.

3. The site of synthesis moves along DNA; DNA that has been transcribed rewinds.

4. Transcription reaches the terminator.

5. RNA and RNA polymerase are released and the DNA helix reforms.

Figure 8.6 The process of transcription. The orienting diagram indicates the relationship between DNA, RNA, and protein synthesis.
BETWEEN GENERATIONS OF CELLS

DNA

Parent cell

Replication

Cell divides

Daughter cells

WITHIN A CELL

DNA

mRNA

Protein

Cell metabolizes and grows

Transcription

Translation

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The genetic code based on codons formed by three nucleotide bases.
To summarize,

- genes are the units of biological information encoded by the sequence of nucleotide bases in DNA
- A gene is expressed or turned into a product within the cell, through the processes of transcription and translation.
- The genetic information carried in DNA is transferred to a temporary mRNA molecule by transcription.
- Then, during translation, the mRNA directs the assembly of amino acids into a polypeptide chain:
  - mRNA attaches to a ribosome,
  - tRNAs or transfer RNA deliver the amino acids to the ribosome as directed by mRNA codon sequence
  - and the ribosome assembles the amino acids into the chain that will be the new synthesized protein
4/ Genotype and phenotype

a/ The genotype of an organism is its genetic makeup, the information that codes for all particular characteristics of the organism.

= In molecular terms, an organism’s genotype is its collection of genes
= The genotype represents potential properties, but not the properties themselves

b/ Phenotype refers to actual, expressed properties, such as the organism’s ability to perform a particular chemical reaction

= In molecular terms, an organism’s phenotype is its collection of proteins (enzymatic or structural) and other macromolecules such as lipids or polysaccharides
5/ mutation: change in the genetic material

a) mutation

A mutation is a change in the base sequence of DNA.

Such a change in the base sequence of a gene will sometimes cause a change in the product encoded by that gene.

For example, when the gene for an enzyme mutates, the enzyme encoded by the gene become inactive or less active because its amino acid sequence has changed.

Such a change in genotype may be disadvantageous, or even lethal, if the cell loses a phenotype trait it needs.

However, a mutation can be beneficial if, for instance, the altered enzyme encoded by the mutant gene has a new or enhanced activity that benefits the cell.
The most common type of mutation involving single base pairs is *base substitution* or *point mutation*, in which a single base at one point in the DNA sequence is replaced with a different base.

The effects of such mutation can be dramatic.

For example, sickle-cell anemia is caused by a single change in the gene for globin, the protein component of hemoglobin.

A change from A (adenine) to T (thymine) at a specific site, results in the change from glutamic acid to valine in the protein.

The effect of this change is that the shape of the hemoglobin molecule changes under condition of low oxygen, altering the shape of red blood cells, such the movement of the cells through small capillaries is greatly impeded.
b) mutagens

Mutagens are any environmental agents that can directly or indirectly bring about mutation.

They are:

= chemical mutagens such as *nitrous acid* (HNO2) which makes a specific base pair change in DNA. Like all mutagens, it alters DNA at random locations.
= \textit{X rays or gamma rays} are forms of radiation that are potent mutagens because of their ability to ionize atoms and molecules. Some of these ions can combine with bases in DNA, resulting in errors in DNA replication and repair that produce mutations.

= an other form of mutagenic radiation is \textit{ultraviolet (UV)} light, a nonionizing component of ordinary sun-light. The most important effect of direct UV light on DNA is the formation of covalent bonds between certain bases; such as to form thymine dimers. Such dimers, unless repaired, may cause serious damage or death of the cell because it cannot properly transcribe or replicate such DNA.
Ultraviolet light

1 Exposure to ultraviolet light causes adjacent thymines to become cross-linked, forming a thymine dimer and disrupting their normal base pairing.

2 An enzyme cuts out and removes the damaged DNA.

3 DNA polymerase fills the gap by synthesizing new DNA, using the intact strand as a template.

4 DNA ligase seals the remaining gap by joining the old and new DNA.

Figure 8.17 The creation and repair of a thymine dimer caused by ultraviolet light. After a exposure to UV light...
c) frequency of mutation

- Spontaneous mistakes in DNA replication occur at a very low rate, perhaps only once in $10^{9}$ (a mutation rate of $10^{-9}$).

- Because the average gene has about $10^3$ base pairs, the spontaneous rate of mutation is about once $10^6$ (a million) replicated genes.

- A mutagen usually increases the spontaneous rate of mutation by a factor of 10-1000 times.

- In other words, in presence of a mutagen, the normal rate of $10^{-6}$ mutations per replicated gene become a rate of $10^{-5}$ to $10^{-3}$ per replicated gene.
Genetic materials can be transferred between bacteria in several ways.

In all of the mechanisms, the transfer involves a *donor cell* that gives a portion of its total DNA to a *recipient cell*. Once transferred, part of the donor’s DNA is usually incorporated into the recipient’s DNA.

The transfer of genetic material between bacteria occurs in only 1% or less of the entire population.

The specific types of genetic transfer are:
- *transformation* in bacteria
- *conjugation* in bacteria
- *transduction* in bacteria
a) transformation in bacteria

During the process of transformation, genes are transferred from a bacterium to another as “naked” DNA in solution.

The initial experiment on transformation was performed by GRIFFITH in England in 1928 while he was working with two strains of Streptococcus pneumoniae.

One, a virulent encapsulated strain causes pneumonia; the other, an avirulent strain, lacks the capsule and does not cause disease. Next figure presents the Griffith experiment.

Only after years of research, USA’s researchers announced in 1944 that the component responsible for transformation harmless S. pneumoniae into virulent strains was DNA.
Figure 8.21 Griffith's experiment demonstrating genetic transformation. (a) Living encapsulated bacteria injected into mouse: mouse died, colonies of encapsulated bacteria were isolated from dead mouse. (b) Living nonencapsulated bacteria injected into mouse: mouse remained healthy, colonies of nonencapsulated bacteria were isolated from mouse; phagocytes controlled bacterial proliferation. (c) Heat-killed encapsulated bacteria injected into mouse: mouse remained healthy, no colonies were isolated from mouse. (d) Living nonencapsulated and heat-killed encapsulated bacteria injected into mouse: mouse died, colonies of encapsulated bacteria were isolated from dead mouse.
b) Conjugation in bacteria

- Another mechanism by which genetic material is transferred from one bacterium to another is known as **conjugation**.
- Conjugation is mediated by one kind of **plasmid**, a circular piece of DNA that replicate independently from the cell’s chromosome.
- In *Escherichia coli*, enteric gram-negative bacilli, the **F factor (fertility factor)** was the first plasmid observed to be transferred between cells during conjugation.
- In gram-negative bacteria, the plasmid carries genes that code for the synthesis of **sex pili**, projections from the donor’s cell surface that contact the recipient and help bring the two cells into direct contact.
- Donors carrying F factors (F+ cells) transfer the plasmid to recipient (F- cells), which become F+ cells as result. Next figure illustrates conjugation in *Escherichia coli*.
When F+ factor integrates into cell’s recipient chromosome, it makes the cell a high frequency of recombination (Hfr) cell.

(a) When an F factor (a plasmid) is transferred from a donor (F+) to a recipient (F-), the F- cell is converted into an F+ cell.

(b) When an F factor becomes integrated into the chromosome of an F+ cell, it makes the cell a high frequency of recombination (Hfr) cell.
c) Transduction in bacteria

- A third mechanism of genetic transfer between bacteria is **transduction**

- In this process, bacterial DNA is transferred from a donor cell to a recipient cell inside a virus that infect bacteria, called **bacteriophage** or **phage**

- To understand how transduction works, we will consider the life cycle of one type of transducing phage of *Escherichia coli*; this phage carries out **generalized transduction**
Figure 8.25 Transduction by a bacteriophage. Shown here is generalized transduction, the transfer of genetic material from one bacterial cell to another.

1. A phage infects the donor bacterial cell.

2. Phage DNA and proteins are made, and the bacterial chromosome is broken down into pieces.

3. Occasionally during phage assembly, pieces of bacterial DNA are packaged in a phage capsid. Then the donor cell lyses and releases phage particles containing bacterial DNA.

4. A phage carrying bacterial DNA infects a new host cell, the recipient cell.

5. Recombination can occur, producing a recombinant cell with a genotype different from both the donor and recipient cells.
7) Plasmids

- Plasmids are self-replicating gene-containing circular pieces of DNA about 1-5% the size of the bacterial chromosome.

- Recall that **F factor** is a plasmid that carries genes for sex pili and for the transfer of the plasmid to another cell.

- Under certain conditions, genes carried by plasmids can be crucial to the survival and growth of the bacterial cell.

- For examples:
  = some species of *Pseudomonas*, aerobic bacilli gram-negative enteric bacteria, carry **dissimilation plasmid** which code enzymes that trigger the catabolism of such exotic substances as toluene, camphor, and hydrocarbons of petroleum.
Strains of *Escherichia coli* that cause infant diarrhea and traveler’s diarrhea carry plasmids that code for *toxin production* and *bacterial attachment* to intestinal cells. Without these plasmids, *E. coli* is a harmless resident of the large intestine; with them it is a pathogenic.

*R factor (resistance factor)* are plasmids that have significant medical importance. They were first discovered in Japan in the late 1950s after several dysentery epidemics. In some of these epidemics, the infectious agent was resistant to a number of usually antibiotics. Researchers soon discovered that resistance was acquired by these bacteria through the spread of genes from one organism to another. The plasmid that mediated this transfer are *R factor* which confer upon their host cell resistance to antibiotics, heavy metals or cellular toxin.
In some cases, the accumulation of resistance genes in a single plasmid is quite remarkable. For example:

*plasmid R 100* carries resistance genes for sulfonamides, streptomycine, chloramphenicol, tetracyclines, and many other antibiotics, as well as genes for resistance to some heavy metals. This particular plasmid can be transferred between a number of enteric species, including *Escherichia*, *Klebsiella*, and *Salmonella*.

*R factors* present very serious problems for the treatment of infectious diseases with antibiotics.

Population of resistant bacteria grow larger and larger; because the widespread use of antibiotics in human, veterinary medicine and agriculture (many types of animal feed contain antibiotics) has lead to the preferential survival (selection) of bacteria that have *R factors*.
The transfer of resistance between bacteria of different genera, also contributes to the problem.

A bacterial species can conjugate and transfer plasmids to other species.

*Neisseria* (specifically *Neisseria gonorrhoea* causative agent of gonorrhea) may have acquired its penicillinase-producing plasmid from *Streptococcus*.
section 3 recalling

1/ the microbiota and the host

=fetus of human (and animals) are free of microbes, protected by the placenta
=more microorganisms are introduced to the newborn’s body from environment when breathing begins and feeding starts

=a typically human body contains $10^{13}$ body cells, yet harbors $10^{14}$ bacterial cells

=normal microbiota or normal flora consists of commensals bacteria that don’t produce disease under normal condition
=normal flora is located: in nose and throat, in mouth, into the large intestine, on skin, in urogenital system, and on eyes conjunctiva
we must keep in mind that under certain conditions, commensal relationship can change. Therefore *Escherichia coli*, harmless in large intestine, may cause urinary tract infections.

2/ Microbial mechanisms of pathogenicity

most pathogens must **gain access to the host, adhere to the host’s tissues, penetrate and evade host defenses, and damage host’s tissues to cause disease**

= the portal entry for pathogens are:

a) Mucous membrane of respiratory tract, of gastrointestinal tract, of genitourinary tract, and of conjunctiva  
b) Skin  
c) Parenteral route
Once pathogens gain entry of host, almost all of them means of attaching themselves to host’s tissues.

This attachment with fimbriae containing adhesins is also called adherence that is a necessary step of pathogenicity.

Although some pathogens can cause damage on the surface of tissues, most penetrate tissues to cause disease.

When a microorganism invade a body, it encounters immune defenses and firstly the phagocytes.

Real strong pathogens can overcome the host defenses that produce extracellular enzymes, or have a capsule, or secrete toxins.
=thus virulence of some bacteria is thought to be aided by producing extracellular enzymes such as leucocidins, collagenases.

=only encapsulated *Streptococcus pneumoniae* strains are virulent and cause pneumonia; without capsule, *S. pneumoniae* is avirulent unable to cause any disease

=toxins are poisonous substances that are produce by certain microorganisms. They are two types of toxins:

a) **Exotoxins** which are produced inside mostly gram-positive bacteria
b) **Endotoxins** which are part of outer portion of the cell wall of gram-negative bacteria
Exotoxins may be grouped in three principal types based on their modes of action:

a) Cytotoxins which kill host’s cells such as diphtheria toxin of *Corynebacterium diphteriae* causative agent of the diphtheria.

b) Neurotoxins such as tetanus neurotoxin or tetanospasmine

c) Enterotoxins such as Vibrio toxin of *Vibrio cholerae*, causative agent of cholera

Endotoxins are liberated when gram-negative bacteria die and their cell walls undergo lysis.
3/ defenses of organism

=our ability to ward off disease is called resistance
=vulnerability or lack of resistance is called susceptibility

=in discussing resistance we will divide our body’s defenses into two general kinds:
   ▶ Nonspecific immunity
   ▶ Specific immunity

=nonspecific immunity is also called innate or natural immunity that it acts immediately and non-specifically
=innate immunity forms the first line of host’s defenses. =first, innate immunity recognizes non-self antigens such as bacteria; and then reacts using:
a) **Mechanical factors** such as intact skin, mucous membranes or epithelial cilia
b) **Chemical factors** such as sebum, lysozymes, acidity of gastric juice
c) **Commensal flora** that inhibit pathogen implantation
d) **Complement system** which can lyses foreign cells, or induces inflammation and/or opsonization
e) **Phagocytosis** the main mechanism of innate immunity

=that’s phagocytes, monocyte-macrophages and dendritic cells, which deal phagocytosis. They engulf, ingest and digest non-self antigens like bacteria and other microorganisms
Phagocytes have receptors for a variety of molecules; for antigens, for components of the complement system, for Fc cristallizable fragment of IgG, immunoglobulins gamma that are antibodies.

But pathogens can evade phagocytosis in many ways; such as *Mycobacterium tuberculosis* inhibits the fusion of lysozymes’s vacuole and phagosome, for forming a phagolysosome.

In addition, phagocytes are CPA or cells presenting antigen to the specific acquired immunity; that’s one of the link between innate and specific immunities. But in fact, there are only one global immune response to all antigenic stimulations.
specific resistance or acquired immunity,

- is specific to an antigen
- takes a delay to response
- has an immunological memory

it’s sustained by:

- Humoral immunity
- Cell-mediated immunity

humoral immunity involves production of antibodies or immunoglobulins which are proteins of the serum

B cells or B lymphocytes are responsible for the production of antibodies
Humoral immunity is primary involves against extracellular bacteria, bacterial toxins and virus that are circulating freely in the body’s fluids.

Antibodies are Y shaped proteins composed by two light chains and two heavy chains. They specifically recognize antigens, by their Fab or fragment antigen binding. Whereas their Fc for cristallizable fragment has receptor on macrophages and are antibody’s receptors of the complement. We classify antibodies, accordingly to heavy chains chemical compositions, into IgG, IgM, IgA, IgE, and IgD isotypes.

Indirect biological diagnostics are based on screening of antibodies that are performed into serological assays so as we do to diagnose syphilis infections.
In primo-infections we usually detect IgM which are relatively short lived.

Screening detection of IgG, relatively long lived, indicates an acquired immunity in the more distant past.

Mother’s IgG (not IgM) can be transferred through placenta to the fetus, and through colostrum to newborns when breast-feeding.

Cell-mediated immunity involves activities of specialized T cells or T lymphocytes.

Antigens, that stimulate cell-mediated immunity, are mostly intracellular in nature such as virus inside their hosts or *Mycobacterium tuberculosis* into alveolic macrophages.
the two main functional types of T cells are:

a) Th or helper T cells, or CD4+ T lymphocytes
b) Tc or cytotoxic T cells, or CD8+ T lymphocytes

cytotoxic T cells destroy target cells on contact
helper T cells are prolific producers of cytokines which aid monitoring of all other immune effectors cells (monocyte-macrophage, B cells and cytotoxic T cells)
each specific immune response involves in effectors cell actions and establishing of immunity memory
antigens are trapped inside peripheral lymphoid organs mainly lymph nodes, that they react with mature B and T cells, the immune effectors cells.
4/Microbial genetics

- Bacteria have a single circular chromosome consisting of a single molecule of DNA.
- Chromosome contains genes which are segments of DNA that code for functional produces.
- DNA is a macromolecule composed of repeating units called nucleotides which consist a nitrogen base (adenine, thymine, cytosine and guanine), a pentose desoxyribose and a phosphate group.
- DNA molecule consists of two long strands of nucleotides helded together by hydrogen bonds between their nitrogenous bases.
- Base pairs always occur in a specific way: the purine base adenine always pairs with the pyrimidine base thymine, the pyrimidine base cytosine with the purine base guanine. So that the base sequence of one strand determines the sequence of other strand.
- The two strand are therefore complementary.
Chargaff coefficient means A+T/ C+G or GC% that indicates the specie’s DNA bases composition

GC% is constant for each species therefore a powerful criteria of taxonomy or classification

Genes, portions of chromosomal DNA, are expressed into products within the cell through processes of transcription and translation

By transcription genetic information, carried in chromosomal DNA, is transferred to a temporally messenger RNA (mRNA)

During translation mRNA codon sequence directs the assembly of amino acids delivered by a ribosomal transfer RNA (tRNA) into a polypeptide chain or new protein

An organism’s genotype is its collection of genes that represents potential properties

An organism’s phenotype refers to actual expressed cell’s properties such as to perform a particular metabolic reaction.
Mutation is a change in the base sequence of a gene that will sometimes cause a change in the product coded by that gene.
Mutation can be disadvantageous or beneficial.
The most common mutation involve replacement of a single DNA nitrogenous base by an other.
That’s base substitution or point mutation which can be dramatic such as in sickle-cell anemia disease.
Mutagens are environmental agents that usually increase spontaneous rate of mutation by a factor of 10-100 times.
Nitrous nitrogen, ultraviolet light or x-rays are mutagen agents.
Normal rate of DNA mutation is very low about once per million replications of gene.
Bacterial gene transfer between a donor cell to a recipient one occurs in only 1% or less of entire population.
Mechanisms involved in bacterial gene transfer are of three types: transformation, conjugation and transduction.
Transformation refers to transfer of “naked” DNA

Conjugation is mediated by a plasmid that will be transferred through a sex pili between donor and recipient cells

About transduction, bacterial DNA is transferred inside a bacteriophage or phage a virus that infect bacteria

Plasmids are cytoplasmic self replicating genes independently from bacterial chromosome.

Plasmids can code toxins, and various enzymes involving in cell’s pathogenicity and metabolic pathways.

90% or more bacterial antibiotic resistances refer to transfer of R factors or plasmids of resistance from a resistant to a sensitive bacteria of the same specie or different genera.