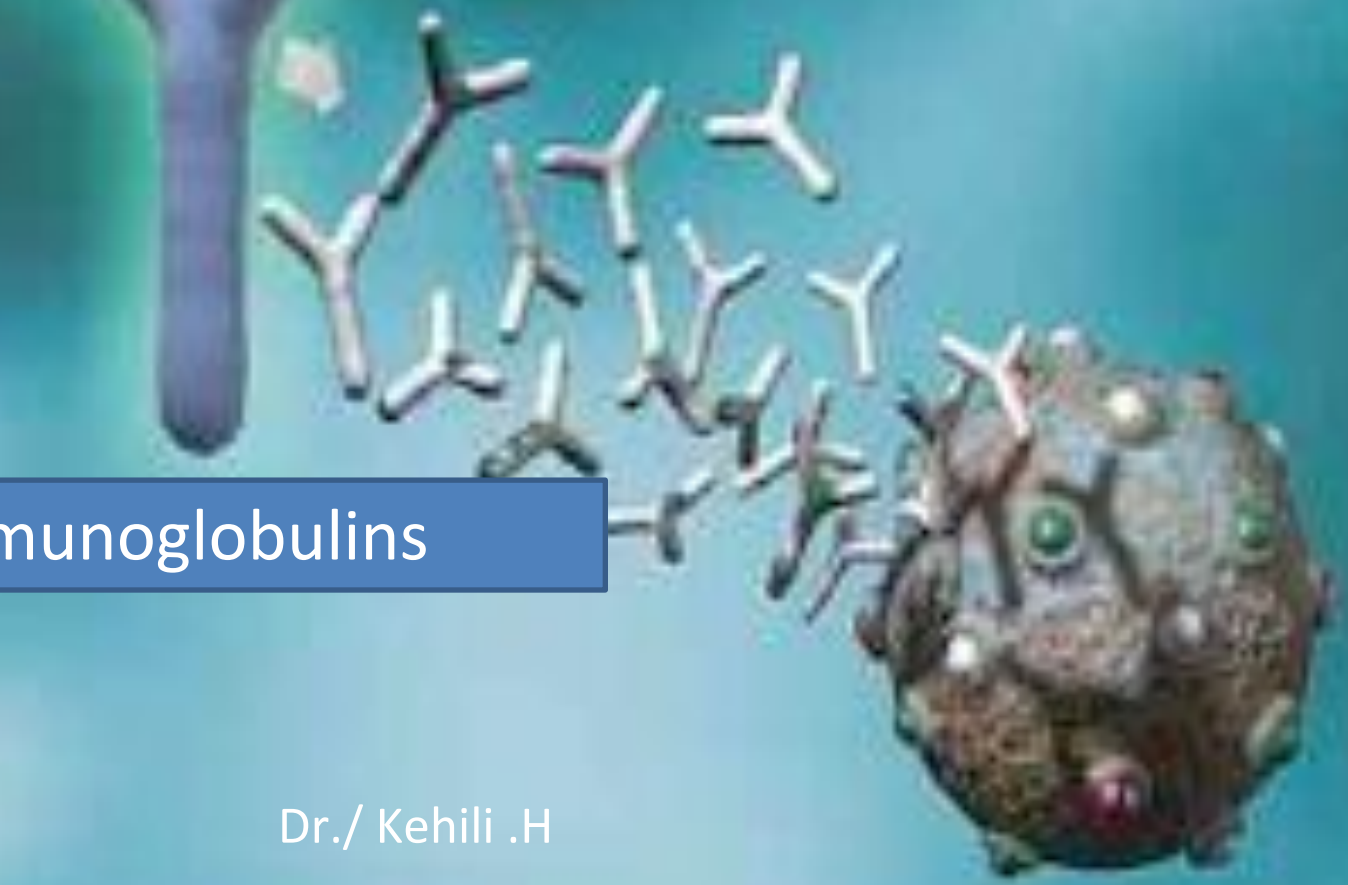
The People's Democratic Republic of Algeria Ministry of Higher Education and Scientific Research
Mila University Center.
Institute of Natural and Life Sciences
Department of Natural and Life Sciences



Immunoglobulins

Dr./ Kehili .H

Introduction:

Antigen recognition is ensured by specific molecules:

→ **Located on the surface of T and B lymphocytes = membrane antigen receptors.**

→ **Soluble = immunoglobulins**

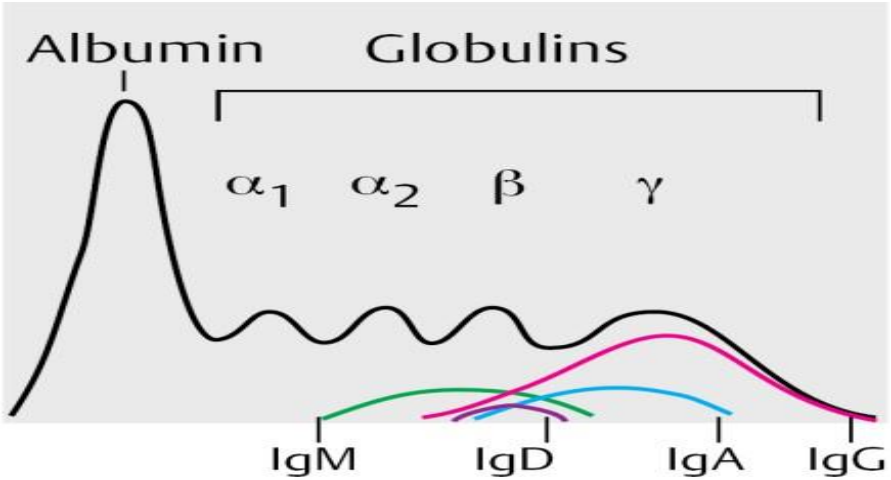
Introduction:

- Family of globular proteins: "globulins".
- Widely represented in vertebrate sera and body fluids.
- Produced by plasma cells derived from B lymphocytes after antigenic stimulation.
- Present on the surface of B lymphocytes for which they are the specific receptors for Ag.
- Humoral specific immunity effectors.

Classification of immunoglobulins:

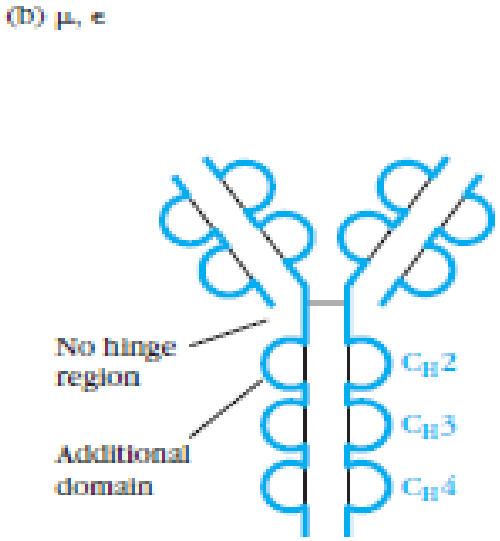
- More than 10^8 varieties of antibodies can be synthesized in response to the many antigenic solicitations to which each individual is subjected.
- Classification according to the international nomenclature recognized by the **WHO** in **5 classes** thanks to the different methods of exploration of plasma proteins:

- IgG
- IgA
- IgM
- IgD
- IgE



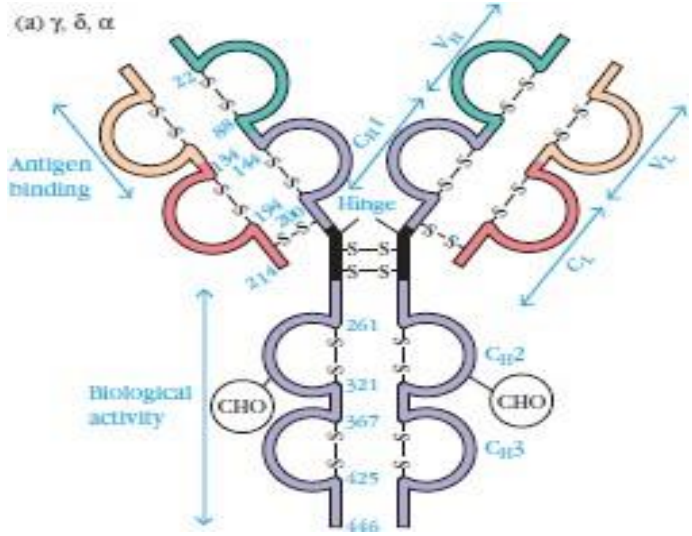
A. Protein electrophoresis

Immunoglobulin structure:



The heavy and light chains contain intracatenary disulfide bridges, each bridge allowing the formation of a peptide loop that represents the central part of a functional region of about 100 aa called the **DOMAIN.**

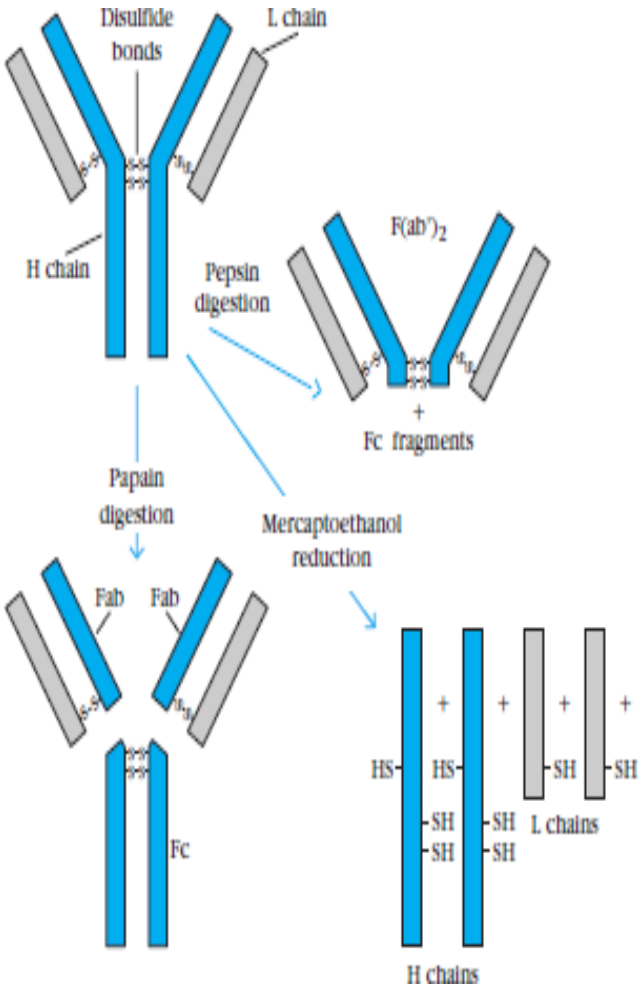
Ig have **4** or **5** domains per **heavy chain H** (a variable domain **VH** and 3 or 4 constant domains **CH**) **2** domains per **Light** chain L (one **VL** and one **CL**)



On heavy chains there is a relatively linear sequence called: **hinge region**), this region is the target of proteolytic enzymes and allows the Ig molecule some flexibility

IgM and IgE are devoid of hinge region and have additional domain

Immunoglobulin fragmentation:



Action of papain

Papain cuts the IgG molecule at the hinge region into three fragments:

2 identical **Fab "Fragment antigen binding"** fragments of MW = 45,000, corresponding to the N terminal half of a heavy chain and to the entirety of a light chain.

1 " **crystallisable fragment" Fc** fragment that corresponds to all of the remaining two halves of the heavy chains responsible for the effector biological properties of each Ig class.

Action of pepsin

A brief digestion with pepsin gives a single fragment with a MW of 100,000 and composed of two fragments similar to Fab and designated **F(ab')2.**

The Fc fragment, on the other hand, is digested into multiple **fc'fragments.**

Immunoglobulin heterogeneity

1. Isotyping:

- Isotypic characters are **common to all individuals of the same species** and define immunoglobulin **classes** and **subclasses** as well as light chain types and subtypes.

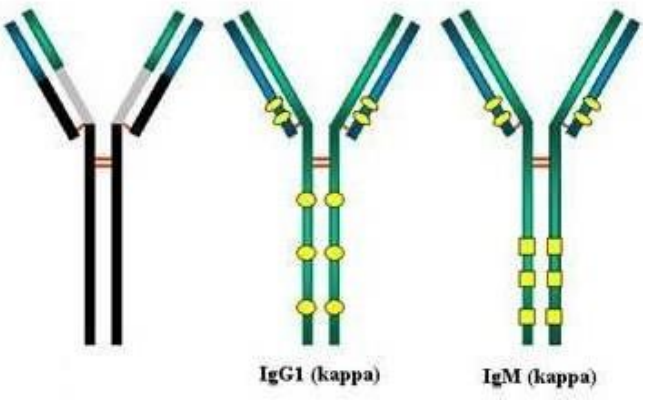
- The isotypic determinants are carried by the **constant** domains of the **heavy** and **light**chains. There are:

  9 different isotypes for heavy chains to distinguish: Ig classes: IgG, IgA,

  - **5** IgM, IgE, IgD including:
  - **4** igG subclasses/classes: IgG1, IgG2, IgG3,
  - **2** IgG4. IgA subclasses/ classes: IgA1, IgA2.

  5 different Isotypes for light chains to distinguish between **Kappa** and

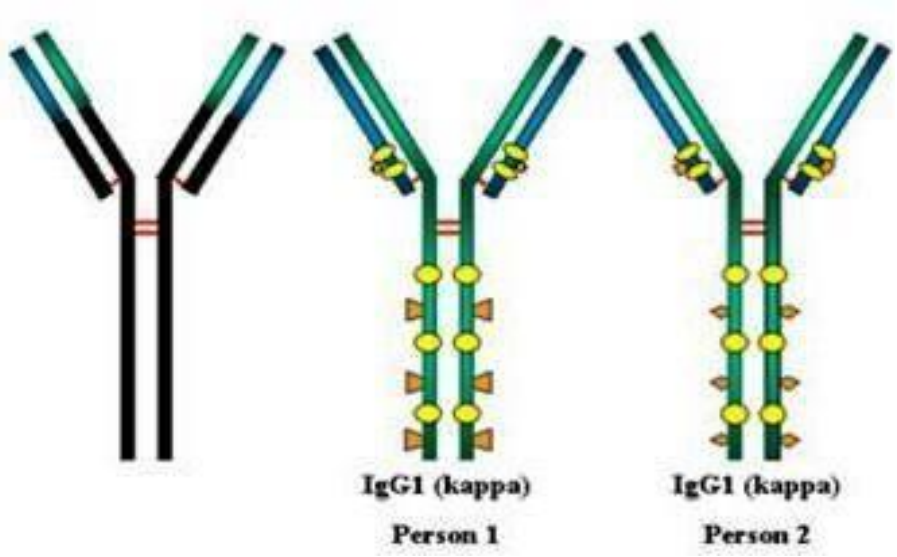
  - **2 Lambda** light chain types including:
  - **4** lambda chain subtypes.



2. ALLOTYPY

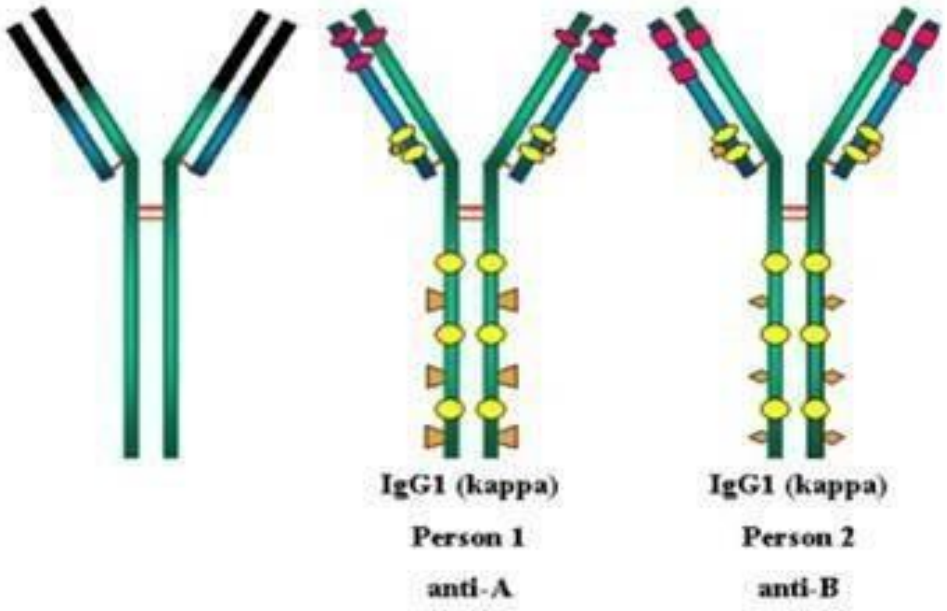
- allotypic specificities, are antigenic determinants that make it possible to **distinguish Ig from two individuals or groups of individuals within the same species.**

- Allotypic determinants are present at the level of **certain regions** on the **constant domains** of **γ-chains, α-chains** and **κ-chains.**



3. IDIOTYPY:

- Idiotypic specificities are antigenic determinants that characterize **a given antibody in a Individual**
- They are carried by the **variable domains** of Ig.



VI. IMMUNOGLOBULIN ONTOGENY

The mammalian fetus has, from the third month of life in utero, a thymus, a lymph node and circulating lymphocytes.

Studies show indisputably cells containing either IgM (incidentally IgG) from the 20th week of fetal life.

The proof of the immune possibilities of the fetus is provided by the qualitative estimation of Ig of cord blood. Following intrauterine infections (congenital rubella, congenital toxoplasmosis, etc.), specific IgM antibodies can be found in the cord blood.

Such a finding signals the immune response of the fetus and makes it possible to distinguish it from the simple passive transmission of maternal antibodies since IgM does not cross the placenta.

1. Ab's production in the fetus

- While it is possible to gather a body of evidence of IgG and IgM production during fetal life, it is not the same for IgAs that are found only at very low concentrations in the good neonatal condition at birth

- IgD and IgE are detectable in trace amounts in cord blood and can only be of origin in fetus since they do not cross the placenta.

- The fetus passively acquires maternal IgG at rates approaching those of the mother at birth.

2. Ab's production after birth

The evolution of the level of Ig synthesized by the child during the first months of life is influenced by:

- The level of maternal antibodies acquired by trans placental transmission;
- The importance of antigenic stimulation of both the saprophytic flora and the pathogenic flora that it is called upon to encounter.

IgG:

The birth rate is equal to or sometimes higher than that of the mother. The rapid decrease in maternal IgG or during the first trimester explains the hypo-gamma-globulinemia observed physiologically around 2 to 3 months.

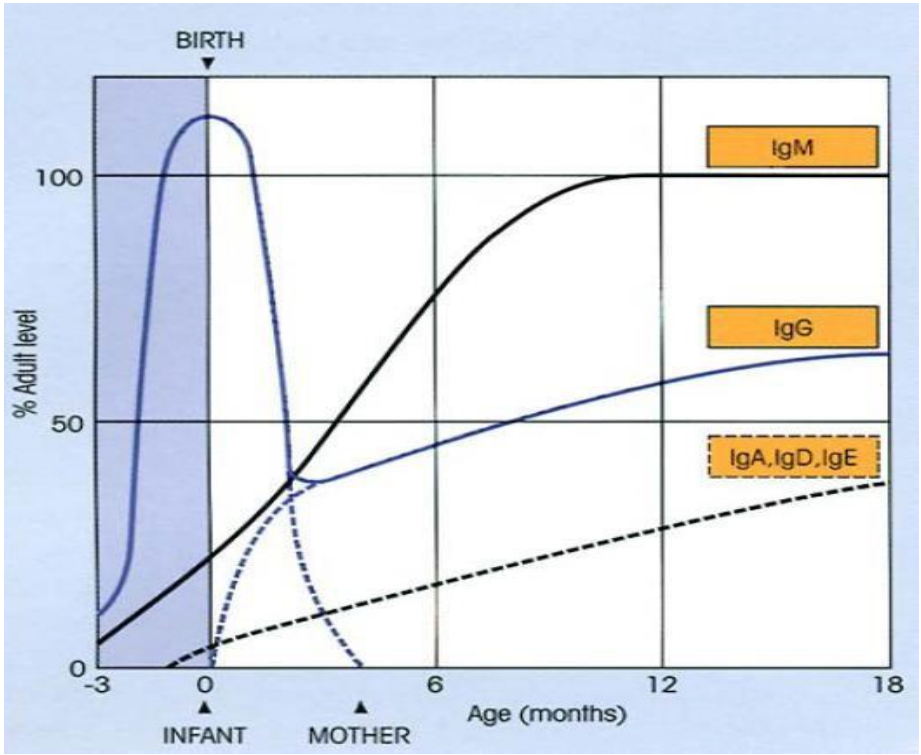
As for the IgG of the child, their level will increase to reach that of the adult after the age of **two years.**

IgM:

The rate has been steadily increasing since birth to reach that of the adult after the age of **one year.**

IgA, IgD, IgE:

They develop more slowly than the previous ones and do not reach adult values until around the tenth year



VII. SYNTHESIS OF IMMUNOGLOBULINS

Immunoglobulin biosynthesis:

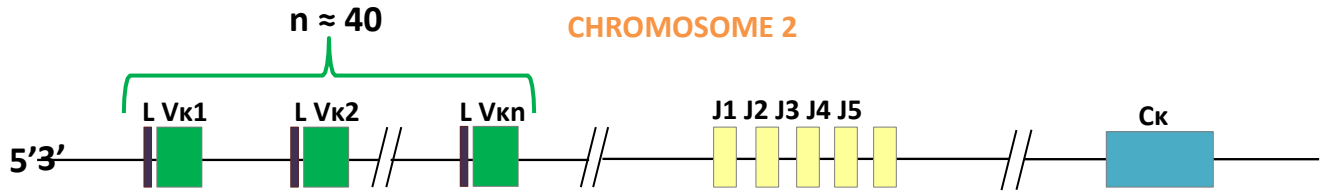
1. GENETIC BASES

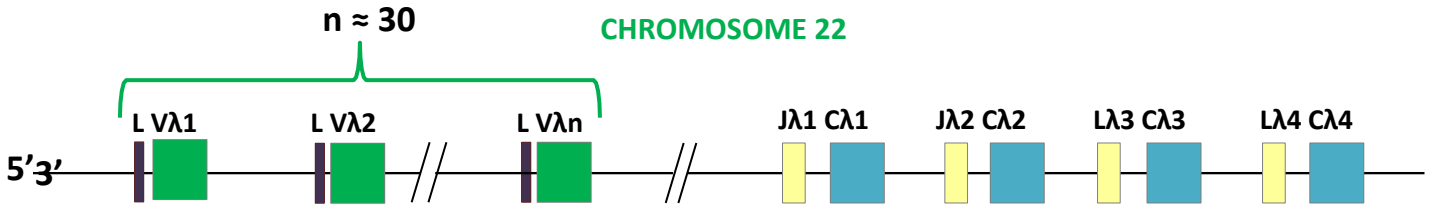
It is estimated that the mammalian immune system can generate more than **10^{10}** different antibodies.

This enormous diversity of Ig structure must necessarily derive from a genetic system capable of creating this innumerable diversity.

The light and heavy chains are encoded by **three distinct multigenic families** located on different chromosomes:

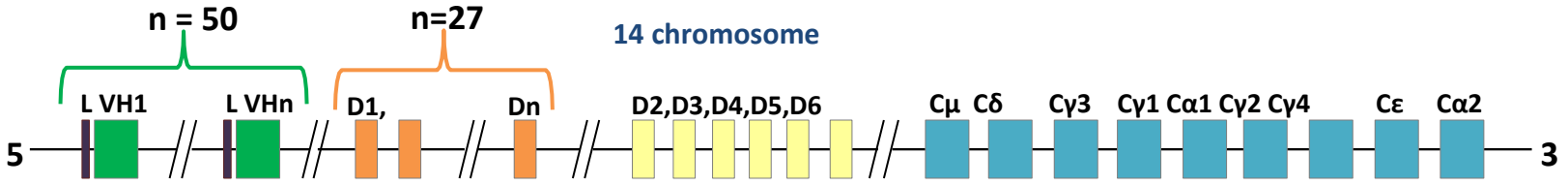
- λ strings → **chromosomes 22**
- chains κ → **chromosomes 2**
- heavy chains → **chromosomes 14**





The multigenic families of **κ and λ light chains** contain 3 separate groups of exons: **V**, **J** (for junction) and **C**

[V and J: code for the variable part, C: code for the constant part]



The multigenic family of **heavy chains** contains 4 separate groups of exons called:
V, **D** (for diversity), **J** and **C** **[V,D and J code for the variable part, C code for the constant part]**

Multigenic organization of Ig genes

The functional genes of light chains are created by the random rearrangement of the gene segments of the Germ line DNA occurring during B-cell maturation.

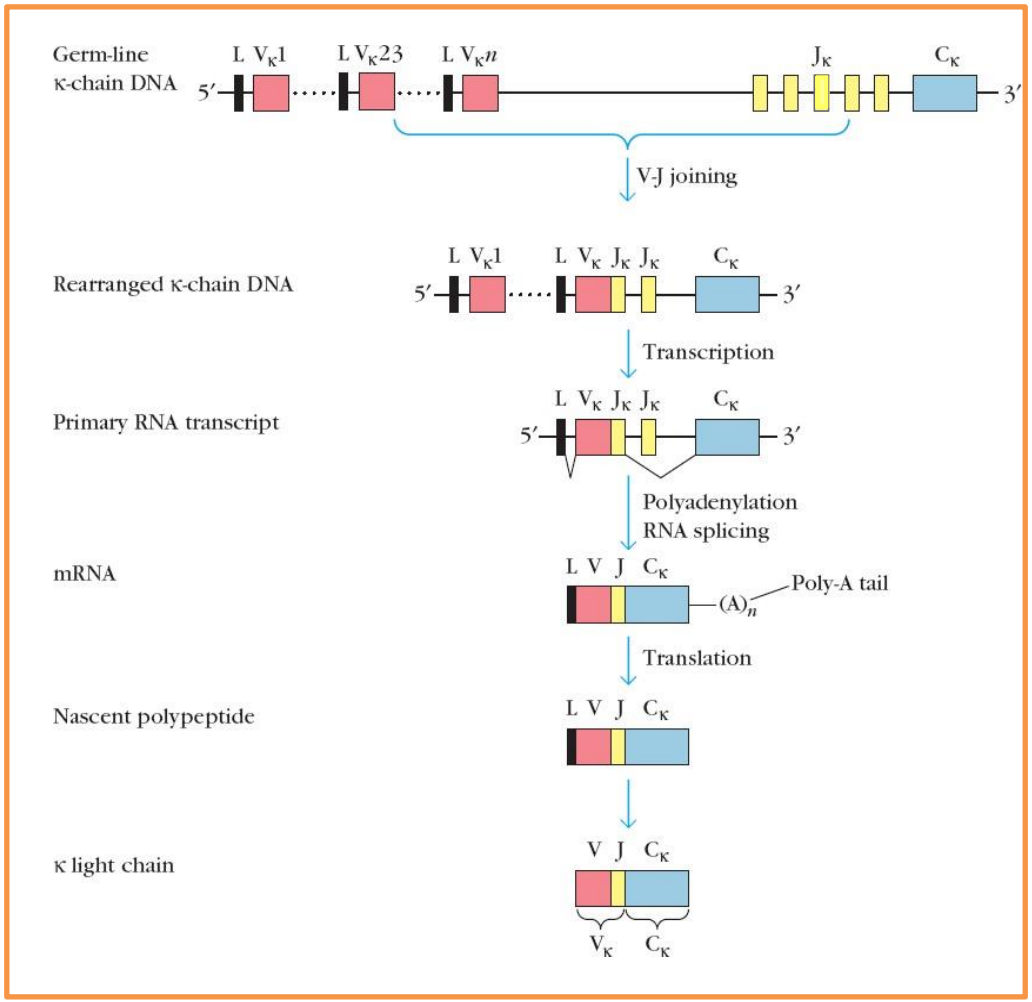
During this rearrangement, **one of the V exons attaches to one of the J exons** forming a **V-J combination**.

This functional gene is transcribed into primary RNA

This primary RNA gives rise to a messenger RNA after excision and splicing of the introns.

The mRNA is subsequently translated into a light chain

Case of light chains:



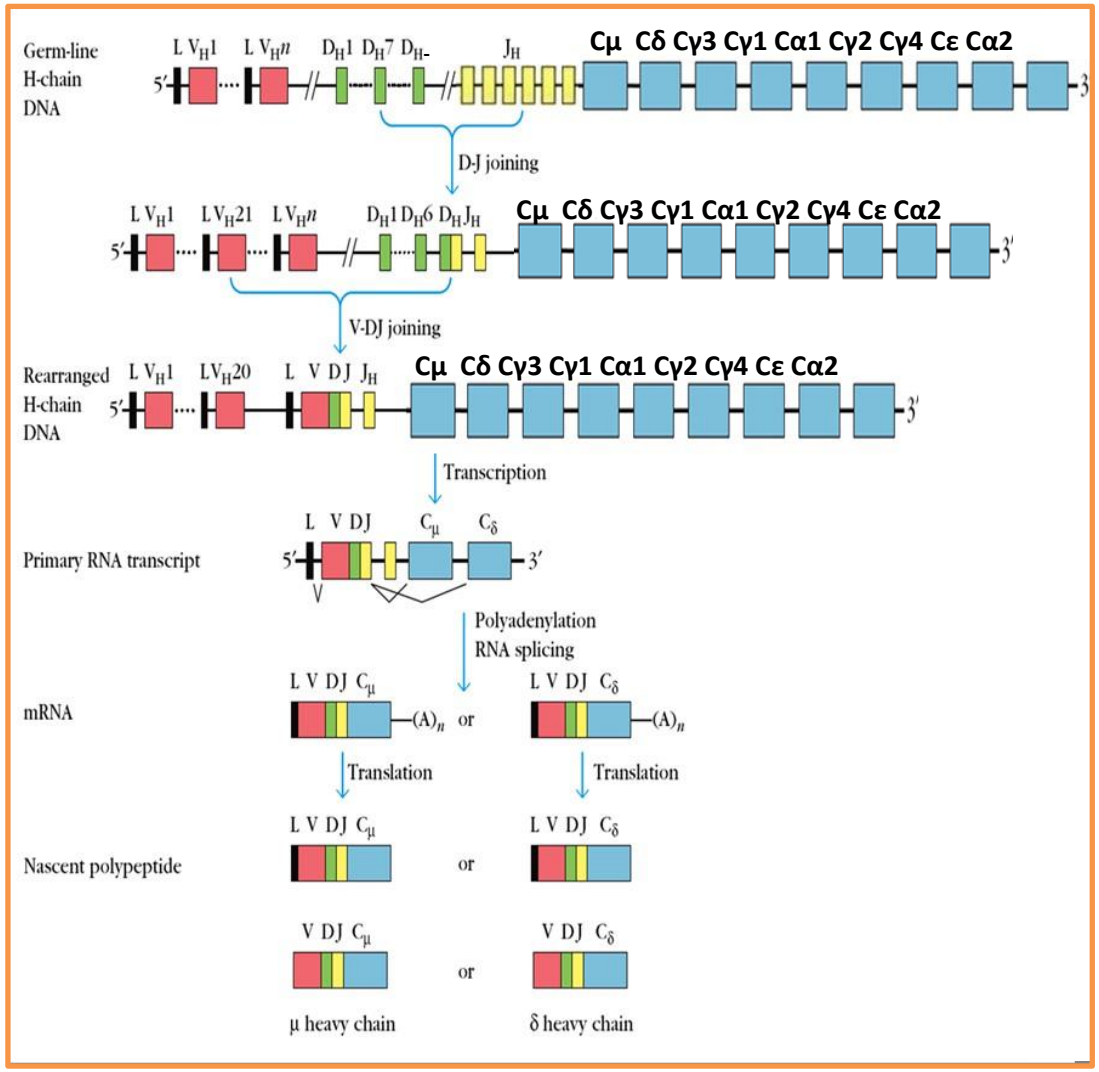
In the case of heavy chains, the creation of a functional gene requires **two successive rearrangements:**

a. **An exon D** randomly joins one of the **exons J** forming a **combination D-J.**

b. **The D-J segment joins one of the V exons to create a functional gene.**

An mRNA includes either the **Cμ** transcript or the **Cδ** transcript to then be translated into either a **μ** heavy chain or a **δ** heavy chain**.**

Case of heavy chains:



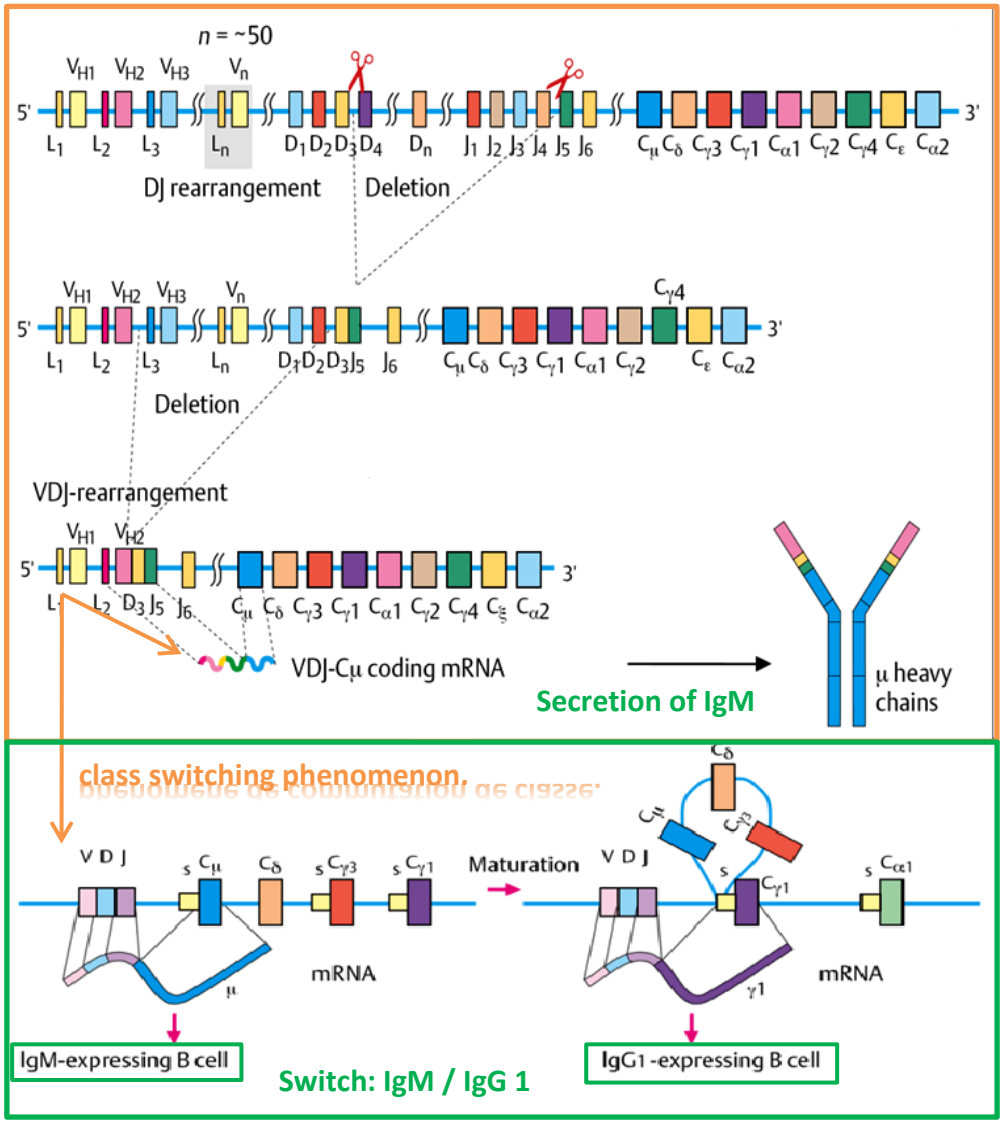
During LB maturation (outside of any antigenic stimulation):
Rearrangements of Ig genes (heavy and light chains) occur, resulting in functional genes encoding **surface Ig** (IgD and IgM)

After antigenic stimulation, the mature B lymphocyte undergoes differentiation into a **plasma cell that secretes antibodies.** There are two possibilities:

- IgM secretion.
- IgG, IgA or IgE secretion: This is **the class switching phenomenon (or switch),**

During this process, the heavy chain DNA undergoes a rearrangement additional time during which **the unit V-D-J** may be associated with one of the CH exons. This results in switching:

- **IgM/IgG,**
- **IgM/IgA,**
- **IgM/IgE.**



At cellular level

Reverse pinocytosis secretion

↑

Glycosylation

↑

Assembly

↑

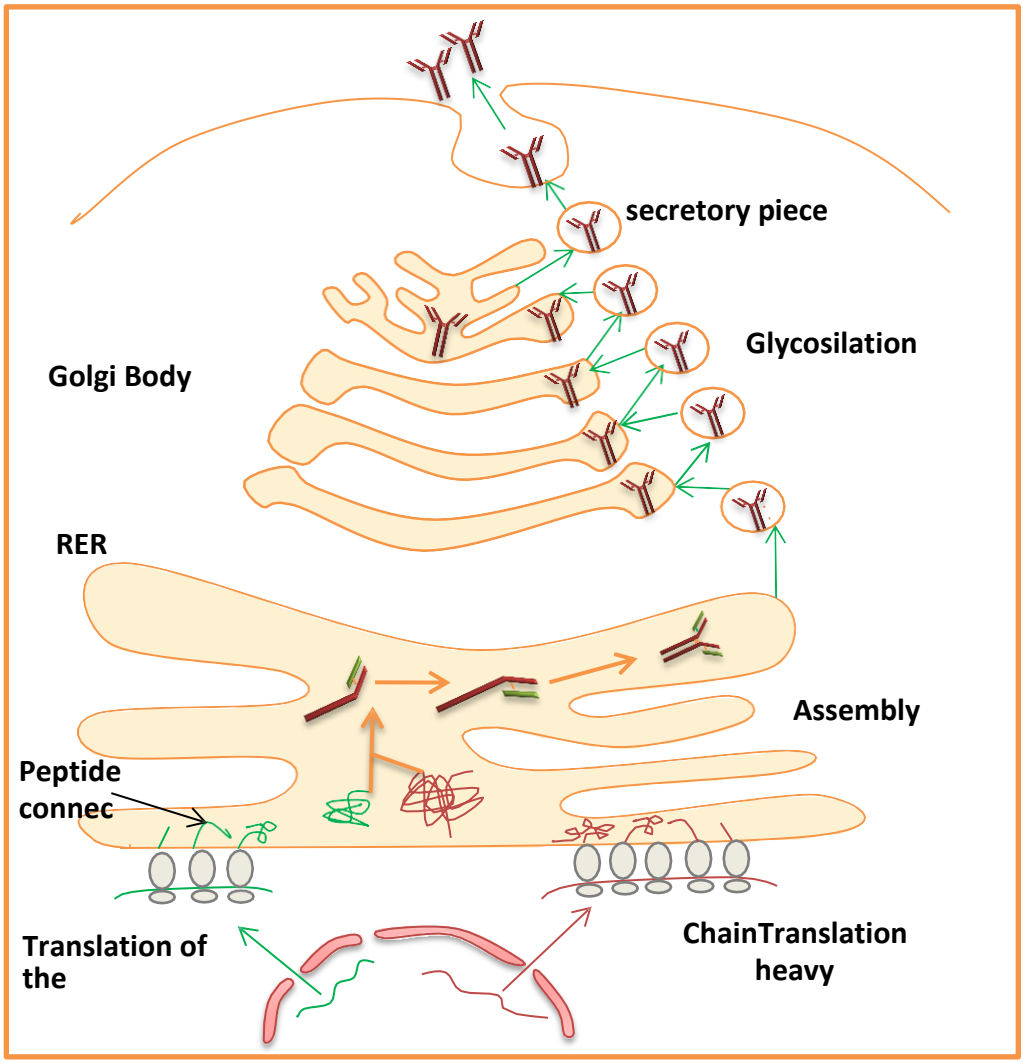
Excision of the signal

↑

Translation of mRNAs, H and L chains in the process of synthesis enter the RER thanks to the peptide signal rich in hydrophobic amino acids.

↑

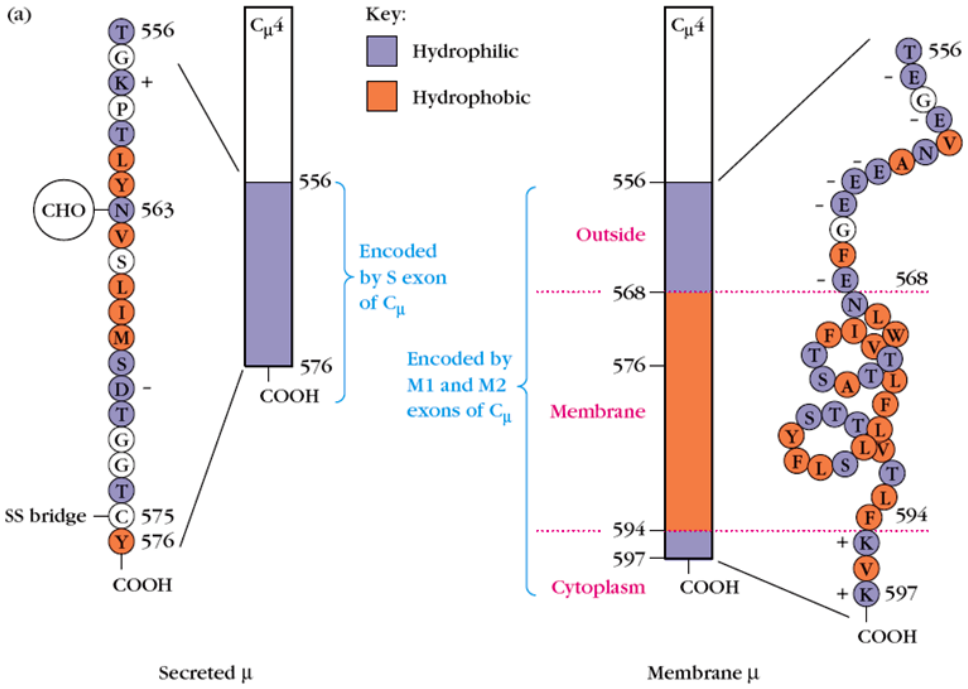
The mRNAs (H+L) leave the nucleus, bind to the Polyribosome



Particular case of membrane Ig

The membrane Ig produced by a cell, which serves as its antigen receptor, is identical to Ig secreted by this same cell with the exception of an amino acid sequence in the C-terminal part of heavy chains.

The Ig of the membrane is therefore longer than its secreted counterpart, the additional amino acids serving to anchor the molecule to the cell membrane.



VIII. PRODUCTION OF acres AFTER ANTIGENIC STIMULATION: Primary Response/Secondary Response

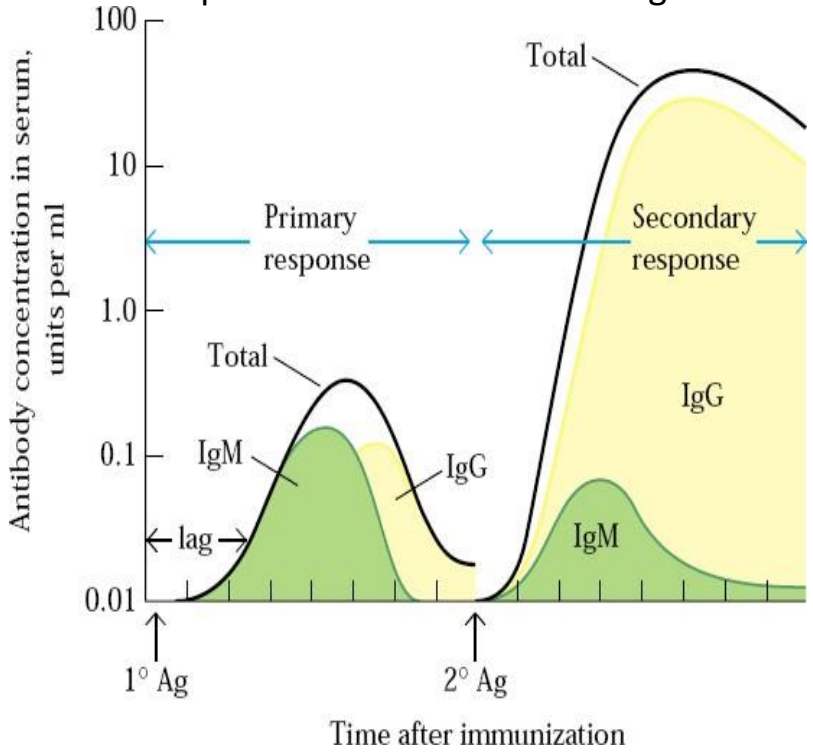
The introduction of a given Ag gives rise to two types of responses:

Primary response: Occurring on first contact with the antigen.

- **Secondary Answer:** Coming after a second contact, or in subsequent contacts with the antigen.

These two types of response are distinguished by:

- The isotype of the Ac products;
- The amount of antibodies produced;
- The time taken for ACs to appear;
- The affinity of the Ac products.



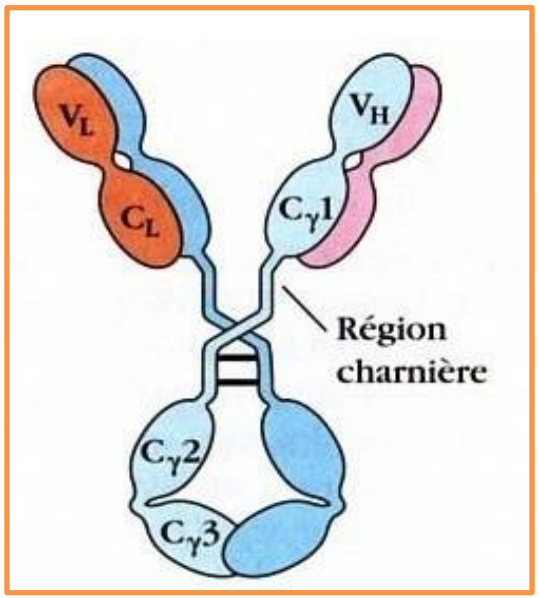
Primary and secondary humoral response

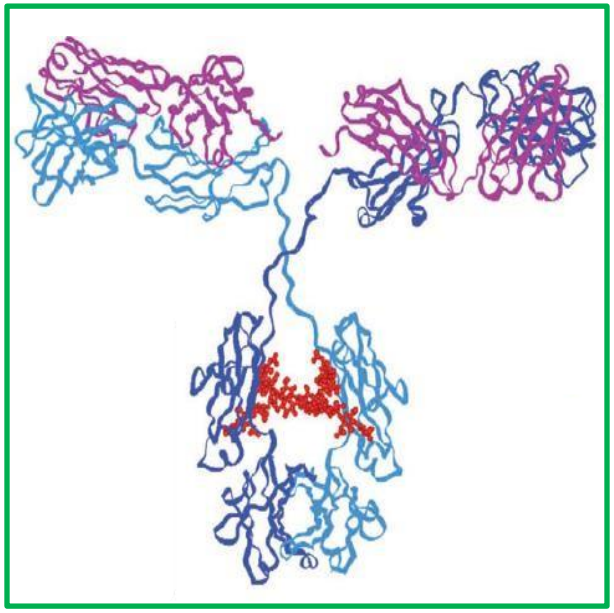
	Primary Response	Secondary Response
Response time	5 to 10	1 to 3 days
amplitude of response	Low	100-1000 times stronger than the primary response
The isotype of the Ac products;	IgM > IgG	Prevalence of IgG under certain conditions: IgA, IgE
The affinity of the Ac products.	Low	High
Nature of Inducing Ag	Ag T dependent and Ag T independent	Ag T independent
Type of immunization required	High dose of Ag, optimally with adjuvants	Low dose Ag, no adjuvant needed
LB responders enabled	and all naive people.	Thesis

Characteristics of the different classes of immunoglobulins:

IMMUNOGLOBULINS G (IgG)

- Represent **about 75-85%** , human serum Ig.
- Serum concentration between **8-12 g/l.**
- Monomeric molecules formed by the combination of 2 chains heavy γ (containing 4 domains) and 2 light chains κ or λ.

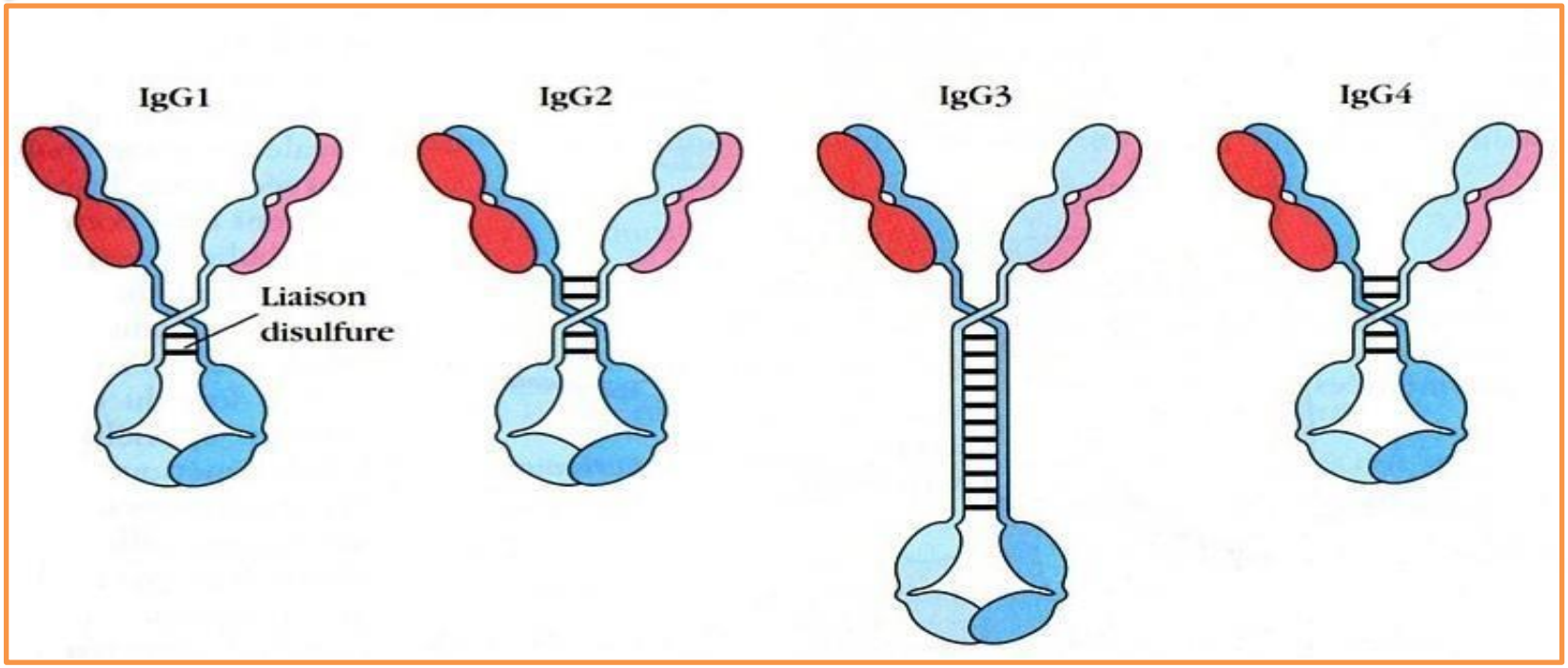




IgG subclasses

4 subclasses called **IgG1, IgG2, IgG3, IgG4** distinguished from each other by:

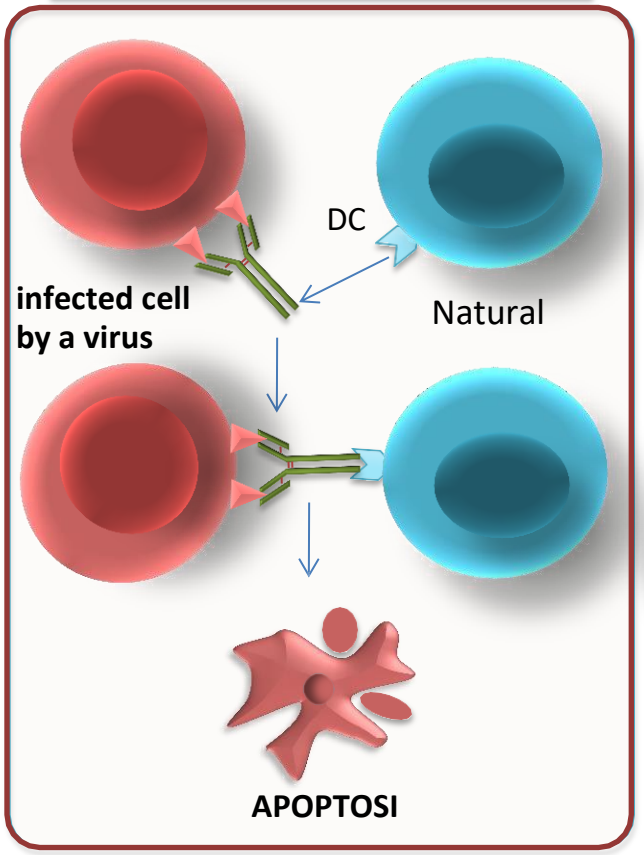
- Distinct sub-class antigenic determinants located in the constant part of the heavy chains (Fc fragment).
- The number of heavy interchain disulfide bridges located in the hinge region.



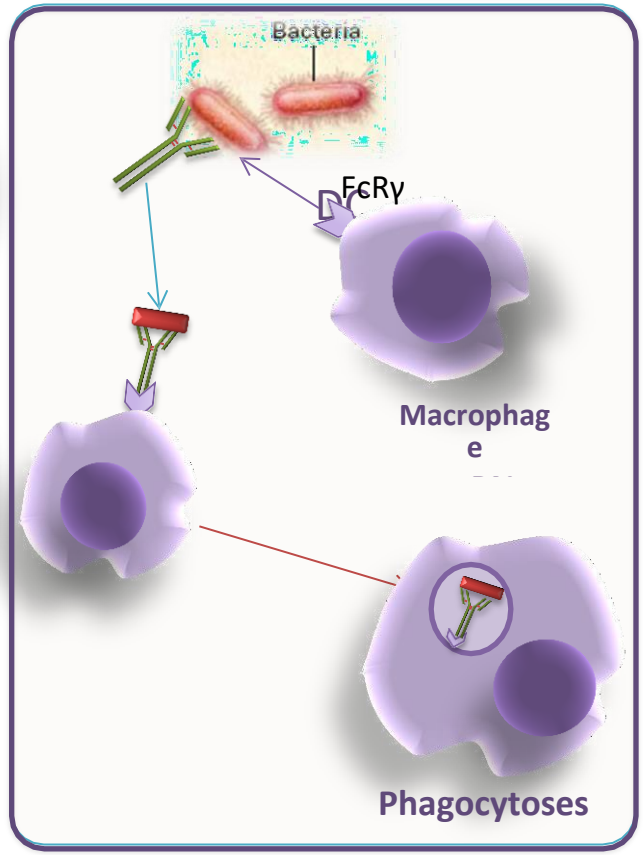
	IgG subclass1	IgG subclass 2	IgG subclass 3	IgG subclass 4
Distribution	**70%**	**(18)**	**8.**	**4%**
½ life (D)	**23**	**23**	**8**	**2**

Some biological functions

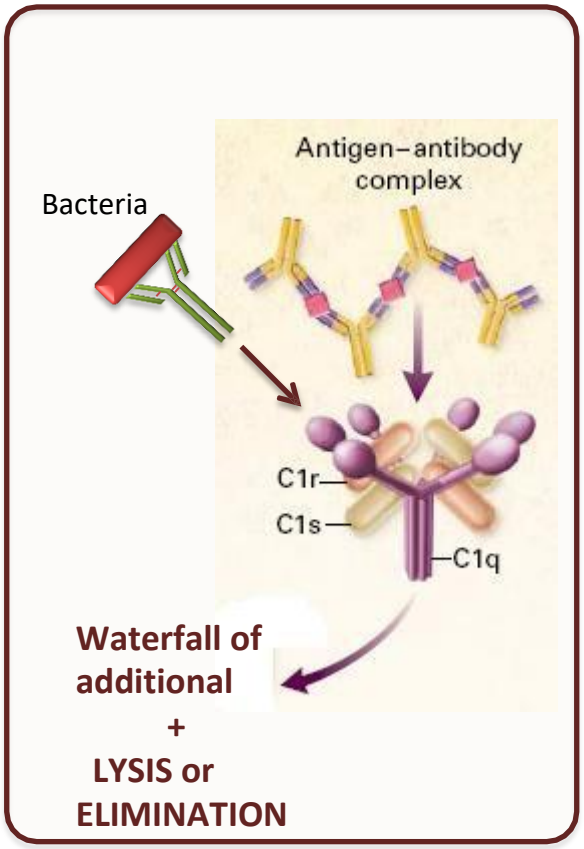
ADCC



Antibody opsonization



Activation of the complement by the classical pathway



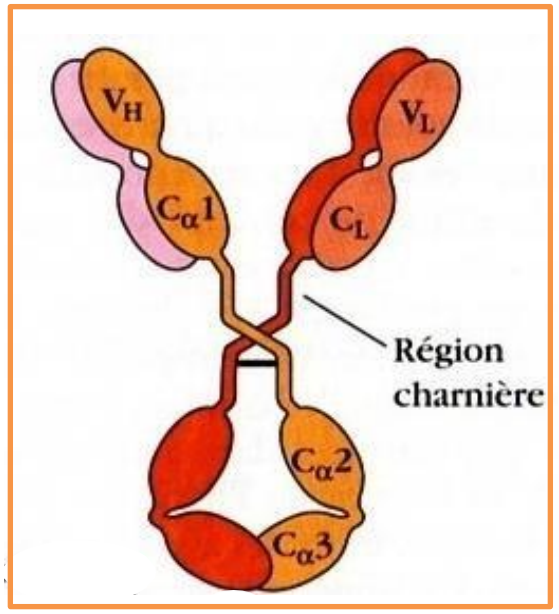
Some biological functions

Placental transfer

IgGs are the only Igs that can cross the placental barrier thanks to a placental crossing site located on the CH2 and CH3 domains of the gamma chain.

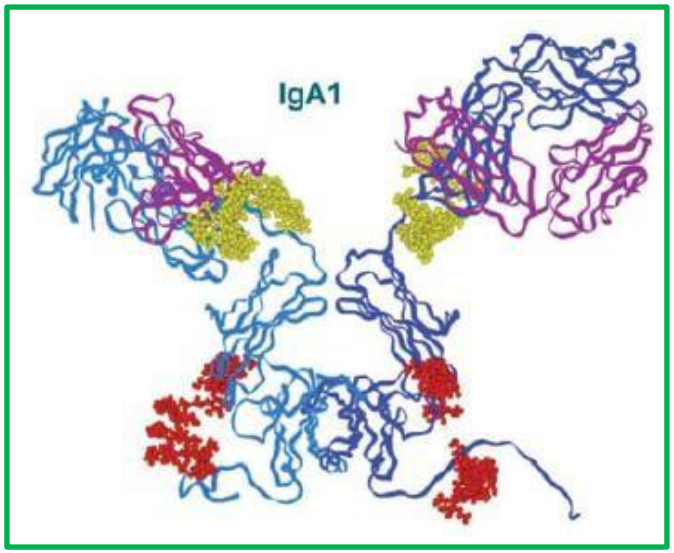
IgG1, IgG3 and IgG4 pass the placental barrier easily and play an important role in protecting the fetus during its development.

IMMUNOGLOBULIN A (IgA)



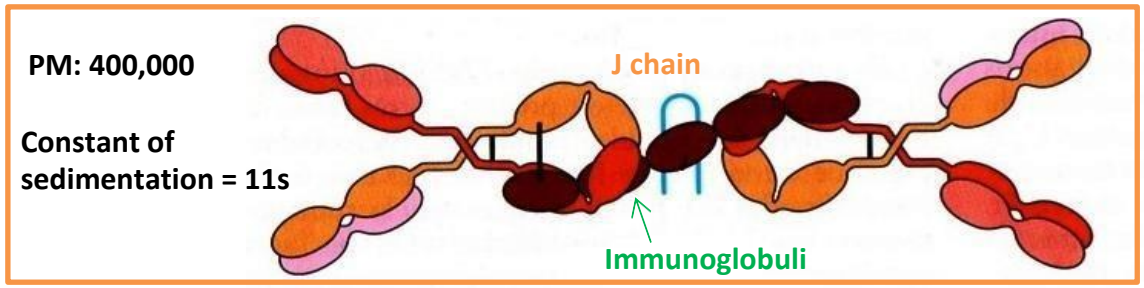
- Represent about **15%** of circulating Ig. Serum
- concentration of **2 to 4 g/l**.

  Relatively low proportion due to rapid catabolism **(½ life = 6D).**
  the daily amount of IgA synthesized **(60 mg/kg/day) >** IgG (30mg) **>** IgM (8mg), which makes this protein a **"major Ig".**
- Monomers built on the model of IgG molecules: two light chains (κ or λ), attached to two heavy chains **α**.
- Two subclasses: **IgA 1** (80%) and **IgA2** (20%), which differ from each other in the structure of their α chains.
- They tend to polymerize forming disulfide bridges between α-chain cysteine residues.



Secretory IgAs (IgAs)

In humans, most of the Ig present in saliva, tears, colostrum, milk, bile, nasal, bronchial and gastrointestinal secretions.



- **The J chain (Joining)** : Junction glycoprotein of MW = 16,000,synthesized by the Ig-producing cells and binds to them just before excretion.

- **The secretory component:** Synthesized independently of the molecules of IgA by the epithelial cells of the mucosal and glandular surfaces.

The secretory component masks sites susceptible to protease cleavage in the region hinge of IgA allowing it to exist in the protease-rich mucosal environment.

Some biological functions

IgA:

Do not fix the complement by the conventional route; Do not cross the placental barrier.

Serum IgA:

A wide variety of specificities have been found for serum IgA (antibacterial, antiviral, etc.) but this class never represents the essential fraction corresponding to a precise antigen.

IgAs:

Immune barrier role:

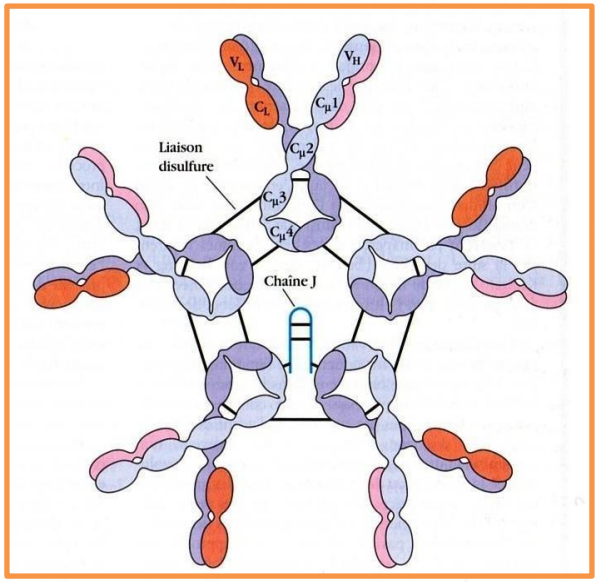
- Decrease the adhesion of bacteria **(salmonella, vibrio cholerae, neisseria gonorrhoeae ...)** to the mucous membranes, facilitating their mixing with the mucus and therefore their elimination.
- Neutralize viruses **(polio virus)**, preventing their attachment to target cells.
- By preventing the absorption of non-degraded food immunogens (milk proteins, beef proteins, etc.) through the gastrointestinal tract and which may cause type **I** or type **III hypersensitivity reactions.**

Regulation of bacterial flora:

Bacteriostatic action in synergy with lactoferin; May increase the bacteriolytic action of lysozyme.

IMMUNOGLOBULIN M (IgM)

5-10% of all Ig. Mean serum concentration = **2g/l**. Mean half-life = **5 d**



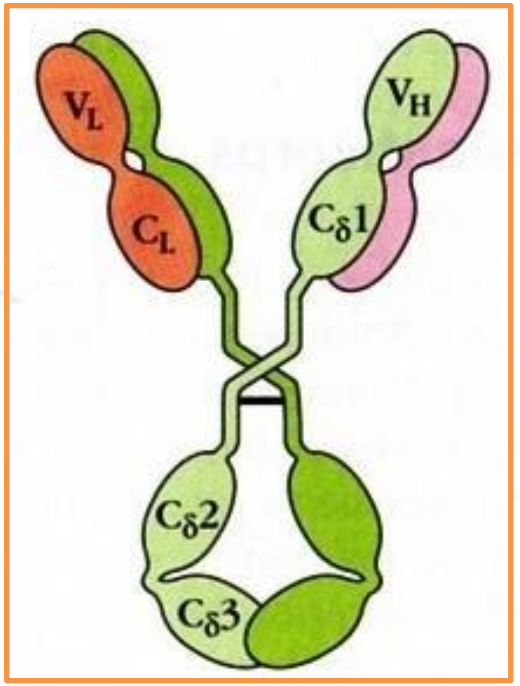
- Exist in serum, in the form of pentamer whose basic unit is constituted, on the IgG model, by two light chains (κ or λ) and by two heavy chains **μ** which comprise **5 domains:** CL ,CH1, CH2, CH3 and CH4.
- The 5 monomers are linked together by disulfide bridges and by J chains, similar to those found in IgAs.
- This particular architecture gives the molecule a characteristic star structure, with two Fab fragments at the end of each of the 5 branches.
- The number of active sites varies between 5 and 10 depending on the size of the complementary antigenic determinant.

Some biological functions

- Appear early in fetal life.
- IgM is the first antibody to be synthesized during a humoral immune response.
- Mainly confined to the intravascular compartment (Do not diffuse well due to their large size) Particularly active in the
- following processes:
  - **So-called natural antibodies** such as **anti-A** and **anti-B** intravascular iso-agglutinins of the blood groups;
  - **Immune antibodies** (Gram-negative bacteria);
  - **Auto-antibodies (rheumatoid factor, cold agglutinins)**.
- Multivalent macromolecules constituting an edifice perfectly adapted to the capture of large antigens, Do not cross the placental barrier.
- Play an important ancillary role as a secretory immunoglobulin.

IMMUNOGLOBULIN D (IgD)

- Discovered par **ROWE** & **FAHEY** en **1965.**
- IgD have the same general structure as IgG, with heavy chains δ consisting of 4 domains and featuring a very long hinge region of about 50 amino acids.
- Low serum levels (25 to 40 mg/l), less than 1% of serum Ig.



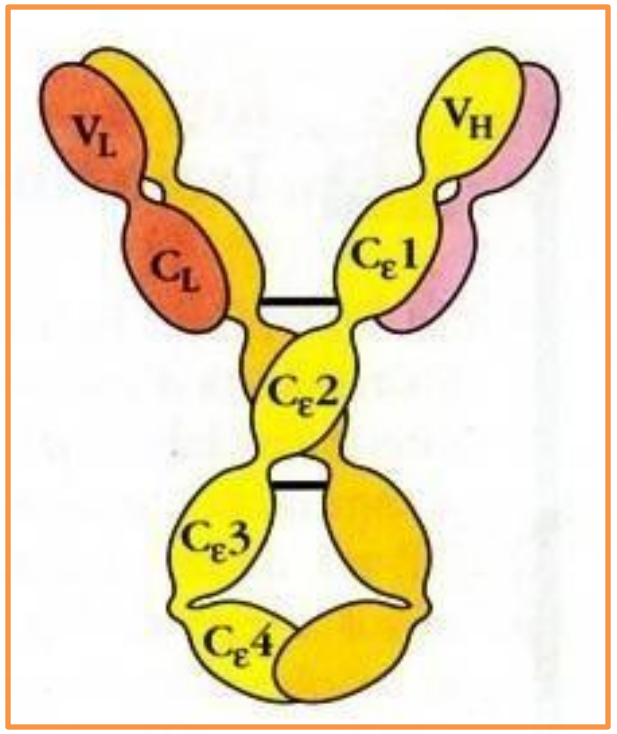
Some biological functions

- IgD is present on the surface of B lymphocytes in peripheral blood. They constitute, at this level, (with surface IgM) the specific receptors by which these cells recognize antigens.

- In addition, they seem to play a facilitating role in pregnancy during which high serum levels are found.

IMMUNOGLOBULIN E (IgE)

- Last Ig class to be discovered **(ISHIZAKA-1966)**
- Constituted, like other Ig by two light chains (κ or λ) , and by two heavy chains **ε**.
- The ε chains have, like the μ chains, **five domains** of which a variable.
- Very low concentration **(3 mg/l on average in adults).**
- Very short half-life: **2 to 4 days**



Some biological functions

- Do not fix the complement by the conventional route.
- Do not cross the placental barrier.
- The most important biological property of IgE is its ability to attach to tissues of the same species. They are said to be **homocytotropic.** This particularity explains:

The role of IgE in allergic manifestations:

the interest in pathology of these IgE lies in the mediation of atopic reactions in humans.

The cytotoxic role of IgE:

This is a protective function for the body a certain parasites.

IgE contributes to immune destruction of parasites through eosinophils.