INTRODUCTION TO MEDICAL

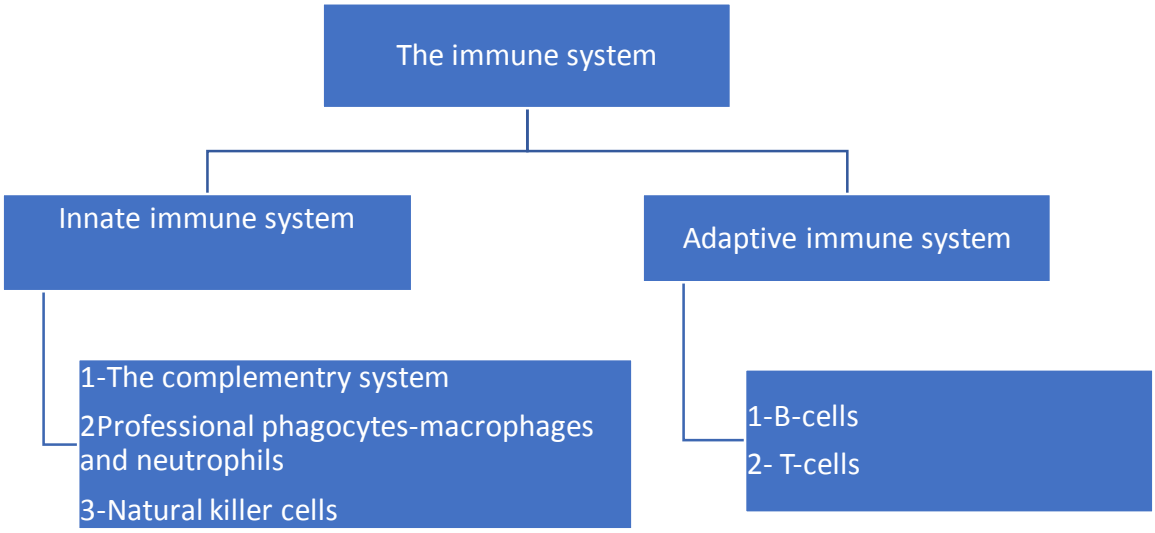
IMMUNOLOGY

SLIDE SHEET

NUMBER	5
DONE BY	Dania Qarqash
CORRECTION	Rahaf AL-Qaryouti
DOCTOR	**Issa Abu-Dayyeh**

B-cells and Antibodies

Quick revision



The adaptive immune system evolved at level of vertebrates -to be more specific at level of fish- where T-cells and B-cells were first seen .

B-cells are made in bone marrow.

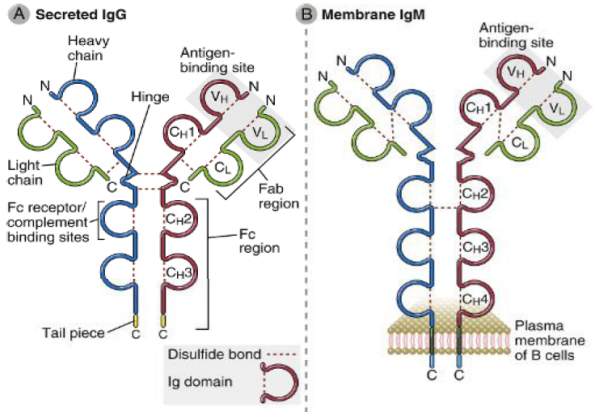
They produce immunoglobulins and these immunoglobulins are either:

1-inserted on cell membrane called B cell receptors , OR

2-secreted form antibodies

Antibody structure

Antibody is a Y shaped molecule that has **2 heavy** chains and **2 light** chains .



Regions of antibody :

Fab : antigen binding site

Fc : fragment crystallizable region

Between Fab and Fc there is a **hinge** which give flexibility to the antibody

Each chain of the antibody has two main regions:

1-*Variable* region (V)>> **end** of heavy and light chains represents antibody **binding site** which is **different** from one antibody to another

2-*Constant* region (C) >> the rest of the light and heavy chains

*From the figure, we note that the heavy chains of IgG has 3 constant regions while IgM has 4 constant regions.

According to this region(C),antibodies are divided into five major classes: IgM, IgG, IgA, IgD, and IgE.

-C-terminus is inserted into the plasma membrane if the immunoglobulin is bound to the surface

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For better understanding : (not mentioned by Dr .Issa)

The constant region is not the same as the Fc region. The constant region constitutes nearly ½ of the light chain, and ¾ of the heavy chain. While the Fc region is the tail-like portion (or the stalk) of the Y-shaped immunoglobulin.

The variable region is not exactly the same as Fab region. The variable region constitutes nearly ½ of the light chain, and ¼ of the heavy chain. While the Fab regions represent the arms of the Y-shaped immunoglobulin

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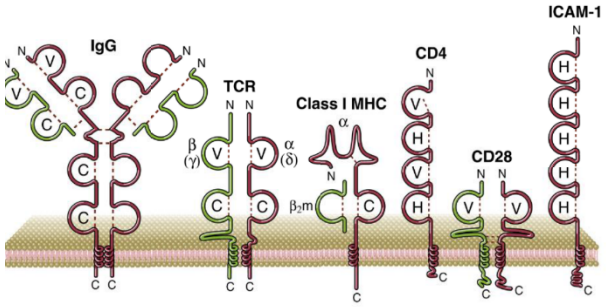
Ig superfamily proteins containing Ig domain

-Immunoglobulin domains are

lobes found on immunoglobulins and different proteins.

-Proteins that have them belong to Ig superfamily proteins like those shown in the figure to the right.



Remember :

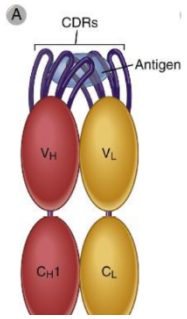
***CD4** and **CD28** are found on **T-cells**

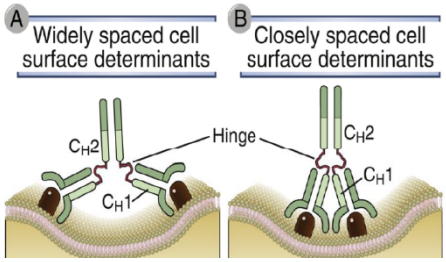
***ICAM-1** for **chemotaxis** and **extravasation**

Antigen binding

CDR : Complementarity-determining regions which are very variable regions and determine the binding of antibody to antigens.

*As you can see in the figure the **hinge** gives the antibody **flexibility** that allows the antibody to bind widely spaced antigens .





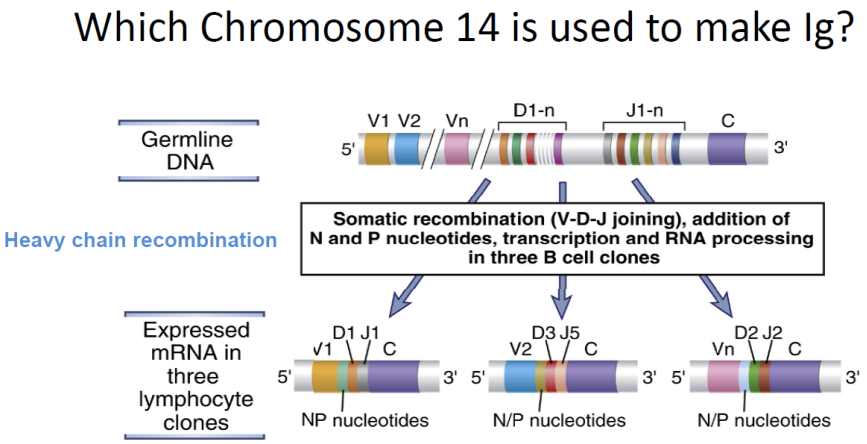
Antibodies Genes

-**Variety** of antibodies is due to **VDJ recombination.**

-Heavy chain genes are found on chromosome 14

-The light chains of an antibody can be classified as either **kappa (κ)** or **lambda (λ)** type so patients with multiple myeloma are interested to know if monoclone is kappa (κ) or lambda (λ).

-Each type of light chain is on different chromosome and heavy chain on chromosome14 .



How to know which copy of chromosome 14 is used, the one from mother or father ???

--**Randomly** , the cell will chose the first allele if it is good then it will continue if not >> the second ,if it doesn't work >> cell will die (commit apoptosis).

#**Heavy** chain germline DNA is divided into 3 segments **V ,D,J** while **light** chain V&J **only** .(important)

Different copies for V, different copies for D, and different copies for J, ant the cutting process is random with specific signals, then after the cuts they get combined forming different mRNA with different antibody's specificity .

***Gene Rearrangement test* :**

The cell asks itself if it formed heavy chain completely >> yes >>

same question with light chain >> yes >>

after forming both chains >>did it join them together? if yes >> then did it upload them perfectly on the cell surface? (because the antibody at first is B -cell receptor then if there is activation it will be secreted) if all answers were yes then the cell will inactive the other copy of chromosome 14

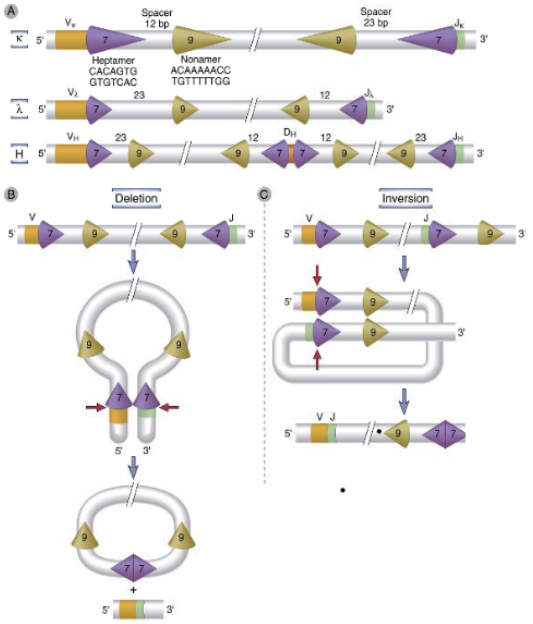
but if answers were no >> the cell will use the other copy and if answers from the other copy also were no >>B cell dies by apoptosis .

So Gene rearrangement test:

Full Heavy/light chain? Did it load on cell surface? >>>>> If yes, inactivate other copy of chromosome 14

If both copies fail: B cell dies, by apoptosis.

V(D)J Recommendation or Somatic Recommendation



First D and J combines then they are added to V.

--How to stop cutting segments ??

by **RSS** : **R**ecombination **S**ignaling **S**egment

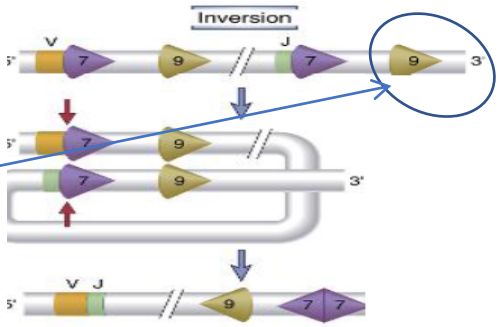
which is a non coding segment found between every V,D,J .

*RSS is composed of heptamer (highly conserved 7 base pairs) and nonamer (highly conserved 9 base pairs)with space region between them either 12 base pairs or 23 base pairs .

--Why ? 12 because it can form one lobe of DNA and 23 for the loop to be cut .

--This RSS is **recognized by RAG genes** (recombination activating gene) how ??

RSS forms loop between V& J this loop make the two heptamers closer then RAG1and RAG2 cut this RSS.



IMPORTANT

(if the heptamer is found on 3` there will be inversion)

Sequence of Events in VDJ Recombination

1- RSS forms a loop >>
So heptamers get closer

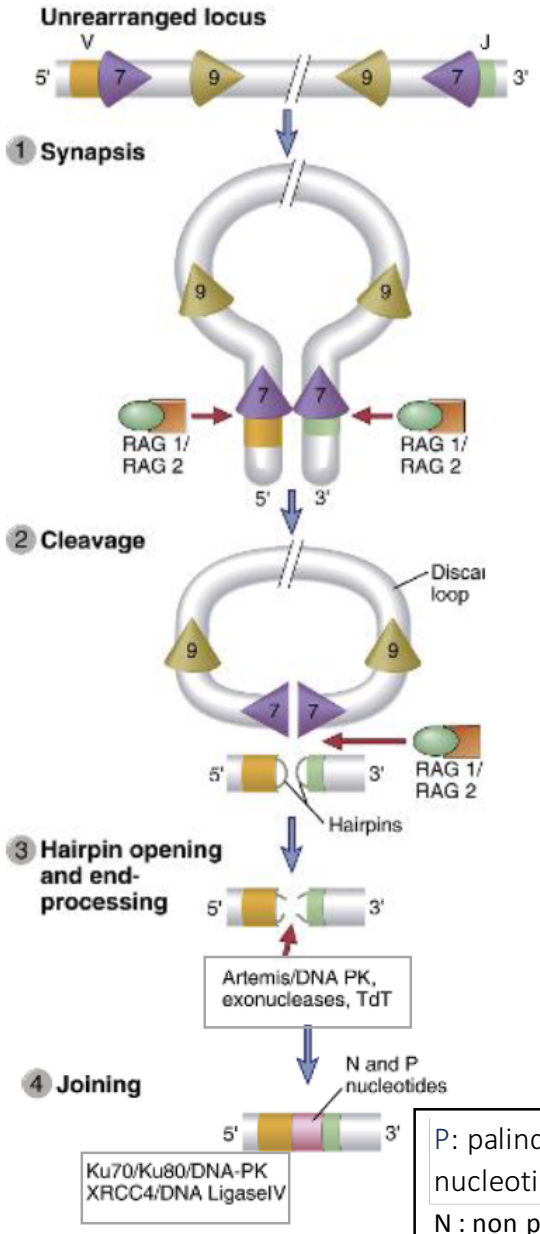
2- Heptamers are docking sites for RAG 1 and RAG2 >> resulting in cleavage
3- After cleavage >> formation of two pieces with hairpin like structure
4- Opening hairpins by **Artemis**
5- **DNA PK** : adds nucleotides
Exonucleases: remove nucleotides
TdT :adds nucleotides

*While removing and adding

Random nucleotides are added (N and P) in

the joining area to increase variability of antibody.

6- Joining V and J using
7- complicated adaptor proteins and ligase>>forming mRNA transcript .



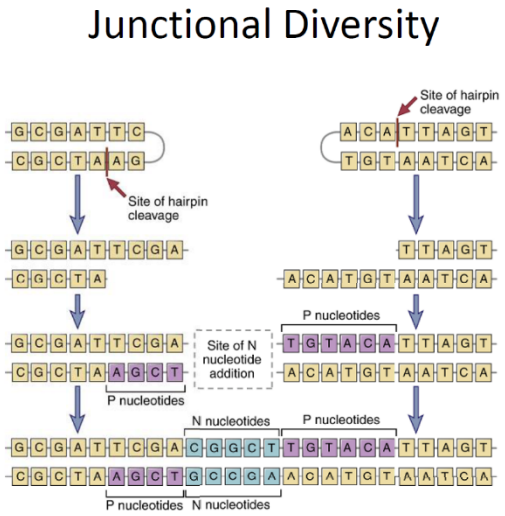
P: palindromic nucleotide
N : non palindromic

Any deficiency in RAGs or Artemis or DNA Protein Kinase will cause SCID (Severe Combined Immunodeficiency)
In case of Artemis deficiency >> no B cells + T cells maturation (same maturation mechanism for T cell receptor) >> SCID palindromic

Junctional Diversity

When Artemis cuts the hairpin , it generates palindromic sequences (reading from right to left or left to right give same sequence like “DAD” word) after that, adding P nucleotides between them N nucleotide >> this gives more **diversity** - junctional diversity -

That’s why there is huge diversity among antibodies .



One B cell >>> One antibody (specificity)

One B cell→ **One** antibody (one heavy and one light chains)

Recombination options so many! We can produce antibodies to **every** organic molecule available.

If a B cell recognized a Malaria antigen it will produce antibodies against Malaria antigens for the whole this B cell life (keep specifity toward malaria)

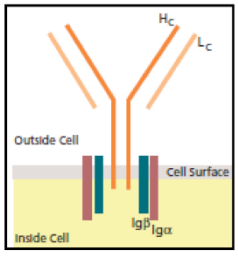
BCR signaling

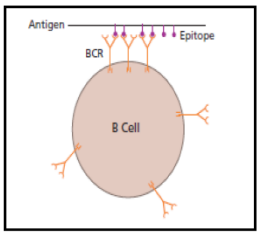
After forming the antibody and uploading it on the surface , the cytoplasmic tail is short that can`t transmit signal to the nucleus in case of binding an antigen so how the signal will be transmitted to the nucleus ??

By the accessory proteins in B cell receptor .

Accessory proteins are Igα and Igβ ,when an antigen binds to an antibody they are recruiting with other accessory protein to form signaling cascade reaches the nucleus to transcript certain genes and enzymes and to become active.

Also , for activation to occur, cross linking must take place ,it means more than one antibody should bind to more than one antigen .

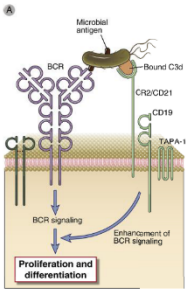




Accessory proteins are required for signaling
but cross-linking of antibodies is key.

Can the complement system interact with the adaptive immune system ??

Opsonization by complement system greatly amplifies BCR signaling



Complement receptor engagement tightens BCR binding and signaling

If the bacterium is opsonized(has complement part) , binding to complement receptor in addition to BCR will increase BCR binding and signaling .

B cell activation

To activate BCR and to guarantee right activation , **two signals** are needed :

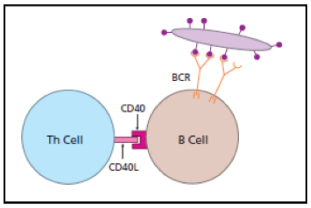
1- Antigen that binds to BCR
2- Co-stimulatory signal
   - T cell dependent : CD40L of T helper cell (patients who born without CD40L have no class switching so all their Ig are IgM and their bodies don't produce memory cells)
   - T cell independent : cytokines : IFN-γ

Two-step activation system:

1- BCR engagement and clustering

2- Co-stimulatory signal

(T-cell-dependent: CD40L)
(T-cell independent: cytokines: IFN-γ)



What is the purpose of T-cell-independent activation??

T cell can recognize proteins on the antigen while the antibody can recognize carbohydrates, proteins and lipids on the antigens

so if the antigen is coated with carbohydrates, just BCR recognizes the antigen ,that's why cytokines are used as other signal .

B cell maturation

After VDJ recombination, B-cells recognize antigens and produce antibodies and they get activated ...now time for maturation

3 main steps occur :

1- Class switching from IgM or IgD to IgG, IgE, IgA (changing of Fc portion)
2- Somatic hypermutation ;mutation in antigen binding site to increase the binding affinity .
3- Career Decision

- **Class switch**

Is changing the antibody class from **IgM** or **IgD** to IgG,IgA,IgE

(the significance of IgD until now is not clearly understood and the degree of its expression is low)

How this change occurs ??

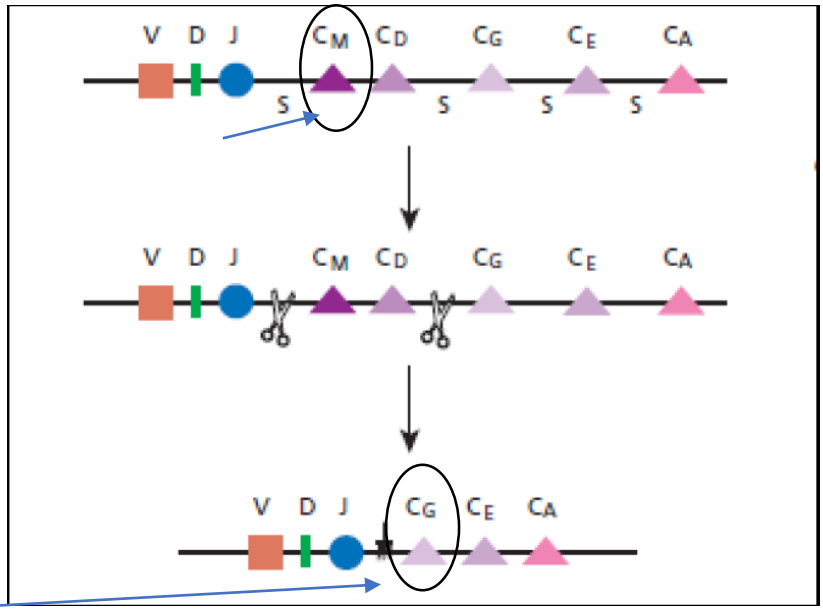
By cutting and joining and according

to the first constant region>> Ig class is determined

*Now by cutting CM and CD

then joining the V D J with the other piece,

the IgM is switched to IgG



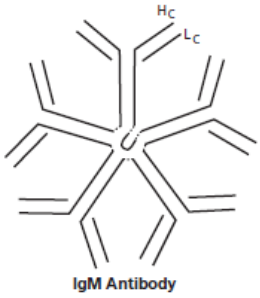
(this gene formed is actually a signal to start producing IgG)

*Further processes of cutting will take place if IgE or IgA is needed .

***AID** is a key enzyme in isotype switching .

Antibody classes and their function

1- IgM Antibodies



IgM Antibody

-Pentamer, first antibody to be produced.

-Can trigger classical complement pathway

through bringing **C1** molecules in close proximity.

C1s bind to Fc portion, get activated and subsequently activate the C3 convertase causing a complement cascade on the surface of the pathogen.

-Why is the classical pathway needed? Because we need to trigger the innate immune system to assist the adaptive immune system in this condition .

-Why IgM not IgG first??

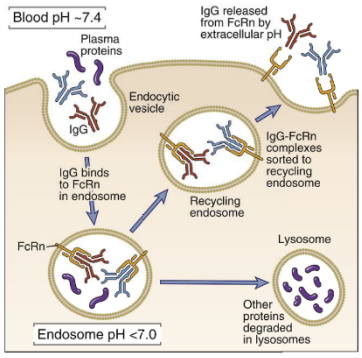
BecauseIgM is a better complement fixer and has a better neutralizing ability.

Half life = 5 days.

2- IgG or Gamma globulins (γ)

-Called Gamma globulins. Monomer .

-Decent complement fixers, good virus inactivators.



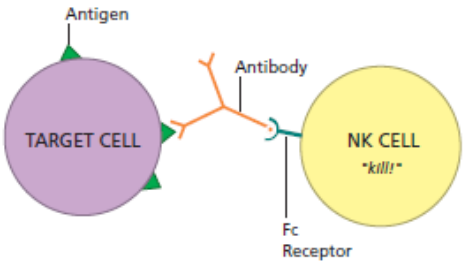
-Can cross placenta (the only class that can provide immunity from mother to fetus in pregnancy)

- half-life 23 days, Why does it live longer ?? because transporting IgG through the blood to the uterus then to the fetus needs long time and to live more in the fetus, for this purpose there is a receptor called **Neonatal Fc receptor** that binds to Fc of IgG and protect it from degradation in lysosomes of endothelial cells .(scientists used this mechanism in making drugs, for example : rheumatoid arthritis drug has Neonatal Fc receptor to protect it from degradation)

-Four subclasses: IgG1, IgG2, IgG3, IgG4

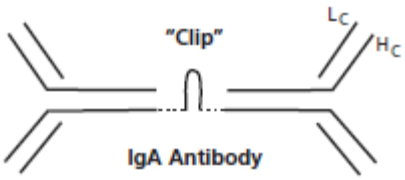
-IgG1 is a good opsonizer. Macrophages and neutrophils have receptors for IgG1-Fc

-IgG3 fixes complement better than other subclasses. NK cells have receptors for it.



>>The NK Cells get activated when binding occurs ,this process is known as

"Antibody-dependent cellular cytotoxicity (**ADCC**)"



3- IgA class

-Main Ab class that guards the mucosal surfaces of the body(like GI tract ,Urogenital tract)

-Its structure facilitates its transport to intestines, and makes it resistant to acids and enzymes

-Dimeric structure with clip in the middle ,that helps clump bacteria together to be swept out with mucus or feces.

-Secreted into the milk of nursing mothers. Why? IgA provides immunity to the newborn in his first few months because newborn are incapable of producing their antibodies until the 6^{th} month of age .
when baby drinks milk ,the antibodies will go to the GI tract and IgA is ideal for mucosa of GI

-Good or bad complement fixers?? Bad complement fixer and this is actually a good thing because we want to calm immune response in the intestine .If IgA is a good complement fixer, severe inflammations in the intestines will easily occur .

*Which class is the highest in number in **blood** ? **IgG** in **tissues** ? **IgA**

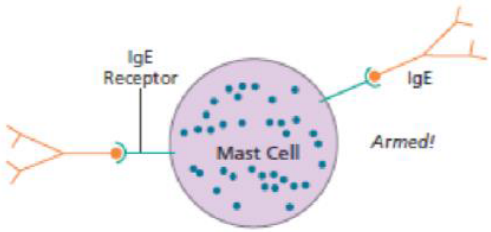
*IgM is the second highest in serum .

4- IgE class

1-In Parasitic Infections: IgE is made, Fab binds to parasite and Fc binds to mast cell
Mast cell releases histamine and cytokines such as TNF and IL-3,4,5 to kill parasites.
2- In Allergies:
Allergic reaction, anaphylactic shock in some cases!
Allergy can be considered a disadvantage or a side effect of IgE class of antibodies.

-When we talk about allergies, we usually separate between first exposure, and second exposure.

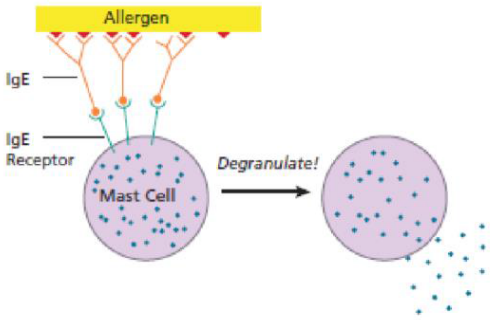
- If a person has allergy towards certain substance, the **first exposure to this substance will not result in a severe allergic reaction**. For example, if a person is allergic to peanuts, he will not develop an anaphylactic shock directly after the first time he eats peanuts. Why?
Because the mast cells are not primed (armed) yet.



AFTER FIRST EXPOSURE

The first exposure to the allergen causes the B cell to "see" the allergen and form antibodies against it. These antibodies will bind -via their Fc portions- to "Receptors of Fc portion of the IgE" on the surface of the mast cell, then the mast cell is now armed.

-Upon second exposure to the same allergen, the antibodies -that are already bound to the mast cell – bind to the allergen leading to:
activation of the mast cell and degranulation
allergic reaction occurs, and if the reaction is very exaggerated, anaphylactic shock may occur.

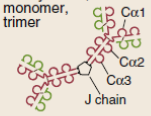
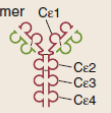
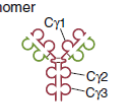
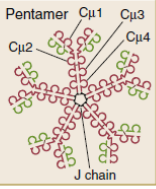


The table below summarizes antibodies classes and functions .

This table is from Basic Immunology -Abbas

Ab Classes and function

ANTIBODY CLASS	ANTIBODY PROPERTIES
IgM	Great complement fixer Good opsonizer First antibody made
IgA	Resistant to stomach acid Protects mucosal surfaces Secreted in milk
IgG	OK complement fixer Good opsonizer Helps NK cell kill (ADCC) Can cross placenta
IgE	Defends against parasites Causes anaphylactic shock Causes allergies

Isotype of antibody	Subtypes (H chain)	Serum concentration (mg/ml)	Serum half-life (days)	Secreted form	Functions
IgA	IgA1,2 (α1 or α2)	3.5	6	Mainly dimer, also monomer, trimer 	Mucosal immunity
IgD	None (δ)	Trace	3	Monomer	Naive B cell antigen receptor
IgE	None (ε)	0.05	2	Monomer 	Defense against helminthic parasites, immediate hypersensitivity
IgG	IgG1-4 (γ1, γ2, γ3 or γ4)	13.5	23	Monomer 	Opsonization, complement activation, antibody-dependent cell-mediated cytotoxicity, neonatal immunity, feedback inhibition of B cells
IgM	None (μ)	1.5	5	Pentamer 	Naive B cell antigen receptor (monomeric form), complement activation

What triggers class-switch?

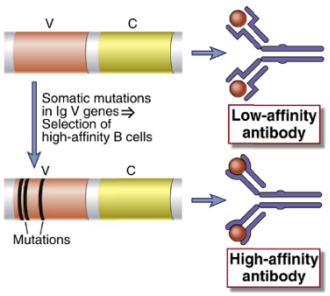
1. Cytokines produced by Th cells
2. **IL-4** and **IL-5** favour a switch to **IgE** (Parasitic infections)
3. **IFN-γ** favours switch to **IgG** (Fights bacteria and viruses)
4. **TGF-β** favours a switch to **IgA** (Common colds, intestinal infections).

- **Somatic Hypermutation**

-In our bodies the mutation rate in our genome is approximately 1:100,000,000 b.p. per replication cycle because of correcting enzymes and etc. Otherwise cancer occurs .

-After VDJ segments have been selected, this region undergoes very high mutation rate:

(As high as 1:1000 b.p. per generation). This high mutation rate is on purpose to **increase antibodies variability** .



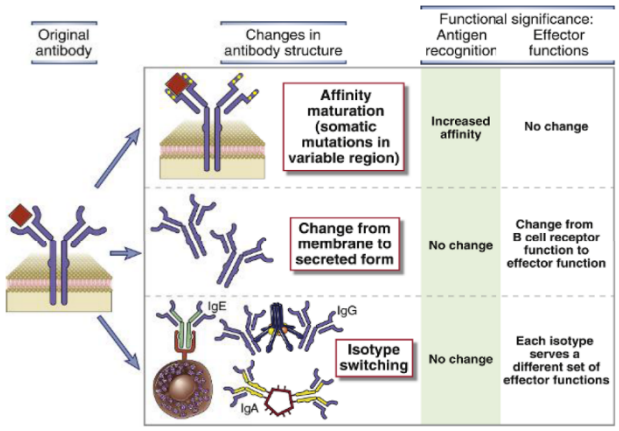
-This somatic hypermutation affects affinity of Fab region of B cell antibody.

-B cells with higher affinity are better stimulated by antigen presented by follicular DC and can present it to TFH which rescues B cells from apoptosis and promotes proliferation.

Changes in Ab structure during humoral response

----In other words, the result of somatic hypermutaion is selecting B cells that are able to exert better binding to the antigen

This antibody can bind antigens but not perfect .



B cells can change both their Fc region (Class-switch) and Fab region (somatic hypermutation) to become better adapted to fight invaders.

- **Career choice**

cells make a career choice

Plasma Cell

Go to spleen or bone marrow and secrete Abs

(up to 2000 Abs/sec)

Short-lived (few days) because it works and secrets a lot of antibodies .

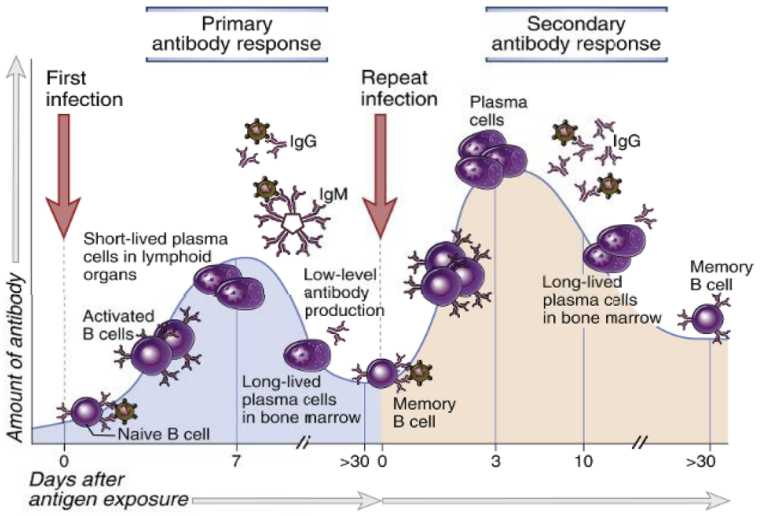
Memory Cell

Cells that remember the first exposure

Defend against subsequent exposure

Need Th cells to develop (CD40L)

Primary and Secondary Humoral Immune Responses



This graph summarizes the immune response

Naive B cells (after VDJ recombination and insertion of B receptors) go to circulation and lymph nodes >>> at the first infection ,their antibodies can recognize the antigen >> signaling cascade >>activation of B cells >> conversion into short lived plasma cells >> secretion of IgM mostly and after a while little IgG>> no more infection >>plasma cells decrease in number and antibodies titers decrease >> signal to some of these cells to convert into memory B cells that can live for years.

<u>Notice that in order to have plasma cells, it takes a week that's why innate immune system is important because the adaptive immune system needs time to be active .</u>

Second exposure >> recognition of antigens by B memory cells(no need of innate immune response to recognize and activate B cells)>> Mature B cells (that have class switch done ,IgG ready, somatic hypermutation occurred) >> proliferation and conversion to plasma cell(takes less time than first infection ,faster) >> production of more antibodies than first infection(many times we get a second infection and we don't even know about it because the quick immune response and a lot of antibodies)these antibodies are IgG>> conversion to long lived plasma cells in bone marrow that produce little amount of antibodies (circulatory titers of antibodies if they decrease below certain level we need to make a booster which means the immune system need to see the antigen again)

In pregnancy , women need to measure titers of Hepatitis B, Hepatitis C ,Rubella IgG .

If a pregnant woman with low titers of Rubella IgG got infected with rubella >>>abortion or fetus with abnormality .

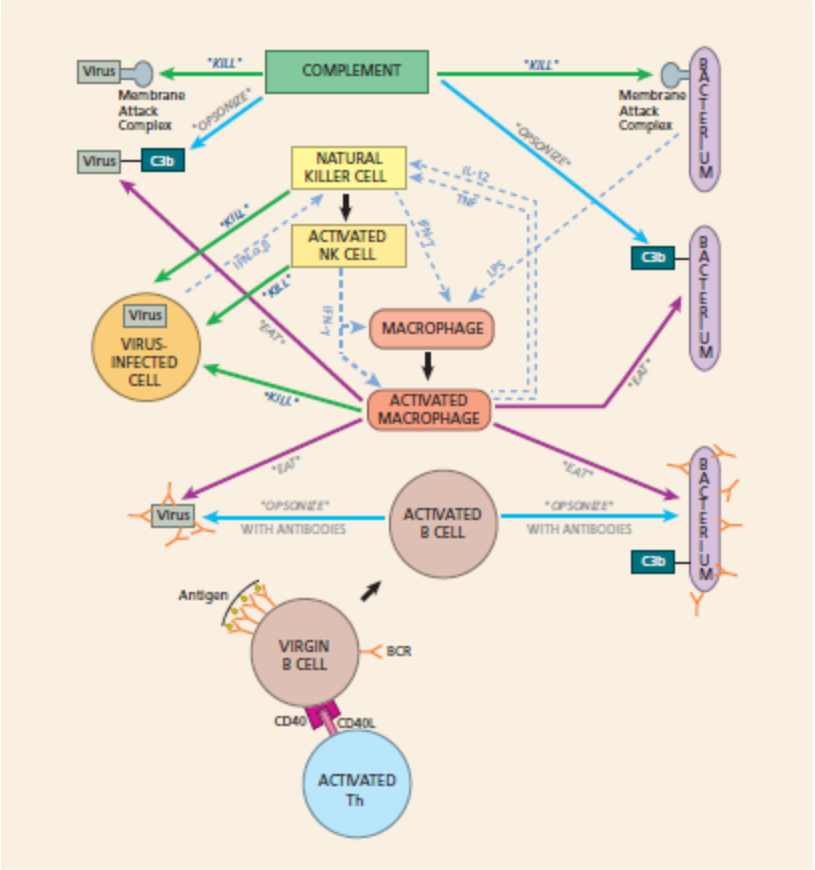
*Complement immune system can kill the pathogen through MAC or opsonization that allow macrophages to see the pathogen and kill it .

*And NK can kill the pathogen directly or by activating macrophages to kill it.

*If a virgin or naive B cell encounters its antigen (Binding = the first signal), and then gets co-stimulated by the activated T helper cell (Co-stimulation via CD40-CD40L interaction = the second signal) or the second signal independently from a cytokine.

The B cell will become activated and will start producing antibodies that can coat bacteria and viruses (opsonization) so that they can be recognized by macrophages, complement, or NK cells (remember the ADCC)

*Or the antibody can neutralize the antigen (neutralizing =prevention its binding to its receptor).



The end

Test yourself:

1. Plasma cells are
   a) lymphocytes which are short lived
   b) lymphocytes which are long lived
   c) lymphocytes involved in non-specific defense
   d) none of these

2. Which of the following statement is incorrect regarding plasma cells
   a) plasma cells are the effector cells
   b) plasma cells secretes antibodies
   c) The precursor of plasma cell is B cell
   d) plasma cell has surface receptors

3. B cells upon activation by antigens
   a) undergo clonal expansion followed by clonal selection
   b) divides continuously
   c) undergo clonal selection followed by clonal expansion
   d) secrete antibodies

4.The specificity of an antibody is due to
   a) The heavy chains
   b) The variable portion of the heavy and light chain
   c) It's valence
   d) The Fc portion of the molecule

5. The variable region of the heavy chain and the variable region of the light chain in a given antibody molecule:
   a)are identical to each other in primary amino acid sequence.
   b)are each encoded by single germ-line gene segments.
   c)in combination with one another, define the specificity of the antibody for an antigen.
   d)contain the hypervariable and constant region portions of an antibody molecule.

6. Which of the following is not a feature of a secondary immune response to an antigen, when compared to the first response to the same antigen?

a) Antibody is generated without T-cell help.
b) More antibodies are produced.
c) The antibody produced has greater affinity for the antigen .
d) The antibody is generated faster.

7. Which of the following immunoglobulins is responsible for most allergic and hypersensitivity reactions?

a)IgA
b)IgG
c)IgE
d)IgD

8. Fc region is involved in

a) cell surface receptor binding
b) complement activation
c) determining diffusivity of antibody molecule
d) all of these

9. Which is the Ig that can cross placenta and provide passive immunity to new borns and which is the first to reach the site of infection?

a) IgM , IgG
b) IgG , IgM
c) IgA , IgM
d) IgE , IgG

10. Which of the following are antigen-independent steps in B cell differentiation?

a) Expression of surface IgM.
b)Expression of surface IgG.
c)Affinity maturation.
d)Secretion of large amounts of soluble Ig molecules.
e)Both A and C are correct.

11. Which of the following statements concerning Ig genes are true?

a)Each light chain isotype is encoded in a separate gene cluster.

b) For the heavy chain and the kappa light chain, the V-region contains DNA segments for many different V-domains.

c)The constant regions for the heavy chains are encoded on a different chromosome from the variable regions of the heavy chain.

d)The complete protein sequence of a VL or VH domain is encoded in a single gene segment.

e) Both A&B are correct

1	2	3	4	5	6	7	8	9	10	11
A	D	C	B	C	A	C	D	B	A	E

Best of luck!

For better understanding of VDJ Recombination (from Dania)

m-line DNA on **Progenitor B cells** provide H and L chain locus – directing the synthesis of Heavy and Light chain respectively. They have V, (D) and J segments in un-rearranged form.

- H chain (chromosome 14) locus has 3 regions – V, D, and J
- L chain (chromosome 2 for κ and 22 for λ) locus has 2 regions – V and J (no "D")

VDJ rearrangement occurs during the maturation of B cells.

- VDJ rearrangement on 'H' chain occurs in **Pro-B cells** to produce Heavy chain.
- VJ rearrangement on 'L' chain occurs in **Precursor B cells** to produce Light chain.

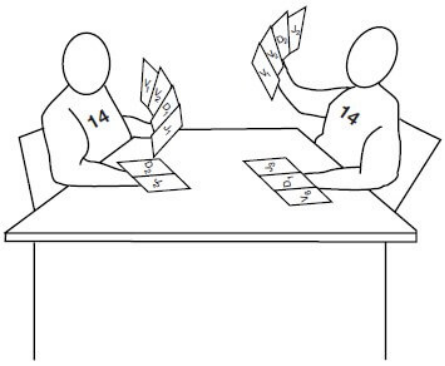
After the re-arrangement, the B cells are now called **Immature B cells**.
Trascription of Immature B cell DNA to RNA followed by *RNA splicing* of introns occur. This brings the Combined V(D)J together with "C" segment that encodes for Constant region of heavy or light chains and forms mRNA.
This is followed by Translation to produce proteins – specific heavy chain and light chain.

V(D)J Rerrangement

Let's start with the full forms for the "V", "D" and "J" segments:

- Variable (V) segment
- Diversity (D) segment
- Joining (J) segment

Now, let us simulate a card game between player no. **14** from two different teams (chromosomes).



- Each players have several variety of **V**, **D** and **J** printed (example: V1, V2, D3, J2, etc.) on the cards. Both have the same **C** printed card.
- Each player tries to rearrange 4 cards each from V, D and J and C (gene segments) until it finds an arrangement that works.
- The first player (chromosome) to do wins and the losing player chromosome stops.
- The players first choose one each of the possible D and J cards and keep them together (by deleting the DNA sequences in between them).
  - **DJ rearrangement occurs first**
- Then one of the many V cards is chosen, and this "card" is kept together with the D and J cards previously matched (again by deleting the DNA in between).
  - **VDJ rearrangement occurs second**
  - **VDJ-C rearrangement occurs at last**
- The player to make these arrangement first, shows the card to the referee and the referee analyzes if the arrangement works or not. If the arrangement is wrong, the referee asks the player to **STOP** (protein translation stops when ribosome meets one of the three stop codons). Hence, the result of this arrangement is useless.

- In fact, you can calculate that each player only has about 1 chance in 9 of assembling a winning combination of gene segments that will produce a full- length Hc protein. Immunologists call such a combination of gene segments a **productive rearrangement**.
- If one of the chromosomes (players) that is playing this game ends up with a productive rearrangement, that chromosome (player) is used to construct the winning Hc protein.
- This heavy chain protein is then transported to the cell surface, where it signals to the losing player (chromosome) that the game is over and the he stops.
    - **Allelic exclusion:** Once a functional product has been achieved by one of the rearrangements, the cell shuts off the rearrangement and expression of the other allele on the homologous chromosome.

Each gene segment (V, D, and J) has an adjacent **Recombination Signal Sequence** (**RSS**)

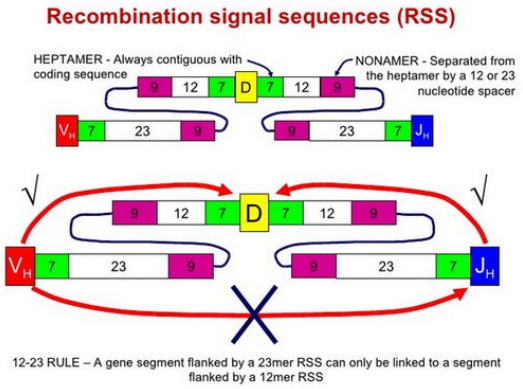
- at the 3′ end of each V segment
- at both ends of each D segment
- at the 3′ end of each J segment

12/23 rule

The configuration prouced by RSS acts as a target for Recombinases. These are recognized by two proteins encoded by two **R**ecombination **A**ctivating **G**enes:

- ***RAG-1*** and
- ***RAG-2***

The RAG-1 and RAG-2 proteins cut through both strands of DNA at the RSS forming double-stranded breaks (DSBs).



The cut ends are stitched together (ligated) to form:

- a **coding joint** (D-J or V-DJ for heavy chains; V-J for light chains)
- a **signal joint** (usually a loop of DNA deleting all the intervening DNA initially present between the 2 gene segments chosen)

Source: https://free.facebook.com/l.php?u=http%3A%2F%2Fepomedicine.com%2Fmedical-students%2Fvdj-somatic-recombination-made-easy%2F&h=ATOGhx_VaPjVrRU2pn3w1m1u9_b85A2q0Erze_kXt6xN6p3S4AdhP0tOSIyw0pF5mIGYGZxiZT12SI-yi-r5taYdEzKtBw1jCNbJ-gINzv-rgY4RrF-KLo7NBlMq-XG_aJF2IeqCJEzw&s= 1