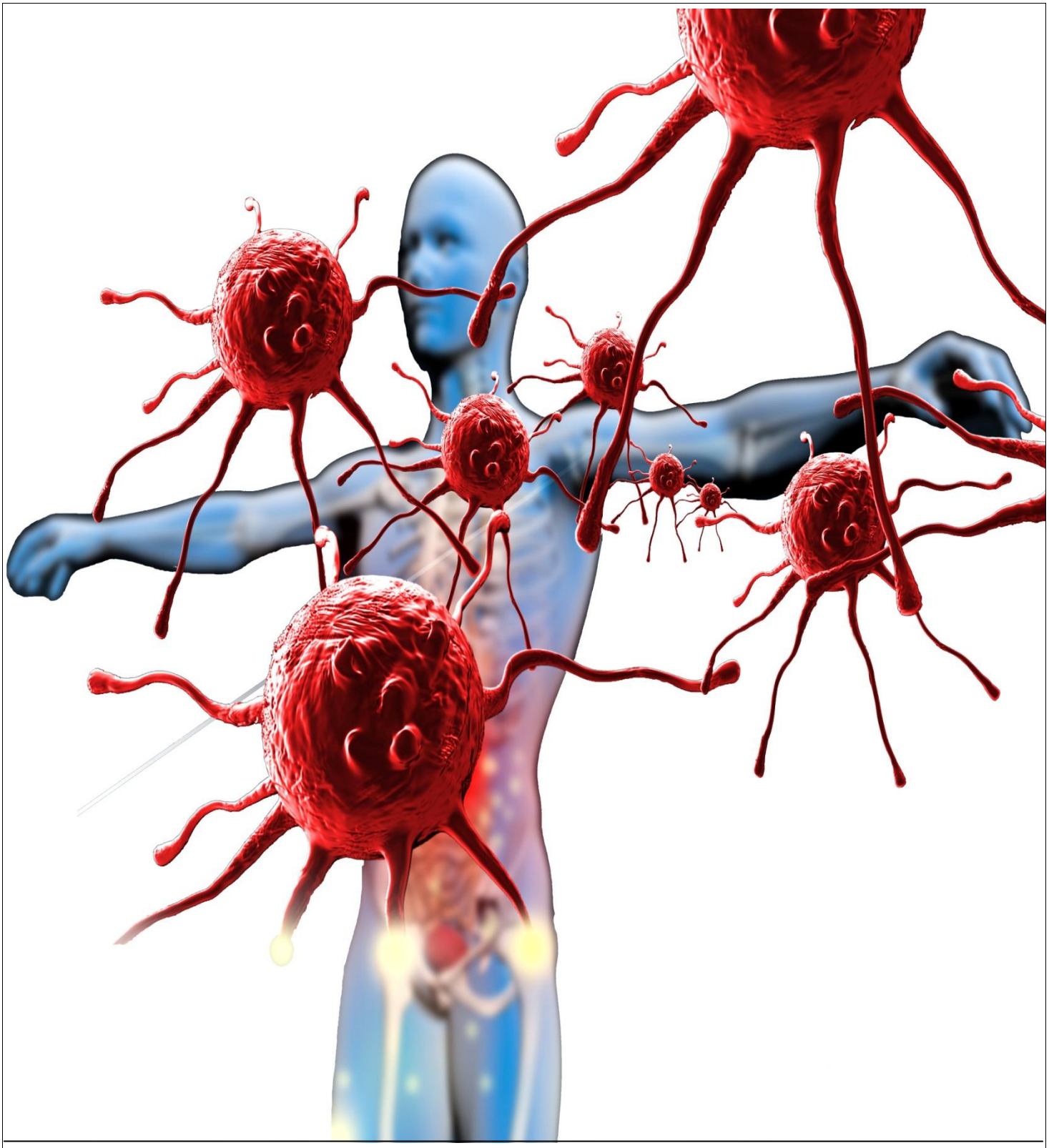


IMMUNOLOGY

Done By: Noor Hammad

#4

Dr. Hassan Abu Al-Ragheb

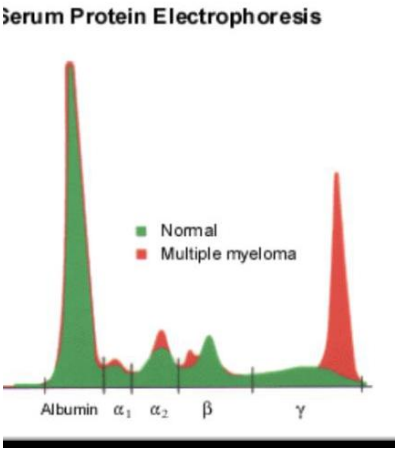
By Mohammed Nawasreh

Immunoglobulins

- **Immunoglobulin (Ig) has a common name which is Antibody (Ab)**
- **Immunoglobulins have two parts of the name**
  - ✓ **Immuno :** since they're involved in the immune system
  - ✓ **Globulin** : because Proteins, which are involved, are actually globular proteins "known as globulins"
- Immunoglobulins have two varieties
  - ✓ Secreted in serum
  - ✓ Attached on the surface of B.cell [Igs are receptors of B.cells]
    [We have 2 types of immunoglobulins ; cell bound & secreted form , the cell-bound Igs are slightly larger than secreted Igs].

- In plasma proteins electrophoresis, we'll find that MOST of the Igs are found in **gamma region** of migration so we called Ig "Gammaglobulin "

  - **Serum protein electrophoresis**: laboratory test that examines specific proteins in the blood called **globulins**
  - We use electric current to separate the serum protein components into five major fractions: serum albumin, alpha-1 globulins, alpha-2 globulins, beta globulins, and **gamma globulins**



Structure of immunoglobulins:

✓ An immunoglobulin molecule is a molecule made of two **identical** heavy chains and two **identical** light chains; these are mirror Images of one another.
  ✓ The light chain is the small one, the other (the larger one) is the heavy chain.
  ✓ Light chain (small) 22-23 KDa whereas the heavy chain(Big) 55-60 KDa

✓ These chains are connected together by means of S-S bonds (**disulfide bonds**)
  ✓ Ig is represented as **Y-shaped**.

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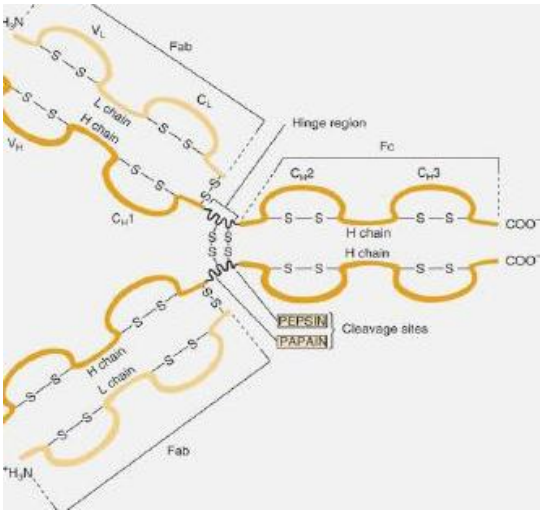
- When you look at the structure of the immunoglobulins ,we notice that ,there is a specific sequence of amino acids known as **DOMAIN**
- **DOMAIN : Special pleated structure called the immunoglobulin fold, 7 pleats and folds**



- ✓ Domain is actually a schematic representation. Each domain is made of β-pleated structure. The polypeptide runs in strands and loops.
- ✓ This structure of strands and loops is maintained by **disulfide bond** and is known as-**β pleated structure**, which is considered the base of the domain.
- ✓ Around 100 -110 amino acids per one domain
- ✓ Many molecules have this domain structure and they're referred to as members of the **super immunoglobulin family**.
- ✓ The end (-NH2) terminal domains are called variable domains on both light and heavy chains, all the rest are constant domains.

- The light chain is made of 2 domain, one domain at the NH2 terminal and the other at the COOH terminal. [Light chain: one variable and one constant domains]

- The light chain is made of 2 domains, the domain which is on the **outside** Variable domain (there are changes in the a.a between one immunoglobulin and the other) but the one that's **inner** to that Constant domain (fixed, NOT changed).
  - VL: Variable domain of light chain
  - VH: Variable domain of heavy chain

- Heavy chain depends on the class of the Ig.

- IgM & IgE: have **5 domains** "so the total is (5), (1) Variable domain & (4) constant domains."
- IgG, IgA & IgD: have **4 domains** "so the total is (4), (1) Variable domain & (3) constant domains."

- Light chain: One variable and one constant domain: VL and CL.

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- Heavy chain: one variable and 3-4 constant domains: VH and CH
  -Most of the reactions like "constant region activation "are related to second constant domain

- **In order to compensate the extra domain, there's a Hinge which is a bit elongated, it's found in IgG, IgA, IgD and it is absent in IgM & IgE (because they have an extra domain 4th constant domains). Some people say that the hinge region is a remnant of a domain, so if it doesn't have 4 constant domains, there must be a hinge which is a remnant of the 4th one that isn't present.**
- **Hinge region: short additional segment is present between CHI (constant heavy region 1) and CH2 domains. It affords flexibility between the two Fab fragments of the Ig molecule.**

- The enzyme **papain** cleaves the Ig molecule into **three** fragments: **two Fab** (antigen binding) and **Fc**. (Crystalline).

- **FAB(ANTIGEN-BINDING FRAGMENTS)**

✓ Is the area where the contact between the **paratope** and **epitope** occurs.
-REMEMBER: **paratope**: area on the Ig which contacts with the epitope on the antigen
✓ There are two (Fab) fragments with the same specificity (they are the same, because both of them have the same light and heavy chains)
✓ The function of this fragment is to recognize the antigen

- **FC. (CRYSTALLINE)**

✓ When we leave it in a solution, it actually crystallizes
✓ It is the stem of (Y) shaped Ig.
✓ The function of Fc is mediating the biological effect

- Immunoglobulins firstly recognize the antigen, after that they do an effect which is biological is mediated by the Fc portion.

- **Variable domain**

✓ There's variability in the sequence of a.a between one immunoglobulin and the other.

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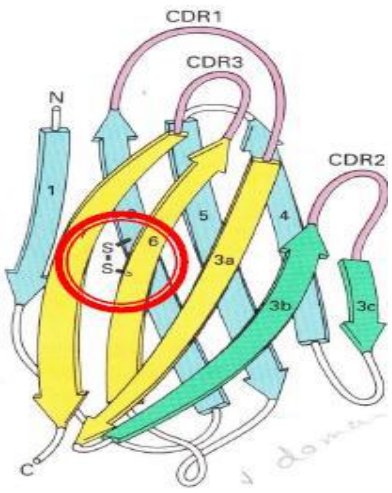
- ✓ The variability in the a.a sequence will produce variability in the electrostatic charges, hydrophobic forces, hydrogen bindings, Van der Waal's.
- ✓ There're 3 areas in these variable regions; one at **26 a.a** ,2nd one at **53 a.a**, the last one at **96 a.a**

- ➢ These regions (26/53/96 amino acids) are called **HYPERVARIABLE REGIONS** and vary a lot between one immunoglobulin and the other and produce varied specificities of antibodies. They form the tips of the paratope and combine and react with the antigen.

- ➢ Another name for hypervariable regions is **Complementarity Determining regions (CDR)** (because they are complementary to the antigen with which they combine)

- ➢ There are 3 CDRs on each variable domain.
  CDR1…..[a.a #26] CDR2…..[a.a#53] CDR3 [a.a#96]
  Most variable one is **CDR3**



- ➢ The CDRs from **light** and **heavy** chains form the **cleft** whose fit determines the epitope with which it is to combine and with which affinity.(The (3) CDRs of the light chain with the (3) CDRs of the heavy chain **come together** to make the **paratope** that's going to determine the complementarity between the Ab and the antigen)

- ➢ Hypervariable regions are far away from each other, but in the conformational status of the domain, they'll be close to each other.
  - ➢ The light chain can be of two classes : Kappa and Lambda, in humans, 60% of Ig contain kappa and 40% contain lambda chains. Only one type of light chain is present in a typical antibody, thus the two light chains of an individual antibody are identical.

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- The heavy chain can be of five different classes: lgM, lgD, IgG, IgA and IgE, these are present in all individuals and are called **isotypes.**
- The class of the heavy chain determines the biological activity of the immunoglobulin.
- There are also subclasses of lgG and IgA
- We have 4 subclasses of IgG & 2 subclasses of IgA (IgA1 & IgA2)
- IgG1, IgG3, IgG4 ……..> pass through the placenta but IgG2 cannot
- IgG1, IgG2, IgG3……..> activate the classical pathway of complement but IgG4 doesn't do that

Class	Heavy chain
IgM	μ
IgG	γ
IgA	α
IgD	δ
IgE	ε

- The differences between the subclasses are very small in comparison to that in the classes of Ig

- The constant domains of the heavy chain bear slight differences from the others. What determines the isotype of the Ig is the heavy chain.{Determine the class and subclass of Ig]

Note: the biological activity of the Ig lies in the Fc fragment, which is mainly constant.

- ✓ We want variability for the detection of antigens but we do NOT need it for the performance (biological activity), which would be the same.
- ✓ (Performance should be reserved)
- ✓ Fc constant domains with constant function.

❖ Diversity of immunoglobulins :

How do these varieties of different specificities actually occur?

-**AT THE BEGINNING**, they used to think that the B cell or T cell took up the antigen, examine it, then decide to make an antibody which is specific to that antigen, in order to make that you would need millions and billions of genes to produce such a diversity, and of course the whole DNA of the cell will not be enough to produce this varieties of diversity

- ✓ This route can't explain this huge diversity

- **LATER THROUGH THE STUDIES**,

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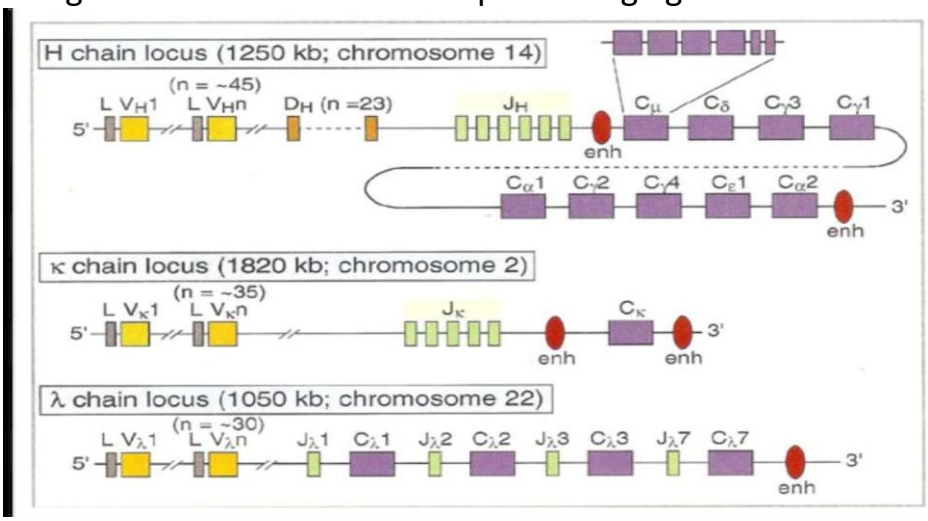
- ✓ They studied the **stem cells and the early lymphocytes** & measured how much DNA there is (by weighing the cells), after that they made a study about the **amount of DNA in mature T and B cells**,
- ✓ They found that, there is a difference **in DNA between mature and immature lymphocytes**

- ✓ THE amount of DNA in mature lymphocytes is less than that of immature ones.

- ✓ During the development of these cells, some of the DNA is rearranged and lost, and this rearrangement occurs at the immunoglobulin's genes in the DNA.

- ✓ The immunologists look for "clonal selection theory", which means that Antigens select B cells that can produce Ig against them



- We notice from the pic

  - ✓ Gene of **heavy** chain is present on **chromosome 14**,
  - ✓ Genes **of kappa** chain are on **chromosome 2**, and **lambda** chain is on **22**.
  - ✓ The gene of each chain is **Not** present as <u>one big gene</u> ; instead it's in the form of **<u>segments</u>** (gene segments)

  - ➢ Firstly, **KAPPA CHAIN GENE**

- ✓ Kappa chain is made of 2 domains, one domain is the variable domain, and the other one is the constant domain

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- ✓ Each domain is 110 amino acids, so the genes of kappa chains should code for 110 amino acids in variable and 110 in constant.
- ✓ IN the picture above , there's one gene segment known as **kappa constant** and abbreviated as **(Cκ)** ,this gene segment codes for 110 amino acids of the constant domain of kappa chain.

- ✓ .For the 110 amino acids of the variable domain:

- We find that the genetic material up in the chromosome are present in **segments,** and we refer to them as **V segments** (variable segments), those V segments are of two kinds:

1) J segments 'joining gene segments"

- They are usually (1-4) , differ in their numbers in different sources, and each J segment codes for about (10-15) amino acids

2) Variable gene regions

- In humans there is about (**35-40)** variable regions, some sources says it can reach 100, however , you have to know the number 100
- There's a big number of these V regions and we refer to the number as' n'
- Each V segment (variable gene regions) codes for about (**90-95)** amino acids.

- ✓ One segment from V region will join one segment from J region to form the variable domain
- ✓ We add amino acids of the variable with those of the J region, and then we will have 110 amino acids of the variable domain.

- ✓ Activation of genes known as Recombination activating genes ; **RAG1 AND RAG2** , these genes are responsible for sorting out gene segments and fusing them together

- ✓ One V segment will make a recombination with One J segment **RANDOMLY**
- ✓ Intervening DNA will be deleted (will not code anymore)

➢ **For example** we get fusion of V3 with J2 randomly through the process of RAG activation and **from V4- to J1** is deleted, this deletion can explain why mature stage of lymphocytes have less DNA B than immature stage

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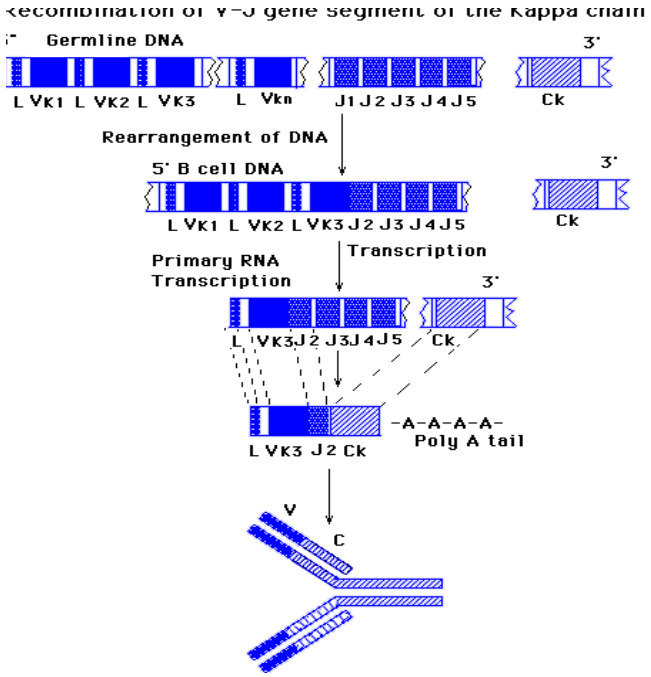
- ✓ After VJ rearrangement takes place, they fuse with the constant to produce VJC rearrangement.[**VJC will code the light chain two domains**]
- ✓ Note that **J3-J5** are not deleted (not disturbing the fusion of V3 to J2, so no need to delete them), the same is **with V1-V2**.[ONLY the intervening DNA will be deleted]



- ✓ In the **variable domain** there are the 3 CDR's (hyper variable regions)
- ✓ Each variable gene segment (V1, V2, V20 …..ETC) has 2 CDR's; CDR1 & CRD2.
- ✓ CDR's **amino acid sequence differ from each other and also differ between different variable and joining segments.**
- ✓ CDR3; the most variable; it's found in **J segment**.

NOTE: the deletion of intervening regions occurs after the VJC DNA formation, transcription, translation to form m.RNA
So, the deletion occurs at the maturation stage of m.RNA

➢ **LAMBDA CHAIN PRODUCTION**

- ✓ Lambda is a bit more complicated than kappa, because in kappa we have only one constant gene unlike lambda, which has **6 constant genes,** every J segment, has its own constant gene segment.

➢ The presence of many genes of constant domain does not increase the variability

Because the constants of lambda **are not changed** **(**the same always)

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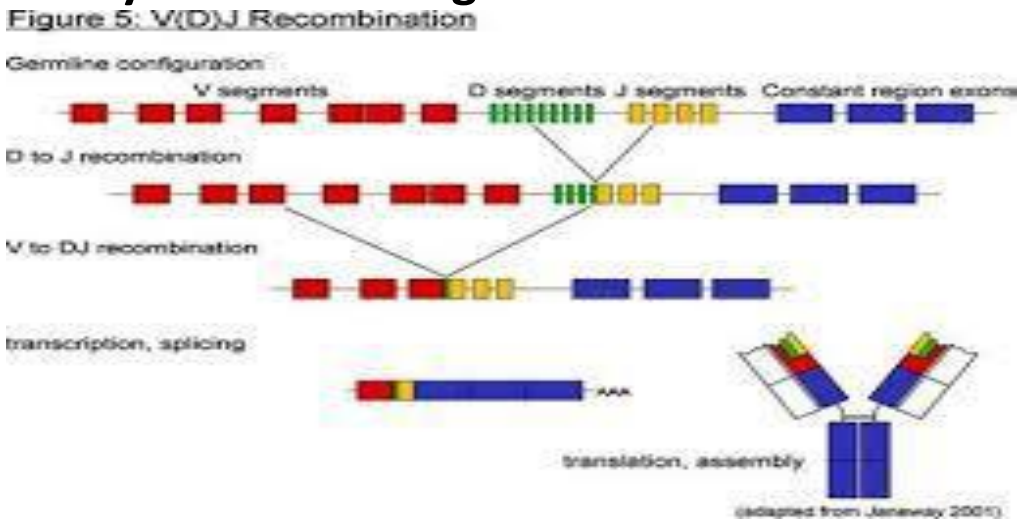
- After joining one V segment with one J segment, and each J has constant already fused , you'll get VJC recombination which form lambda chain

Note: **In B lymphocytes, heavy chain assemble first then light chains** .

Heavy chain rearrangement:



A) Heavy chain variable domain

- The region of variable domain, there are V segments ,D segments (diversity) & J segments
- In the rearrangement, first you get D and J rearrangement **forming DJ**
  - ✓ In the picture D4 joins J2 , and the intervening **D5-J1 is deleted**
  - ✓ Then one of the V segments joins DJ rearrangement to produce **VDJ,** in the picture above V3 joins DJ.

[So we get joining of V3 with D4 and J2, and these are gene segments that are responsible for production of variable domain (110 amino acids) of heavy chain.]

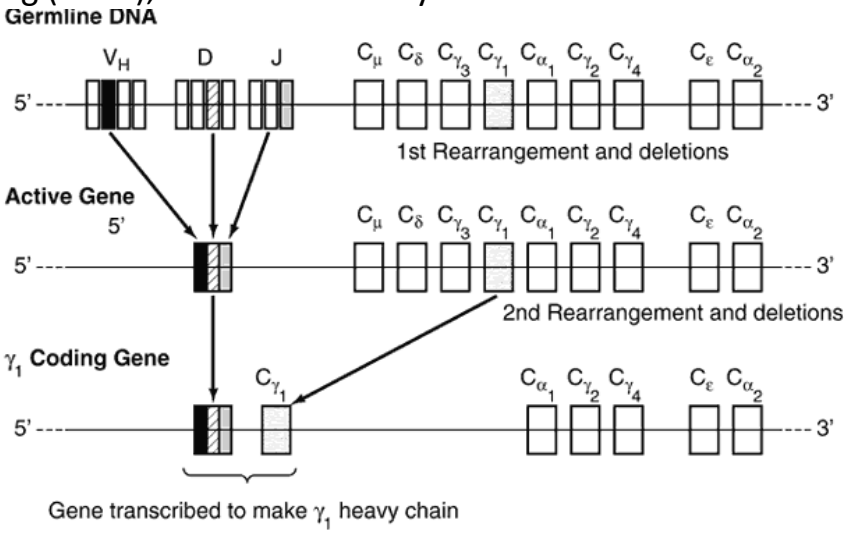
- ✓ After finishing VDJ rearrangement , now constant should join them, and in the case of heavy chain the constant genes are responsible for determining the class of the antibody

Note : **heavy chain variable domain we have more variety than light chain , because we have 3 possibilities(V,D,J) to choose randomly from each of them in comparison with 2 only(V,J) in light chain .**

- CDR1 and CDR2 are produced by V segments
- CDR3 is produced by two gene segments “DJ”and little bit of “V”

B) Heavy chain constant domains:

- ✓ we have many genes, and each one codes for the whole 3 or 4 constant domains of the heavy chain i.e. we don't have for example CH1,CH2,CH3 genes to code for separate domains , instead we have one full constant gene segment that codes for these all domains.
- ✓ Example:
- ✓ Cμ codes for the 4 constant domains of IgM antibody.
- ✓ Cɣ (delta), this delta actually codes for 3 constant domains



- ✓ When the cell rearranges its DNA , it first forms VDJ (variable domain) then you're going to choose a constant , the easiest constant which is directly next to you is Cμ , and that's why the IgM is the first immunoglobulin to appear at the cell as a receptor.

Combinational and Junctional Diversity

-**COMBINATIONAL DIVERSITY**: having many possibilities in joining different segments "Diversity in selection of V, D and J"

-**JUNCTIONAL DIVERSITY:** related to joining of gene segments during the process of V(D)J recombination

- ➢ TO explain the "COMBINATIONAL DIVERSITY":

- ✓ Combinatorial diversity is the combination possibilities if VJ in light and VDJ in heavy chain with the subclasses of heavy chain and we calculate them as follows:

-Kappa chain: 100 V's and 4 J'S; 4*100=400 different kappa chains

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-heavy chain: number of V* number of J * number of D

-Then we add the possibilities of heavy chains and the possibilities of light chain to calculate the "TOTAL DIVERSITY"

- ✓ At the end combinatorial diversity alone will give about 800,000 different specificities, and both junctional and combinatorial will give 10^9 different specificities
- ✓ Combinatorial diversity in heavy chain is more than light chain

➢ To explain the "JUNCTIONAL DIVERSITY" :

- ✓ There are CDR1, CDR2 & CDR3; CDR1 and CDR2 are functions of V's (Variable segments), however, CDR3 which is the most variable (it's bigger) is the function of DJ (diversity-joining in heavy chain {J in light chain}.

- ✓ In heavy chain , the V,D,J combination is **not very accurate** {also J and V in Light chain} , TdT enzyme (terminal deoxynucleotidyl transferase) , which is responsible for joining them together , the problem with this enzyme is that :
  - 1-It adds nucleotides without a frame to the V,D,J regions or it can remove nucleotides during recombination. So the result is **mutations**, maybe breakdown mutations.[If we take away three nucleotides, still preserve coding but we will alter the configuration and the amino acid sequence, subsequently we have altered the diversity.]

  - 2-It can also produce varieties but they are not efficient varieties, and many of these varieties , and many of these end products of this enzyme actually **don't work**

  - NOTE: Only 10% of all T cells and B cells that are produced by bone marrow will complete development and become mature. Most of them will be lost either because rearrangement **hasn't been successful** or the receptors [Igs of B.cells and TCRs of T.cells] were autoimmune and deleted.

➢ **JUNCTIONAL DIVERSITY** is more important than **combinatorial diversity**, This type of diversity is known as in the production of varieties .

Again diversity is both combinatorial diversity and junctional diversity

- Now, you have two chromosomes in your germline (one from the father and one from the mother), the process of rearrangement starts in one chromosome then if it's successful it will complete the stages, if it's not successful it will shut off and move to the other chromosome.

-Example: if rearrangement of heavy chain starts in the chromosome coming from the father, if it's successful it will move to the next stage, if it's not successful it will go to the chromosome coming from the mother, after that if the rearrangement of heavy chain is also not successful, B cell will die .

- -In the previous example, if the father's chromosome rearrangement is successful from the beginning, we don't need the chromosome coming from the mother (it will be excluded because we couldn't have two arrangements one paternal and one maternal; only one rearrangement should take place either maternal or paternal) why?
- -Because if we have two arrangements we'll have different specificities of heavy chain in the same cell, and as you know the heavy chains of antibodies of same B cell are **IDENTICAL**

- -Once finishing successfully with heavy chain, then light chain rearrangement starts, in the case of light chain **always kappa chain has the preference (priority)** to be rearranged.
- -if the allele coming from the father start rearranging kappa chain, if it's successful then all other alleles will be excluded, and IgM consisting of kappa chain will be produced, if it's not successful it will move to the allele of the mother and start rearranging kappa chain, we have also two possibilities if it's successful IgM of kappa will be produced. If not it will switch to lambda rearrangement.
- -If lambda rearrangement is successful in the first allele [from father] then IgM with lambda light chain will be produced , if not, the other lambda allele[from mother] will start rearrangement ,if it's successful again IgM lambda is produced , if not then B cell will die .
- We have more kappa light chains than lambda chains since kappa has the preference, about 60% of all immunoglobulins in your body have kappa chains as a light chain, and 40% have lambda chains
- There's no difference in function between kappa and lambda**.**

Allelic Exclusion: if you successfully rearranged one allele then you would not need the other and they will be excluded and NEVER be activated

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(One chain is produced either one kappa [from mother or father] OR one lambda [From mother or father] when kappa cannot be produced)

وكأن لكل منا ملاكاته الخاصة ان أصر على اخفائها ضاعت بين صفوف الصامتين ,وان جازف وأطلقها قد لا يقدرها الكثيرون ولكن الأهم من ذلك وذاك أنها ملكك وحدك وأنت موجهها فأحسن تقويمها ليحسن بريقها

Written by NOOR HAMAD