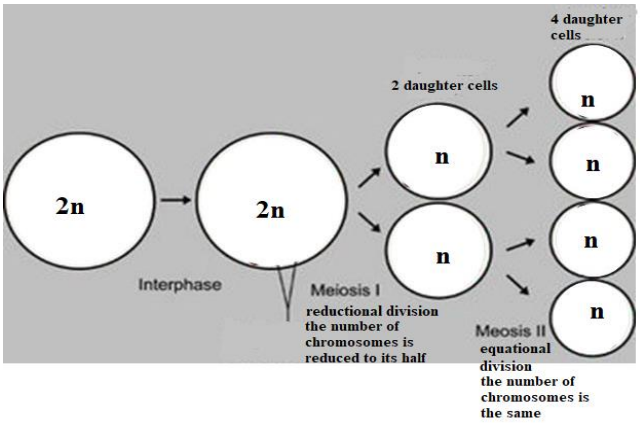
Chapter 1: Basic mechanisms of reproduction

Document 3: Meiosis

Meiosis definition

- Cellular division during which a diploid germ cell gives rise to 4 haploid gametes
- Takes place in the ovaries and testes.
- Includes 2 successive divisions.



Before meiosis:

Interphase occurs during which duplication of DNA occurs: each chromosome of 1 chromatid becomes of 2 chromatids.
No interphase between meiosis 1 and 2.

I- Meiosis I or reductional division

1- Prophase 1:

- Nuclear membrane disappears.
- Centrosome changes into asters that migrate to opposite poles.
- Spindle fibers appear between the 2 asters.
- Chromatin condenses into chromosomes.
- Homologous chromosomes pair up to form tetrads. Crossing over occurs.

2- Metaphase 1:

- Homologous chromosomes pair & line up at the equator, forming the "equatorial plate".

3- Anaphase 1:

- "Polar Ascension":

Homologous chromosomes separate & each chromosome migrates toward opposite pole.

4- Telophase 1:

- Two daughter cells are formed. Each cell contains (1n) chromosomes i.e. half the number of chromosomes of the mother cell.
- Each chromosome is still made up of 2 sister chromatids.

II. Meiosis II or equational division:

5- Prophase 2: Same events as in Meiosis I, but each cell contains (1n) chromosomes.

6- Metaphase 2: Same events as in Meiosis I, but each cell contains (1n) chromosomes.

7- Anaphase 2: Sister chromatids separate & migrate toward opposite poles.

8- Telophase 2: 4 daughter cells are formed: Each cell contains "n" chromosomes, and each chromosome is made of 1 chromatid.

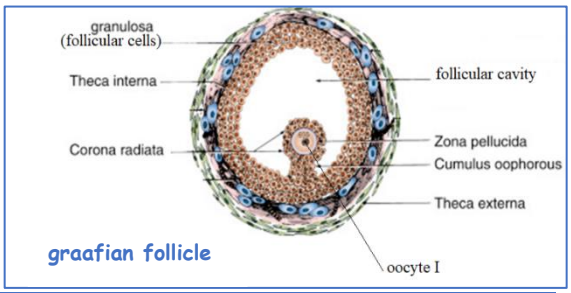
Role of meiosis

- Formation of haploid gametes.
- The gametes are genetically different and this increases diversity at the level of phenotypes in the species.
- Reduces the number of chromosomes to its half.

Document 5: Oogenesis

Definition of oogenesis

Oogenesis is a process that leads to the formation of haploid female gametes from diploid germ cells. It is discontinuous;



starts during embryonic life, stops at birth, then resumes at puberty and finally stops at menopause. This process does NOT end in the ovary.

Folliculogenesis

At birth, the female has primordial follicles in her ovaries.

4 months before the beginning of the cycle: The primary follicle transforms into secondary follicles then into tertiary (cavitary) follicles which has a diameter of 5 mm.

Phases of the female menstrual cycle:

Follicular phase (day1- 13): the tertiary follicle transforms into graafian (20mm).

Ovulation: (day 14): the follicle ruptures and the female gamete is released in the fallopian tube.

Luteal phase: (day 15- 28): the ruptured follicle transforms into yellow body (corpus luteum) and then degenerates at the end of the cycle to white body (corpus albicans).

Phases of Oogenesis (doc. d p. 28)

1- Multiplication:

- Takes place during embryonic life
- Oogonia undergoes mitosis.
- Oogonia (2n, s)

2- Growth

- Takes places during embryonic life
- Oogonium transforms into oocyte I (2n, d).

3- Maturation

- Starts in the embryonic life.
- The oocyte I (2n, d) starts meiosis, but stays blocked at prophase I.
- First stop of oogenesis occurs at birth.
- From birth till puberty, the oocyte I stays blocked at prophase I.
- At puberty, few hours before ovulation, oocyte I blocked at Prophase I resumes meiosis I (reductional division) and gives rise to oocyte II (n, d) and the first polar body (n, d).
- **This polar body** serves to eliminate one half of the diploid chromosome set produced by meiotic division in the oocyte I leaving behind a haploid cell (oocyte II).
- Oocyte II starts meiosis II (equational division) but stays blocked at metaphase II.
- Now the second arrest occurs, waiting for fertilization.
- Oocyte II is released to the fallopian tube.
- If fertilization does not occur, oocyte II stays 24 hours before it dies.
- If fertilization occurs, the oocyte II resumes meiosis II.
- As a result, ootid (n, s) is formed and a second polar body is released.

4- Differentiation:

The fertilized ovum fertilized by the sperm cell gives rise to a zygote.

Oogenesis versus folliculogenesis (doc. e p. 29)

During embryonic life:

- the female germ cells/ oogonia divide my mitosis to give a total number of 700 million oogonia. (multiplication phase)
- oogonia gives rise to the diploid primary oocyte (growth phase)
- each oocyte is surrounded by few follicular cells to form primordial follicle.
- During fetal life, a large number of primordial follicles degenerate. This is called follicular atresia.

- In the other primordial follicles, oocytes I undergo the first meiotic division but stay all blocked at prophase I until puberty.

From birth till puberty

- Follicular atresia continues during childhood.
- At puberty, the ovary will contain 400 000 primordial follicles.

At puberty

- Each month, 10 follicles will unblock. Usually one follicle will mature, while the others will degenerate.
- 4 months before the beginning of the cycle, the primary follicle develops into secondary and then tertiary follicle. During this time, oocyte I is still blocked at prophase I.

Female cycle:

- During the follicular phase, the tertiary follicle develops into graafian follicle.
- In the graafian follicle, a few hours before ovulation, the oocyte I will complete the first meiotic division and gives oocyte II and the first polar body.
- Oocyte II begins the second meiotic division but remains blocked at metaphase II.
- This division will be completed only if fertilization occurs.

if no fertilization occurs

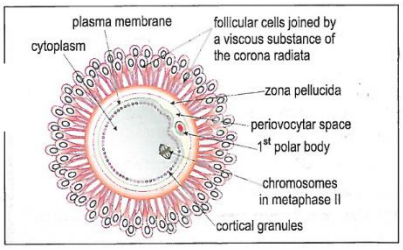
- The oocyte II stays alive for 24 hours then dies.
- The ruptured follicle transforms into corpus luteum which degenerates at the end of the cycle.

If fertilization occurs

- Oocyte II resumes meiosis II and gives ootid and the second polar body.
- A zygote is produced.
- The corpus luteum stays 3 months.

Document 6: Fertilization

oocyte II



1- Capacitation definition

Biochemical changes in the sperm that make them able to fertilize the oocyte.

Pay attention: oocyte lives only for 24 hours.

2- After fertilization

- A zygote is formed
- This zygote undergoes mitosis.

3- Role of fertilization

- Fertilization restores diploidy.

4- Life cycle in humans (doc.d)

In the human life cycle, there are two alternating phases

- Diploid phase which starts with fertilization
- Haploid phase which follows meiosis.

Chapter 15

During the follicular phase (1-13 days):

FSH produced by the pituitary gland stimulates the development of the follicles. The follicles mature and release moderate amount of estrogen which stimulates the proliferation of the superficial layer of the endometrium and exerts a negative feedback on the hypothalamopituitary axis resulting in a decrease in FSH and LH.

At day 12, the amount of estrogen reaches a peak exerting a positive feedback on the hypothalamopituitary axis resulting in an increase in FSH and LH.

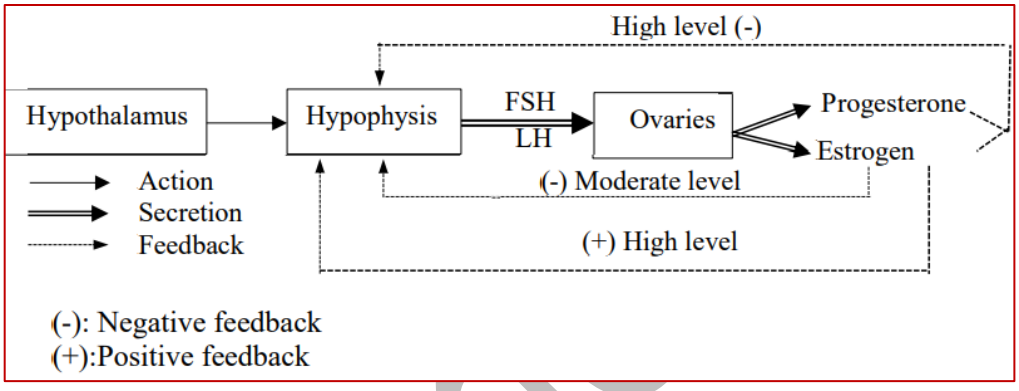
At day 13, a peak of LH triggers ovulation on day 14.

During the luteal phase (14-26 days):

Under the action of LH, the ruptured follicle transforms into corpus luteum, which secretes estrogen and progesterone. These hormones stimulates a further development and thickening of the endometrium and formation of the uterine lace and exert a negative feedback on the hypothalamopituitary axis resulting in a decrease in FSH and LH.

End of luteal phase (26-28 days):

if fertilization doesn't take place, corpus luteum transforms into a non-functional structure called corpus albicans. Now the level of estrogen and progesterone decreases. This leads to the sloughing off of the superficial layer of the endometrium called menstruation.



CHAPTER 3: GENETIC VARIATION AND POLYMORPHISM

1- Definition of mutation: Sudden and unpredictable changes that can affect the DNA molecule.

the types of point mutations: Deletion, insertion, substitution

Effect/consequence of mutation:

- Missense: if one amino acid is changed in the protein
- Silent: if nothing changed in the protein
- Nonsense: if the protein is incomplete (due to stop codon)
- Frameshift (in case of deletion and insertion mutations)

1- What is the difference between the recognition site and the cleavage site?

The recognition site is a double sequence of nucleotides having definite length (base pairs) and recognized by the restriction enzyme. However, the cleavage site is a specific position of cutting in the recognition site.

2- Gel electrophoresis

1- the alleles are cleaved by the restriction enzymes.
2- The normal allele yields 2 fragments a and b while the mutant allele yields 1 fragment only c.
3- The obtained fragments are separated on an electric agarose gel.
4- The DNA fragments migration in the gel depends on:
   - Charge: the fragments of DNA migrate from the negative pole to the positive pole as they are charged negatively.
   - Molecular weight: large fragments migrate slower than smaller ones.
5- Add a dye to stain the colorless DNA bands. This dye fluoresces under UV light.
   The obtained bands pattern is referred to a restriction map.

7- Restriction fragment Length Polymorphism (RFLP):

- Our DNA is made of both coding and non-coding regions.
- Any mutation at the level of the coding regions usually affect the phenotype.

- The non-coding regions are repetitive and abundant sequences in the DNA and have no function in gene expression neither in phenotype. Any mutation at the level in the non-coding regions do not affect the phenotype.
- Cuttings in the whole DNA will produce for each individual unique banding patterns on the gel which is called restriction map.
- The difference in restriction maps between two individuals is called a restriction fragment length polymorphism or RFLP.
- So the aim of RFLP is to ensure the fact that individuals are different at the level of their DNA in both coding and non-coding regions.

1- FISH or Fluorescence In Situ Hybridization:

Aim: used to determine the loci of a specific allele on a chromosome.

Method:

2- List the utilization of DNA fingerprint.

- Ensuring the uniqueness of the genome of each organism.
- Paternity testing.
- Criminal investigations.
- Dead bodies' identification.

Chapter 5: Human genetics

Documents 2 and 3

Determine the localization of a gene:

1- Autosomal (males and females are affected)
   a- Elimination of non-homologous segment of Y: Father- son
   b- Elimination of non-homologous segment of x: Father- daughter
   c- Elimination of homologous segment of X & Y: Father- son & daughter
   c- the non-homologous segment of X.
   d- Therefore, autosomal

2- Gonosomal (only males are affected)
   Start by saying, since only males are affected, therefore the disease is sex linked
   a- Elimination of non-homologous segment of Y: Father- son
   b- Therefore, it is localized on

Notes:

1- Choose the son/daughter having recessive phenotypes (=> affected if the disease is recessive and normal if the disease is dominant)

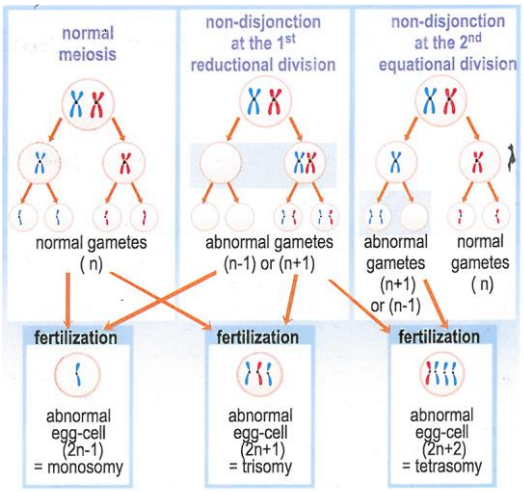
2- When they ask to write the genotype, check first if the disease is autosomal (NN, Nm or mm…) or gonosomal (X^NX^m,X^NY…)

Document 4: Chromosomal mutations

1- Types of chromosomal mutations:

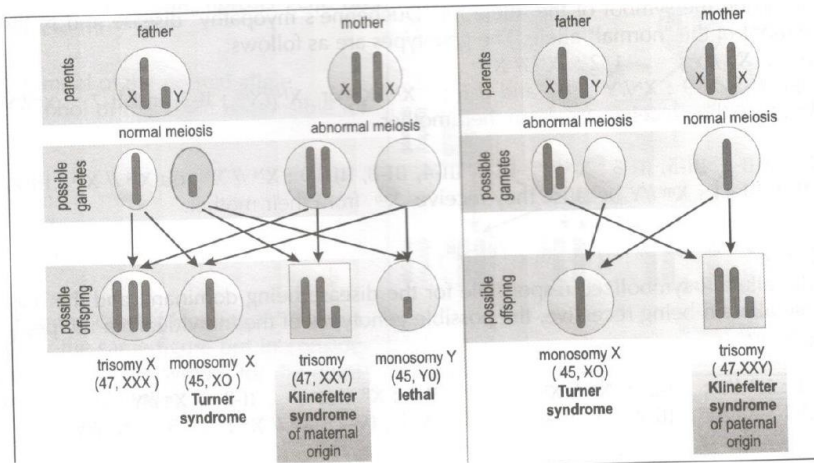
- Numerical abnormalities: abnormalities that affect the whole number of chromosomes. (extra or missing chromosome) (doc.a)
- Structural abnormalities: abnormalities that affect the shape of 1 or more chromosome (translocation, missing part or extra part of segment of chromosome) (doc.c)

2- Schematize the mechanisms that lead to chromosomal numerical abnormalities



Doc.g Mechanisms of occurrence of chromosomal numerical abnormalities.

3- Schematize the mechanisms (meiosis and fertilization) that lead to Klinefelter and down



Document 5: prenatal diagnosis

Definition of prenatal diagnosis: is a genetic technique allowing predicting and detecting the presence of genetic diseases in the fetus before birth.

Method	Chorionic villus biopsy	Amniocentesis	Sampling of fetal blood cells
Procedure	Extract fetal cells from the chorion membrane of the placenta	Extract fetal cells sloughed off from the fetus skin from the amniotic fluid	Withdraw blood from the umbilical cord
Pregnancy time	Starting from the 8th week of pregnancy	Starting from the 16th week of pregnancy	Starting from the 20th week of pregnancy

Immunology

1- Table showing the HLA classes characteristics:

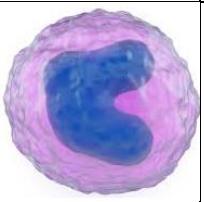
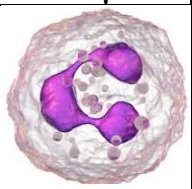
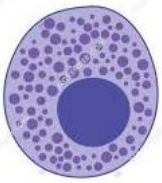
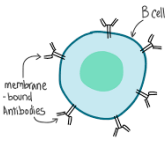
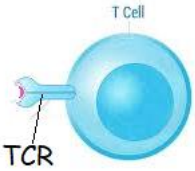
HLA class type	**HLA class I**	**HLA class II**	**Components of complement class III**
Expressed by	All nucleated cells including immune cells	Immune cells	In the blood (plasma)
Coded by loci	Loci A, B and C	Loci DP, DQ and DR	Locus on chromosome 6

Grafts:

- Autograft: is the tissue graft between two different sites in the same body individual

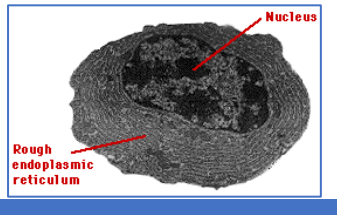
- Isograft: is the tissue graft between two animals of the same lineage. (Isograft in humans: is the tissue graft between 2 identical twins)
- Allograft: is the tissue graft between two individuals of different lineage of the same species
- xenograft: is the tissue graft between two different species.

Different types of leucocytes/WBC

Type of leucocyte	Monocytes	Granulocytes - neutrophils - eosinophils - basophils	Mast cells	B lymphocytes	T lymphocytes	
					T4 or TH cells	T8 or Tc cells
schema						
Shape of nucleus	Horse shoe shape	Multilobed nucleus (granulated cytoplasm)	Round nucleus (cytoplasm rich in histamine vesicles)	Large round nucleus occupying most of the cytoplasm		
Antigenic receptors	No antigenic receptors			Antibodies	T.C.R called CD4	T.C.R called CD8
Function	In the tissues, monocytes transform into macrophages performing phagocytosis of bacteria	Phagocytosis	Role in allergic reactions and phagocytosis of microbes at the level of mucus in the mucosa tissue.	After their activation, they differentiate into plasmocytes that secrete antibodies neutralizing the foreign body.	"Manager" They activate B and Tc cells.	Kill the infected or modified self-cells (cancerous, allograft, virus infected cells.)
Type of immune response	Non-specific immune response			Humoral specific immune response	Activates humoral and cell-mediated immune response	Cell-mediated immune response

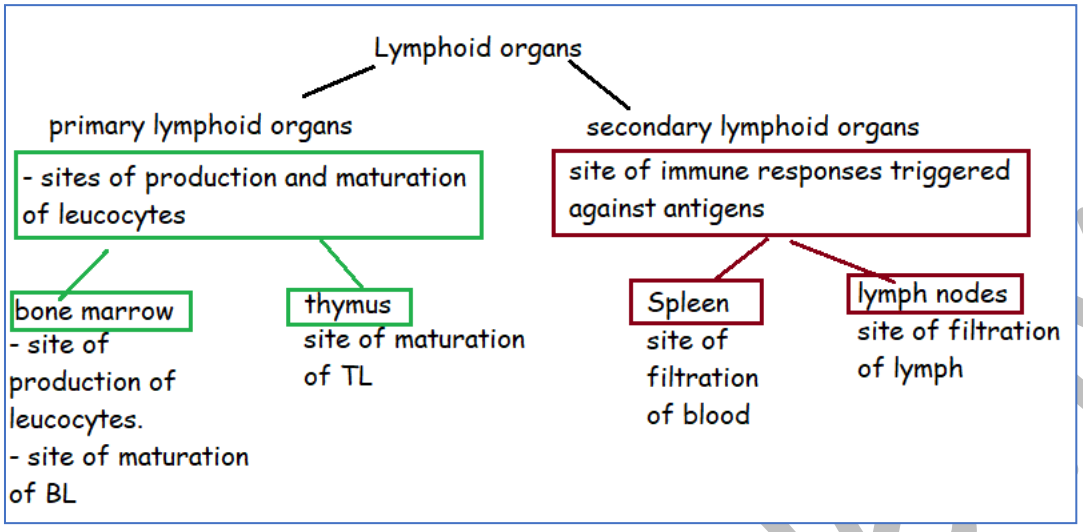
- Graft is more likely to be accepted if both individuals, the donor and the recipient, have a higher no of compatibility at the level of HLA molecules as in the autograft and isograft.

B cells and plasmocytes



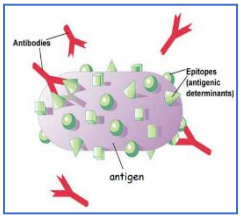
The B lymphocyte transforms into plasma cell or plasmocytes which has a more developed endoplasmic granulated reticulum and a larger cytoplasm. These features help the plasmocytes to secrete antibodies (by protein synthesis, antibodies are proteins)

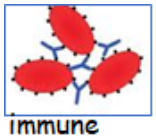
Lymphoid organs



The difference between antigen and antigenic determinant (epitope):

An antigen is the substance recognized by the immune system as being as non-self. While, antigenic determinants or epitopes are molecules (parts) of the antigen. The antigen may carry many antigenic determinants which bind to the antigen binding sites.

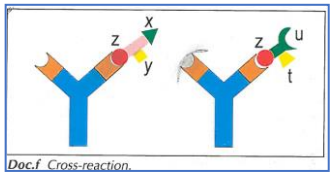




immune complex

Immune complex: is a large molecular aggregate consisting of several antibodies and antigen molecules related to each other.

Cross reaction: is the process of binding of an antibody to 2 different Antigens sharing a common epitope.



Doc.f Cross-reaction.

Differences between antibody and TCR.

	Antibody	TCR
Cell localization	On BL or free in the plasma	TL
Structure	Y shaped with 4 polypeptide chains	Rod shape with 2 polypeptide chains
Antigen recognition	Direct combination (between epitope and antigen binding site)	Double recognition between self HLA-non-self-peptide and TCR.
Number of antigen binding sites	2 binding sites	1 binding site

Double recognition by TCR (T Lymphocytes)

1- Normal cell: self-proteins are fragmented, attached to HLA class I and transported to the cell surface. This self is presented to Tc. However, there is no mature Tc specific for self-peptides.

2- Cell infected by a virus: viral peptides are attached to HLA class I and transported to the surface of the cell where they are presented to Tc. These Tc recognize these viral proteins and are activated.

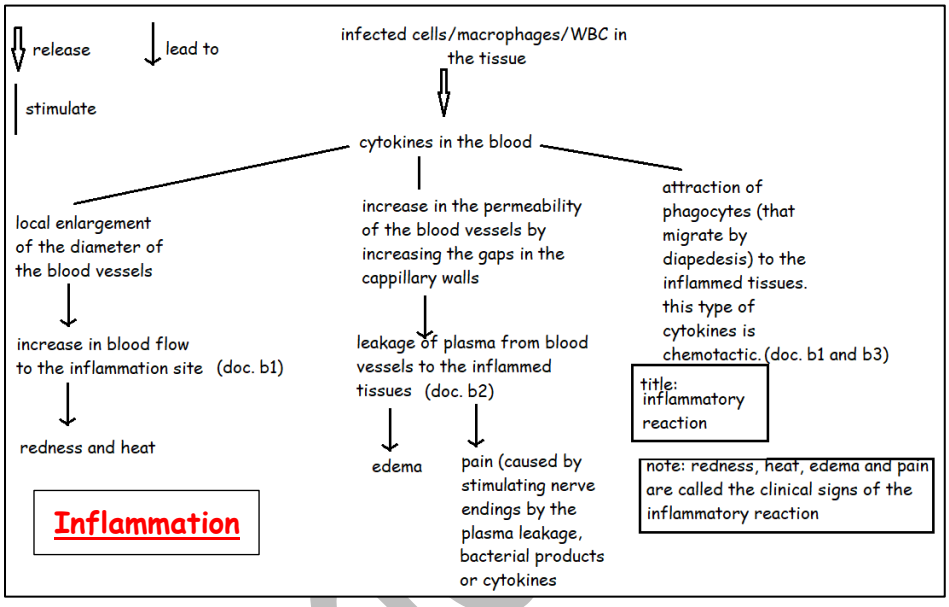
3- Macrophage phagocyte bacteria/virus/antigen: The antigenic bacterial peptides are attached to HLA class II molecules. they are transported to the cell surface and are presented to T_H. These TH recognize these non-self-peptides and are activated.

Note: modified self: HLA (class I or II) attached to non-self-peptide

Non specific immune response

1- **Inflammation:** are the physiological events leading to inflammatory signs such as redness, hotness, pain

2- **phagocytosis:** is the process by which the macrophage kills/ destroys/ digests the microbe. The macrophage does not recognize the antigen, any macrophage can bind to several types of microbes to phagocyte them. This justifies that phagocytosis is a non-specific immune response.



Induction of Specific immune response

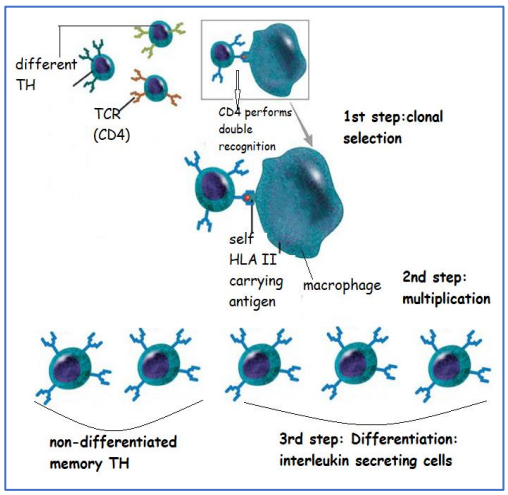
induction of the SIR by macrophages

Macrophages phagocyte an intruder at the site of infection. The resulting peptides are attached to HLA class II and presented on the surface of the macrophage. This macrophage migrates to the closest lymph node and becomes an APC (antigen presenting cell). T_H cells circulate continuously between lymph nodes, where they inspect the HLA- peptide complexes of APCs.

Only T_H cells that are specific for the peptides presented by the APC remain attached to it. They are then activated (clonal selection) and they proliferate. The others leave the lymph node and recirculate.

clonal selection and activation of T_H lymphocytes

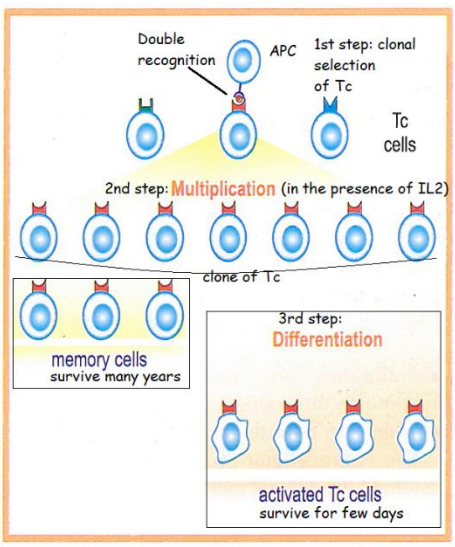
- Clonal selection: is the exclusive activation of the lymphocyte specific of the antigen
- Memory cells: survive many years. Are ready for any future encounter of the same antigen (secondary immune response)
- the cells secreting interleukins survive only for a few day.

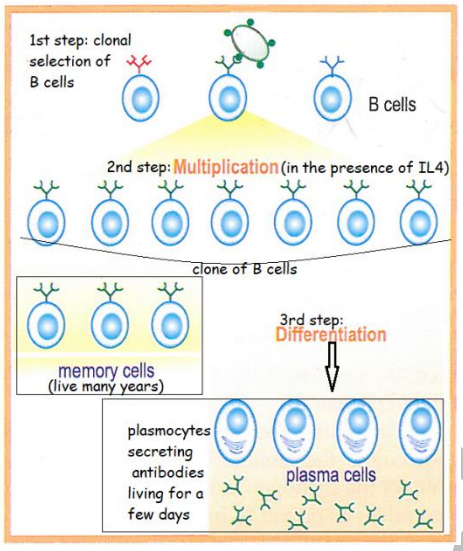


Role of T_H in the SIR: when the macrophages activate the TH, this T_H secrete IL-2 that stimulates the activation and proliferation of Tc cells and secretes IL-4 that stimulate the proliferation of B cells.

why nude mice (having no thymus) don't have a humoral immunity.

Nude mice are devoid of Tlymphocytes, the absence of TH cells leads to the absence of BL proliferation and consequently to the absence of the humoral SIR as a result of the absence of IL4.





Evolution of B and Tc lymphocytes

Humoral Specific immune response (SIR)

Explain how humoral SIR eliminate a small intruder:

- Antibodies cannot kill pathogens. However, they neutralize and inactivate pathogens and their toxins.
- Neutralization: antibodies bind to the soluble antigen (toxin, virus) before this antigen infect cells. This antibody binds to the pathogen attachment site for the target cell. This prevent the pathogen from entering and infecting our cells.
- After neutralization, this small intruder is eliminated by opsonization.
- The antibody neutralizing the antigen binds using its constant region to specific receptors found on the macrophage.Thus antibodies create a molecular bridge between antigens and phagocytes. This mechanism is called opsonization.

Explain how humoral SIR eliminate a large intruder:

- the antibody binds using its variable region to the epitopes found on the surface of the large non-self-cell.
- Activation of the complements: the complement C1 is activated and binds to the constant region of the antibody. Then the complement C1 activates other complements which activate each other (C1 to C9). This chain activation is called the complement cascade.
- The complements form an enzymatic complex that attacks the membrane of the foreign cell causing its perforation and leading to its lysis. The obtained particles are engulfed by macrophages.

specific cell-mediated immune response

Target of Tc: infected or modified body cells (double recognition)

Target of antibodies: circulating antigens, free microbes, antigens on the surface of cells…

Elimination of infected host cells: (doc. a)

- After a double recognition between the Tc through its TCR and the HLA-1- peptide complex on the target cell, the Tc releases perforin. Perforin assembles into polymers that form a hollow channel through the target cell membrane.

- Then Tc releases granzymes in the polyperforin channel. Granzymes trigger an enzymatic chain reaction within the cell, leading to DNA degradation: this causes cell death. DNA degradation is considered a mechanism of cell cytotoxicity since it results in the shutdown of protein synthesis. And since some proteins are necessary for cell survival such as cell division and cell renewal, then this leads to the death of the cell.
- The Tc detaches from the killed target, recirculates and is ready to kill other target cells carrying the same HLA-1-peptide complex.

Cancer: cancer results from an intensive and uncontrolled proliferation of a body cell, following an alteration of its genetic program.

1- Cancer treatment:

Treatment	Surgery	Radiotherapy	Chemotherapy
Procedure	Removal of the tumor by a surgical operation	Irradiation of the tumor tissue with radioactive beams which degrade DNA and kill the cells.	Use anti-mitotic drugs such as colchicine that block cell division
Side effect	If one cancerous cell persists after the surgery, another tumor will be formed again.	Irradiation reaches other normal cells killing them.	The drugs inhibit the division of normal cells also such as stem cells in the bone marrow leading to immunodeficiency.

Immunotherapy:

- Removal of tumor. This tumor contains cancer cells and T lymphocytes specific for these cancer cells. The cell mixture obtained is cultured in the presence of Il-2. Tc cells are activated and proliferate. They kill the cancer cells of the culture.
- After a month, the culture contains activated Tc only.
- Activated Tc are harvested and injected in the patient. They attack the cancer cells and kill them.
- Immunotherapy is a better choice to treat cancer since it is more specific and kills the tumor cells selectively. Theoretically, it has fewer side effects than other cancer treatments.

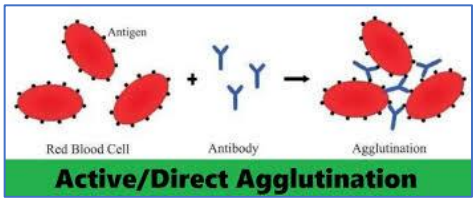
The secondary immune response is amplified, durable (long lasting) and rapid. Explain

- **Rapid**: the primary IR need 2 weeks because of the time needed by the macrophage to recognize the toxin, phagocytes it, transforms into APC to stimulate T_H that also need time to multiply, differentiate, become interleukin secreting cells and secrete IL4 to stimulate B cells to multiply then differentiate to plasmocytes and start secreting antibodies. The secondary immune response has already memory B cells that multiply and differentiate.
- **Amplified:** doc b shows that the no. of effector cells in the secondary immune response is greater than that in the primary IR.
- **Long lasting:** the number of memory cell in the secondary IR is greater than that in the primary IR.

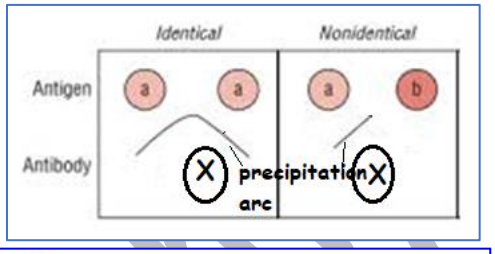
Serological tests

Tests where antibodies are used in the diagnosis of diseases due to their specificity. In these tests, we look for specific antibodies in the serum of the patient.

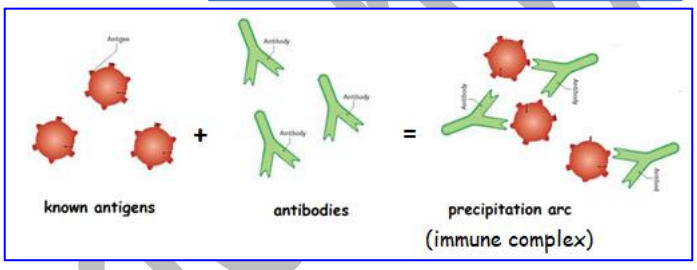
Agglutination reactions: each antibody has two antigen binding sites. When antibodies react with antigens expressed on cell surfaces, they form molecular bridges between the cells causing their agglutination. The aggregates can be seen with the naked eye.



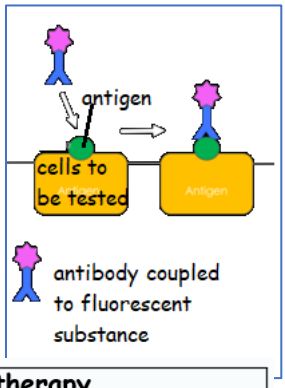
Immunodiffusion in gel: in agar gel, antibodies and antigens are placed in hollowed wells. They can migrate in all directions. The contact between an antibody and its specific antigen constitutes an immune complex that can be observed in the form of a gray arc.



Schematize the mechanism that leads to the formation of the precipitation arc.



Immunofluorescence: is used to detect cellular antigens. **The** cells to be tested are fixed on a glass slide.
Add specific antibodies coupled to a fluorescent substance. The antibody binds to the cells only in the presence of the antigen.
Washing **then** Submit the cells to ultraviolet light beams and observe using fluorescent microscope.
If the medium fluoresces then the antigen is found and the individual is infected.
If the medium doesn't fluoresce, the antigen isn't found and the individual in not infected.



Differences between vaccination and serotherapy

Therapy	Vaccination	Serotherapy
Substance injected	Attenuated toxin or microbes	Prepared antibodies
Origin of antibodies produced	Endogenous, produced by the body	Exogenous, prepared outside the body
Type of immunity	Active	Passive
Latency or delay	1-2 weeks	Few hours
Duration of protection	Several years	2 weeks
Objective	Preventive	Curative
Immunological memory	Yes	No

Non-specific immune response and Specific immune response differences

Immune response	NSIR	SIR	
		HUMORAL SIR	CELL-MEDIATED SIR
Effector	Gran, macro and Mast cells	BL	Tc
Membrane receptor	None	BCR, antibody	TCR
Targeted pathogen	Bacteria virus	- Soluble antigen - Cellular antigen	- Cancer cell - Allograft - Intra-cellular microbes
Mechanism	Phagocytosis	neutralization	cyto-toxicity
Passive transfer	No	serum	blood cells
Latency or delay	Few hours	15 days	15 days
Memory	No	yes	yes
Efficiency	Average	excellent	excellent

Phases of the specific immune response

- Induced in the secondary lymphoid organs closest to the infected site.
- Involves: APC, B and T lymphocytes
- It occurs in 3 phases.

1- The induction phase (antigen presentation to TH by APC)

- Macrophages digest non-self-proteins.
- Non-self-peptides are associated to HLA II and transported to the cell surface.
- The macrophage migrates to the nearest lymph node to the inflammation site.
- The macrophage becomes an APC that activates specific TH.

2- Activation phase (proliferation and differentiation of activated lymphocytes)

- An activated TH proliferates to give a clone.
- Some cells of the clone remain undifferentiated and become memory cells.
- Others differentiate into interleukin secreting cells. (interleukin is a type of cytokines)
- If TH secretes IL-2, Tc is activated (cell-mediated SIR)
- If TH secretes IL-4, BL is activated (Humoral SIR)

3- Effector phase (humoral or cell-mediated) (How Tc and BL eliminate the intruder)

A- Specific humoral immune response (agents: antibodies/B lymphocytes)

- Only B cells that recognize the specific epitopes of the antigen are activated and proliferate in the presence of IL-4, giving a clone.
- Some cells of the clone remain undifferentiated and become memory cells.
- Others differentiate into plasma cells (plasmocytes) that secrete circulating antibodies.
- These antibodies neutralize soluble toxins or antigens on surface of cells by neutralizing interacting with its active site.
- Opsonization or complement cascade.

B- Specific cell-mediated immune response (agents: Tc lymphocytes)

- Only T cells that recognize the complex HLA I – antigenic peptides are activated and proliferate in the presence of IL-2, giving a clone.
- Some cells of the clone remain undifferentiated and become memory cells.
- Others differentiate into effector cytotoxic cells.
- Cell-mediated is essential in fighting intra-cellular pathogens.
- When a Tc cell adheres to the target cell, it first releases perforin that form channels in the target cell membrane. Then granzymes enter the infected cell through this channel and causes DNA degradation. (apoptosis)