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Today we're going to talk about the **generation of diversity** of the receptors of the lymphocytes

The receptors of lymphocytes are :

1. **B cells** : immunoglobulins ; which are cell bound on the surface of B cells , these cell bound Igs have a cytoplasmic extra portion for anchoring it into the cytoplasm.

As you know we have 2 types of immunoglobulins ; cell bound & secreted form , the cell-bound Igs are slightly larger than secreted Igs .

2. **T cells :** TCR (T-cell receptor)

Note that TCR are only cell bound and there's no secreted form

Now coming to the diversity of immunoglobulins :

In your body , you have up to 10^9 different specificities of your receptors (immunoglobulins , TCR)

So how do these varieties of different specificities actually occur?

1. In old days , 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 , bur really 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

Conclusion : this route or this way can't explain this huge diversity

2. Later through the studies , they studied the stem cells and the early lymphocytes & measure how much DNA is there , after that they make a study about the amount of DNA in mature T and B cells , they found that there's a difference in DNA between mature and immature lymphocytes , in a way that the amount of DNA in mature lymphocytes is lesser than that of immature ones .

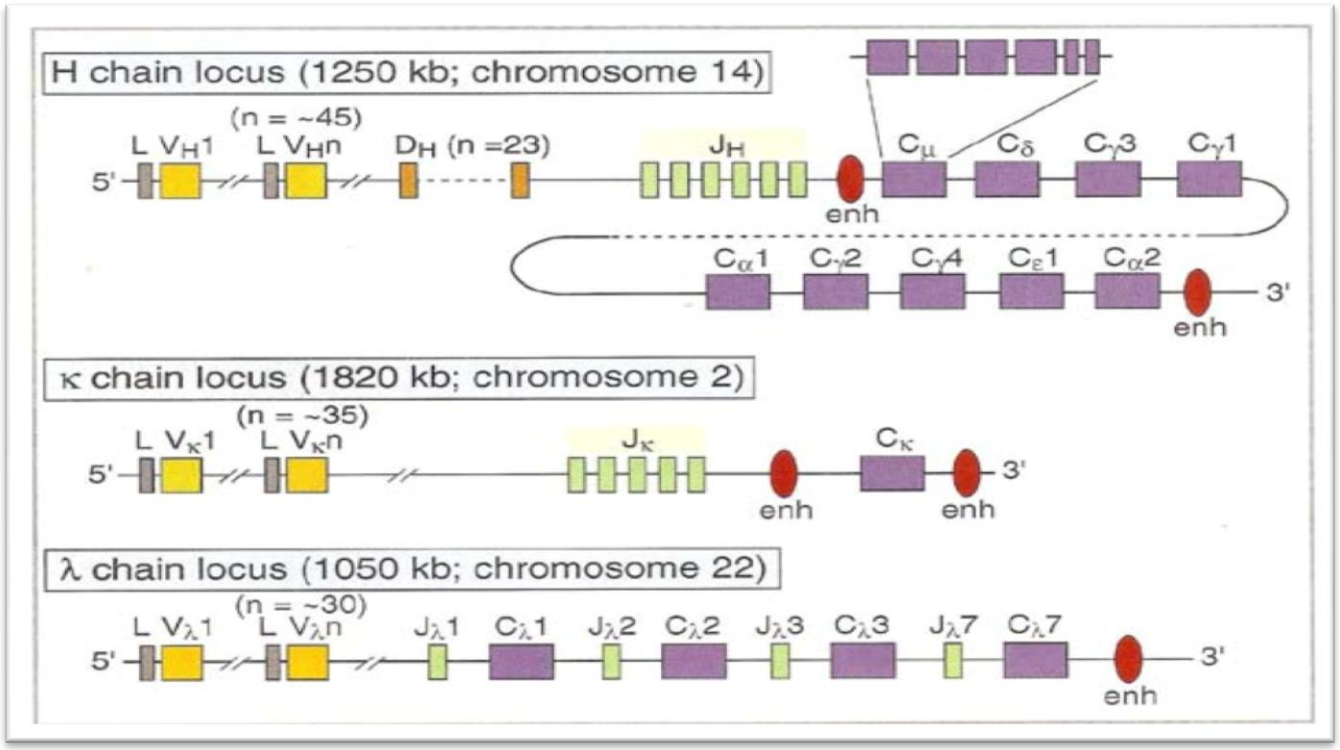
Example mentioned by the doctor : if you find that the DNA in one of them is 1 KILO , the other DNA will be 900 grams.

Why this difference in DNA is found ??

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

The following picture explains the genes of different chains of immunoglobulins , we will not go in details about TCR specificities , since it's somehow similar to Igs specificities and diversity .

As you know the antibody has 2 identical heavy chains and 2 Identical light chains , and also we have 2 types of light chains (alpha & kappa) , and 5 types of heavy chains ; which are responsible for different classes of immunoglobulins .



From previous picture notice that :

1. Gene of heavy chain (H chain) is present on chromosome 14 , genes of kappa chain is on chromosome 2, and lambda chain is on 22.

2. The gene of each chain is not present as one big gene ; instead it's in the form of segments (gene segments)

Now we will start with kappa chain gene segments and how do they form the kappa chain with its final 2 domains

1. kappa chain is made of 2 domains , one domain is the variable domain , and the other one is the constant domain

2. Each domain is a 110 amino acids , so the genes of kappa chains should code for 110 amino acids in variable and 110 in constant .

3. in the picture above , you can see down in the chromosome , 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.

4. Now , for the 110 amino acids of the variable domain , it's summarized as follow :

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 :

A) J segments (joining gene segments)

They are usually (1-5) , differ in their numbers in different sources, and each J segment code for about (10-13) amino acids

B) variable gene regions

In humans there is about (35-40) variable regions , some sources says it can reach 100 , however , you have to know that there's a big number of these V regions and we refer to the number as **n** ; n=35-100 .

Each V segment (variable gene regions) codes for about (90-95) amino acids.

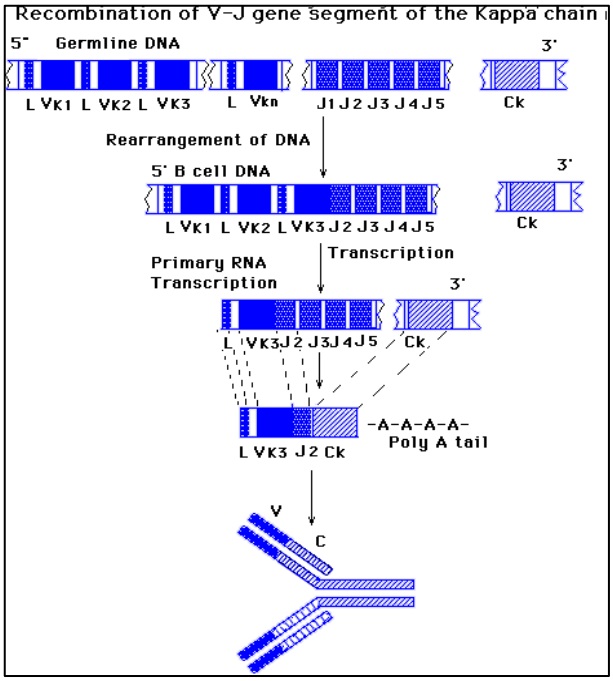
Now one segment from **V region** will join one segment from **J region** to form the **variable domain**

So if we add amino acids of the variable with those of the J region , then we will have 110 amino acids of the variable domain .

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

Early in the development of these chains (kappa chains) in B cells , we get activation of genes known as **Recombination activating genes** ; **RAG1 AND RAG2** , these genes are responsible for sorting out gene segments and fusing them together , i.e.for recombination .

Now in the case of kappa chain , one V segment will make a recombination with one J segment **Randomly** , and the intervening DNA will be deleted (will not code anymore)



For example we get fusion of V1 with J3, V20 with J1 …..etc. randomly through the process of RAG activation .

In the next picture , you can see that that V3 recombine(join) with J2 and from V4-J1 is deleted , this deletion can explain why mature stage of B lymphocytes have **less DNA** than immature stage.

After VJ rearrangement took place , they fuse with the constant to produce VJC rearrangement.

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.

Now VJC will code the light chain two domains (variable and constant)

Variable domain is coded b the J segment and V segment , and constant domain is coded by C segment , in the variable domain there are the 3 CDR's (hyper variable regions)

Now notice that 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.

When we come to CDR3 ; the most variable ; it's found in J segment .

Finished with kappa light chain , now let's move to 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, in a way that every J segment has its own constant gene segment .

So after joining one V segment with one J segment , and each **J** has constant already fused , you'll get VJC recombination.

Question : for lambda chain , does the presence of many constants increase the variability ?

The answer is NO , Because the constants of lambda are not changed (the same always) , and by that not sharing in the variability and diversity of lambda chain .

Heavy chain rearrangement:

As we mentioned earlier , the heavy chain is the first to be rearranged in sequence , then we get rearrangement of the light chain .

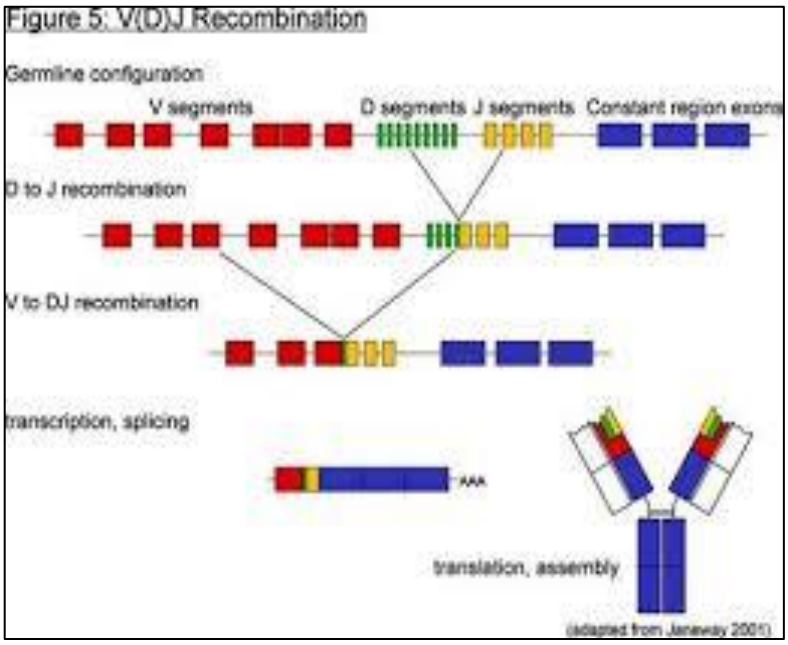
When we come to gene segments that are present in the heavy chain, we find that in the region of variable domain , there are V segments ,D segments (diversity) & J segments

Again the numbers differ according to different sources , in general (27 D's and about 60 V's and 5-6 J's)

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 .



Note that in the case of 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 .

This diversity is referred as **combinatorial diversity**; having many possibilities in joining different segments.

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 .

Look to the picture below and notice that in the constant gene segments , 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 .

After Cμ in the chromosome , there's Cδ (delta),

this delta actually codes for 3 constant domains

and is responsible for IgD antibody.

As you go down , there are Cɣ3,Cɣ1 (gamma),

CƐ2 (epsilon), Cα2.

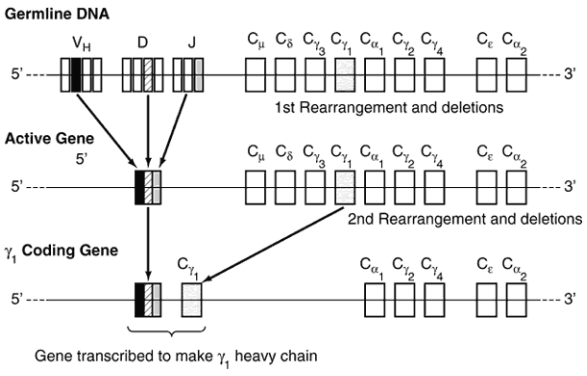
The following note mentioned by the doctor from his slides

<u>Cɣ and CƐ2 are considered as a pseudo genes ;</u>

Which means that they don't have a product (not work) , so when we talked about epsilon that make IgE antibody , we said that there's no subclasses of IgE although there is CƐ2 gene , and that's because this gene is a pseudo gene and doesn't work .

Cɣ is non-functional, but Cɣ1, Cɣ2, 3, 4 are functional, Cα2 is functional too.

When the cell rearrange its DNA , it first form 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.

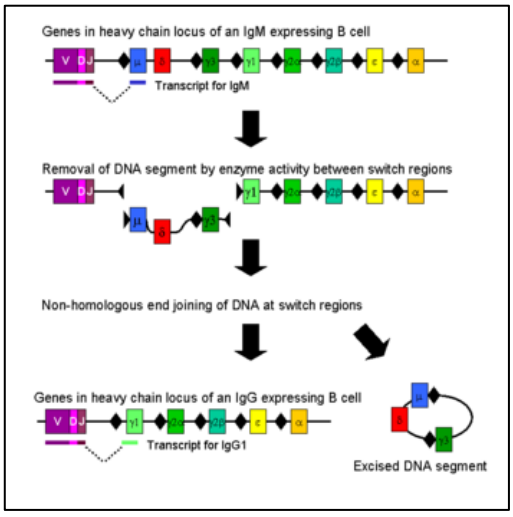


Notice that before Cƍ is free, but before Cɣ (1,2,3,4) there's a sequence of amino acids known as switch region , and if you see that every active gene is preceded by a switch region except Cμ and Cƍ .This switch Region is involved in class switching (we'll talk about it later)

So , in the early stages of development the m RNA will carry the variable domain and the

Constant domain of μ and ƍ ; the messenger RNA is long containing the variable domain then Cμ then Cƍ, after that it undergoes alternative splicing to produce either IgM or IgD , so one minute you're going to produce IgM and other minute you produce IgD depending on splicing .

We conclude that naïve B cell have on the surface both IgM and IgD .



The CDR's of heavy chain variable domain

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).

Because we have more choices in combinations of V with D and J, we're going to have more diversity.

In heavy chain , the V,D,J combination is not very accurate , TdT enzyme (terminal deoxynucleotidyl transferase) , which is responsible for joining them together , the problem with this enzyme is that 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 .

It can also produce varieties but they are not efficient varieties , and many of these endproducts of this enzyme actually don't work , and about 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 was autoimmune and deleted.

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.

This type of diversity is known as **junctional diversity,** and it's more important than combinatorial diversity in the production of varieties .

Again diversity is both combinatorial diversity and junctional 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 follow:

Kappa chain: 100 V's and 4 J'S; 4*100=400 different kappa chains then we add them to the possibilities of heavy chains and the possibilities of light chain (the doctor didn't give definite numbers , so understand the concept and in the exam there will be numbers to refer to them)

At the end combinatorial diversity alone will give about 800,000 different specificities, and both junctional and combinatorial will give 10^9 different specificities.

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**.

This exlusion is known as **allelic exclusion** ; if you successfully rearranged one allele then you wouldn't need the other and it will be excluded and never be activated .

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 produced . if not it will switch to lambda rearrangement .

if lambda rearrangement is successful in the first allele then IgM with lambda light chain will be produced , if not, the other lambda allele will start rearrangement ,if it's successful again IgM lambda is produced , if not then B cell will die .

so , 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.

The switching of immunoglobulin from IgM to IgG is not really well understood , there are multiple factors involved ; we can find some cytokines that enhance and drive switching ; nature of antigens , and of course there's a switch region that's concerned.

Suppose you want to make a class switching to IgE , the switch region before Cε is now in concern , and all of the intervening DNA between VDJ and Cε is removed , and now the cell is committed to produce IgE ,

And can't go back to produce IgM or any of the others (the cell can do only one switch) ;and in this case only IgE is produced .

These switched IgE immunoglobulins are the memory ones , these memory can't go back and produce IgM or any of the others , and the surface receptors of B cell here are made of IgE immunoglobulins only .

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