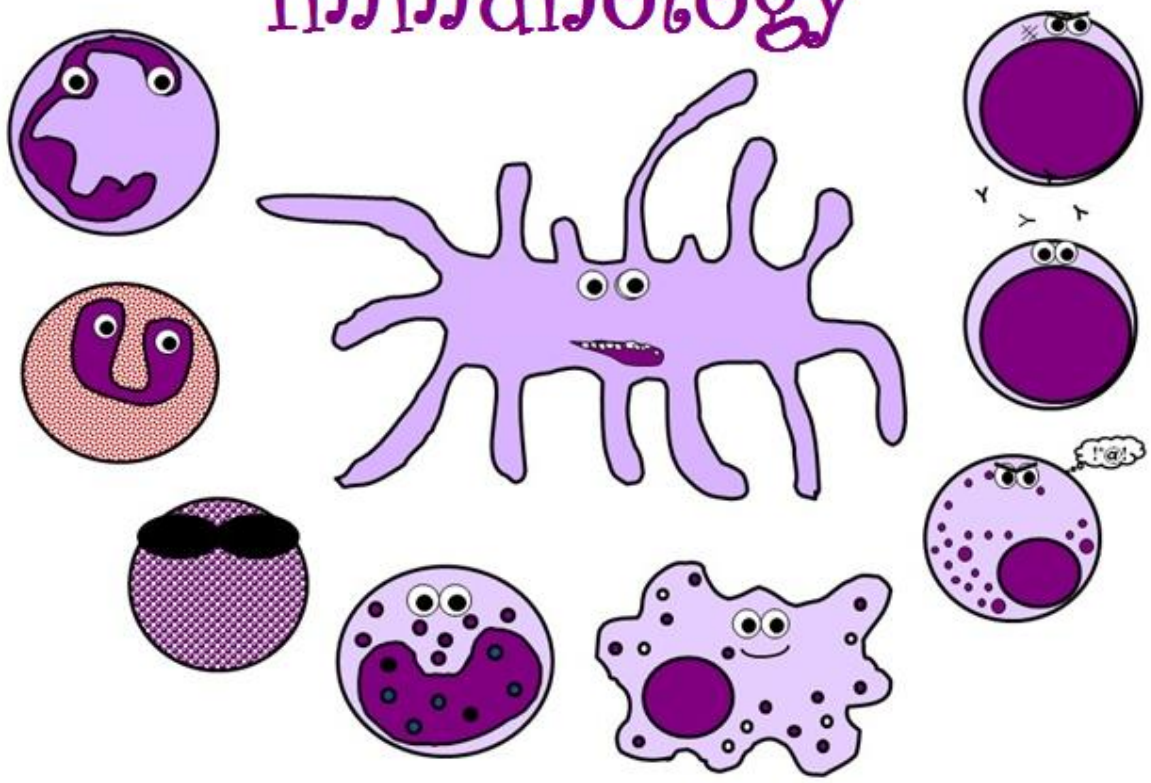
Immunology



◉Sheet ○Slides

Number: 4

Subject: B cells and Antibodies

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**** This sheet was written according to the recording that belongs to section 2.**

**** All the content of the slides of this lecture is included within this sheet.**

❖ Before we start,

Key points from the previous lectures:

- The immune system has two major divisions; the innate immune system, and the adaptive immune system.
- The **innate immune system** consists of:
  - The complement system – Classical, Alternative, and Lectin activation pathways.
  - Professional phagocytes – Macrophages and Neutrophils.
  - Natural killer cells
- The **adaptive immune system** consists mainly of
  - B cells
  - T cells

❖ Topics of this lecture:

* Introduction: the adaptive immune system.

* B cells, BCR signaling, and B cell activation.

* B cell maturation.

❖ Introduction: The Adaptive Immune System

- The adaptive immune system evolved many years after the innate immune system.
- Most invertebrates do not have an adaptive immune system (they only have an innate immune system).
- Due to the complexity of the evolving living organisms, and the complexity of the pathogens that could cause disease to these living organisms, there was a need to develop another immune system –especially, to fight viral infections.

→ The adaptive immune system evolved at the level of vertebrates (to be more specific, at the level of fish, where specialized cells –like T and B cells – were first seen).

Figure (1)

❖ B cells, BCR signaling, and B cell activation

I. About the B cell:

- B cells –like other blood cells – are made in the bone marrow.
- The letter "B" comes from "the **B**ursa of Fabricus".
  (not from "the Bone marrow" as most people think)
- B cells select gene segments to make immunoglobulins (Igs).
  This point was discussed in the first lecture.

Bursa of Fabricus:

- A lymphoid organ in birds.
- It is the first lymphoid organ to discover B cells in.
  → B cells were named after this structure.
- Although other living organisms like human beings do not have "Bursa of Fabricus", the term "B" cell stayed the same.

A quick reminder:

How is it possible to generate around 100 million different antibodies -that are needed to cover antigen diversity- from only a limited number of genes?

The answer is → the **modular design** proposed by Susumu Tonegawa.

Recall: the VDJ recombination, discussed in sheet 1.

- The synthesized immunoglobulins will either
  - get inserted in the plasma membrane (immunoglobulins on the surface of the B cell that can function as receptors). *Think of these immunoglobulins as the eyes of the B cell used to see its antigen.*
  - Or will be secreted into the tissues or circulation in the form of secreted antibodies, *as shown in figure (2).*

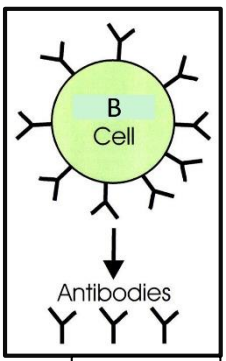


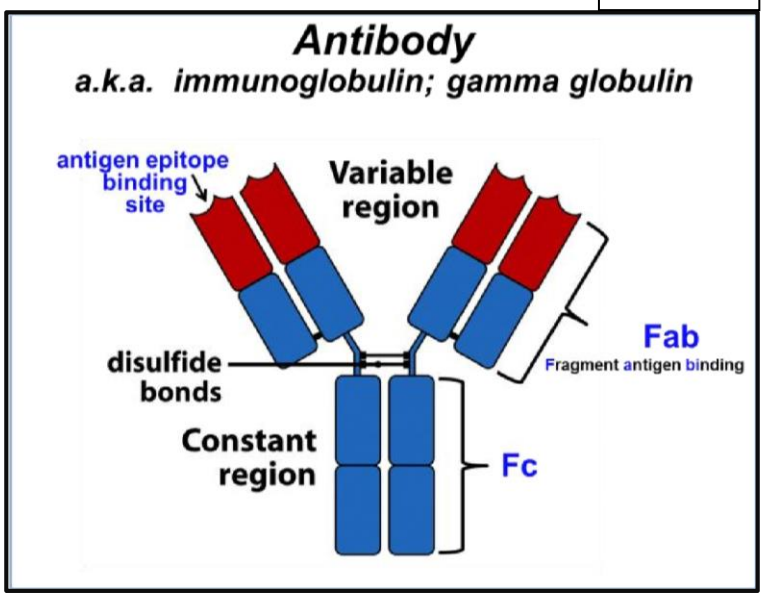
Figure (2)

Note: the state of the B cell is what determines the fate of the immunoglobulins. For example, most of the immunoglobulins of a naïve B cell will be on the surface, whereas most of the immunoglobulins of a plasma cell will be in the form of secreted antibodies.

II. B cell receptor

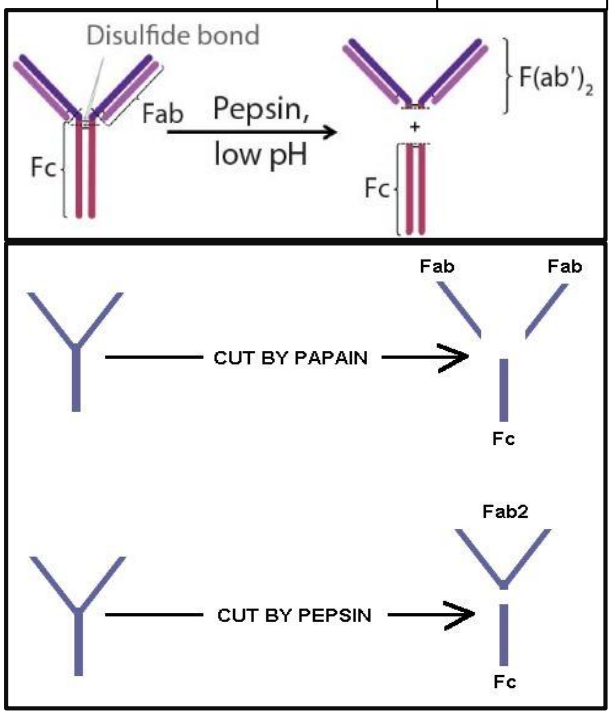
- The B cell receptor (BCR) is an immunoglobulin on the surface of the B cell.
  Figure (3)
- The structure of an antibody (or immunoglobulin):
  - It's bifurcated in shape (a Y-shaped molecule with a stalk and two arms).
  - Consists of two identical heavy chains (the longer chains), and two identical light chains (the shorter ones).
  - The tail like part *(or the stalk)* of the immunoglobulin is called The Fc portion. (the letter "C" refers to "crystallizable" and not "constant").
    While the two arms are termed the Fab portions (fragments of antigen binding).

Figure (3)



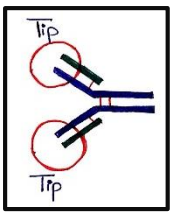
- The enzyme pepsin cuts one of the two sulfide bonds joining the heavy chains. The result of the cut will be two pieces: *(the extra figure)*
  - A piece consisting of the two Fab regions.
  - The second piece is the Fc region.

Extra figure



Note: in the lecture, Dr. Issa mentioned –accidentally I suppose – that the tail-like region (Fc) is the constant region, and the two arms (Fab regions) are the variable regions, but for the purpose of accuracy, please read the following (and keep in mind that Dr. issa also agreed on the following being more accurate):

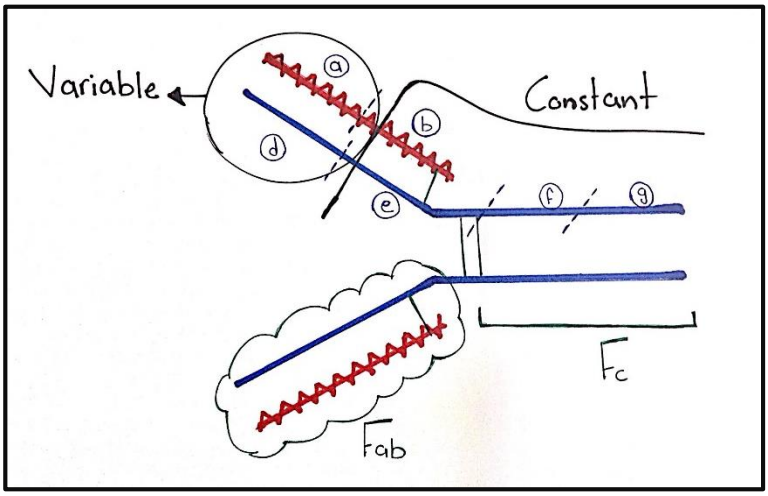
- The antigen binds to the antibody at the tips of the Y-shaped molecule. Each tip is composed of part of the heavy chain, and part of the light chain.



  - Different antibodies differ from each other in the amino acid <u>sequences near the tips.</u>

    ➔ **Variable regions**
  - Whereas the other part of the antibody that does not bind to the antigen is the same in different antibodies ***<u>belonging to the same class.</u>***

    ➔ **Constant regions**
- A general rule (but not 100% accurate):
  - The light chain is composed of 2 domains, one of them is variable (“a” in the figure to the right) while the other one is constant (“b”).
  - The heavy chain is composed of 4 domains. One of them is variable (“d” in the figure), while the other three (“e”, “f ”, and “g”) are constant.



- The previous two points were not mentioned by Dr. Issa in the lecture. They were added for better understanding. So, what you **have to know** is the following:
  - 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.

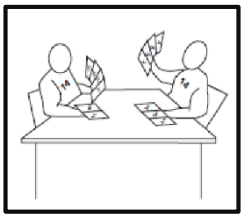
- The constant regions of the heavy chains are <u>constant for each isotype</u> (the IgG has a certain structural sequence as its constant region in the heavy chain. The IgM has a different structural sequence than that of IgG as its constant region in the heavy chain, but it is the same in all IgM molecules, and so on).
  → This principle is used in laboratories. For example, when we want to detect certain IgM molecule against a certain pathogen, but not IgG, we depend on the constant regions that differ between IgM and IgG.

- The variable regions differ from one antibody to another.
  → This is the basis of the ability of a certain antibody to recognize only a single antigen.

- Immunoglobulin genes are found on chromosome 14.

Question: <u>there are two copies of each autosomal chromosome in an individual (one maternal copy, and the other is paternal). Which copy is the one that will be utilized to synthesize the immunoglobulins?</u>

- Blocking one of the copies of the immunoglobulin genes completely and permanently will not result in the optimal benefit, because using both –each at different times- adds to the variety of immunoglobulins that could be generated *(which is a good thing in this context).*
- What happens in reality could be thought of as a card game between the two chromosomes (each chromosome will take its shot and try to form the antibody. It will either succeed or fail).



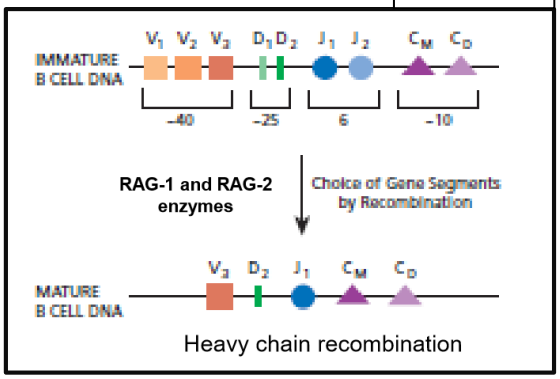
- The first copy of Ch.14 will give it a try.
  - If the series of processes involved in gene rearrangement to form the heavy chain succeed in synthesizing the heavy chain, the same copy of Ch.14 will proceed and try to build the light chain.
  - If the light chain also got synthesized successfully, the following questions should come to mind:
    Did the heavy and light chains associate successfully forming the immunoglobulin? If yes, did the antibody get successfully loaded on the B cell surface?
    - ➔ <u>If YES</u>, the other copy of Ch.14 will be inactivated, and this B cell will continue synthesizing only this antibody.
    - ➔ <u>If NOT</u> (or if any of the previous steps fail), the other copy of Ch.14 will now take its shot and give it a try starting the process all over again.
    - ➔ <u>If BOTH copies fail</u>, the B cell will receive a signal to die by apoptosis.

About the gene rearrangement that takes place in the heavy chain genes during maturing of the B cell: *(figure (4))*

- As mentioned in the first lecture, the location on Ch.14 that contains the genes of the heavy chains of the immunoglobulins in the genome of an immature B cell has the following segments or sites:
  - **V**: variability region – includes 40 different types (or genes)
  - **D**: diversity region – includes 25 different types
  - **J**: joining region – includes 6 types
  - **C**: constant region (determines the class of the antibody)

- One of the copies of Ch.14 will try – *according to the modular design* – to induce VDJ recombination in these genes, so that certain parts will be cut off and removed away, and other parts will be fused together to give in the end (in the genome of the mature B cell) a sequence that includes a single type of V, a single type of D, and a single type of J, followed by the constant region related to IgM and IgD *(note: IgM and IgD are the first antibodies to be synthesized by a B cell).*

Figure (4)



➔ The end result will be the formation of an antibody with certain specificity.

- The two main enzymes responsible for the recombination processes are RAG 1 & RAG 2 enzymes
  (RAG 1&2 enzymes are encoded by the "**R**ecombination **A**ctivating **G**enes 1& 2")
  - RAG 1 and RAG 2 enzymes bind to certain sequences called "RSS (**R**ecombination **S**ignal **S**equences)" found adjacent to the gene segments that can be combined.
  - These RSS sites are restriction specific sites. They are designed to be recognized by RAG1 and RAG 2 enzymes that will bind to them.
    ➔ Then, a loop in the DNA will be formed, and a certain region will be cut, while other regions will be fused to end up with the final arrangement.

- **Note**: Dr. Issa briefly mentioned the previous point and did not mention further details. For better understanding, you can watch the two-minute video in this link https://m.youtube.com/watch?v=QTOBSFJWogE
- **Note 2**: Dr. Issa said that the enzymes RAG 1 and RAG 2 will be discussed in more details in the next lecture (the case study about "Omenn syndrome")

Omenn syndrome results from defects in RAG 1 and RAG 2 enzymes

- ➔ Formation of antibodies will be defective, and the expected variability will not be achieved.
- ➔ Dangerous immunodeficiency

- **Note 3**: an example on a reason that could cause the failure of a copy of Ch. 14 in carrying out the proper rearrangement:
  - ➔ During the cutting processes, a termination sequence in the DNA may be produced
  - ➔ This would result in a premature stop codon in the mRNA
    (Shorter than normal mRNA will be produced)
  - ➔ Heavy chain formation will not be complete
  - ➔ Failure

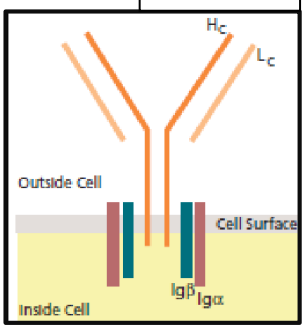
One B cell → One antibody (one heavy chain type + one light chain type)

- In other words, a B cell that produces an antibody against a certain antigen cannot suddenly start producing another antibody against another antigen. (there's some sort of commitment).
- A B cell is designed to recognize one antigen. If it encounters this antigen during its life time, it will form antibodies against it. If not, the B cell will not produce antibodies against another antigen.
- Recombination options are so many. Antibodies can be produced to every organic molecule available.

III. BCR signaling

Figure (5) shows a BCR on the surface of the B cell.

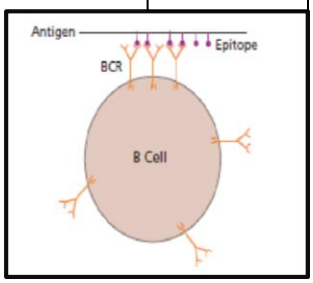
Figure (5)



- Note that a very short part of the Fc portion of the immunoglobulin reaches the interior of the cell. This small part is not enough for transmitting the signal to the nucleus of the B cell and informing it about the engagement when it takes place.
  → That's why Accessory proteins are required.
  (each accessory protein transmits the signal to the following accessory protein –like a chain)
- The accessory proteins in the B cell are of two types; **immunoglobulin α and immunoglobulin β.**
- When the antibody encounters its antigen, certain conformational changes will take place, leading to activation of Igα and Igβ.

→ Igα and Igβ will eventually cause the signal to be transmitted to the nucleus to inform it that the receptor has encountered its antigen and engagement (binding) occurred.

Figure (6)



- Although we said that accessory proteins are required for signaling, **Cross Linking is more important**. *(Figure (6))*
  - The antibody is not fixed in the cell membrane (phospholipid bilayer). The antibody can move freely within this phospholipid bilayer.
  - As you can see in the figure, many antibodies are distributed on the cell surface trying to find their antigens.
  - Although we usually say that "An antibody binds to an antigen", it is more accurate to say that it binds to the **epitope** in the antigen.
    (the antigen is a big unit that includes repeated small units called epitopes).
  - The reason why antigens can trigger immune response is that they have repeated units of epitopes that the antibodies can bind to.
  - The process in which antibodies become in close proximity to each other when they are bound to the antigen –as shown in the figure- is called "Cross Linking of Antibodies".
  - Cross linking of antibodies is essential (but not sufficient alone, as we will see later) for activation of B cell signaling.

In the previous lecture, Dr. Issa talked about the interaction between the innate and adaptive immune systems.

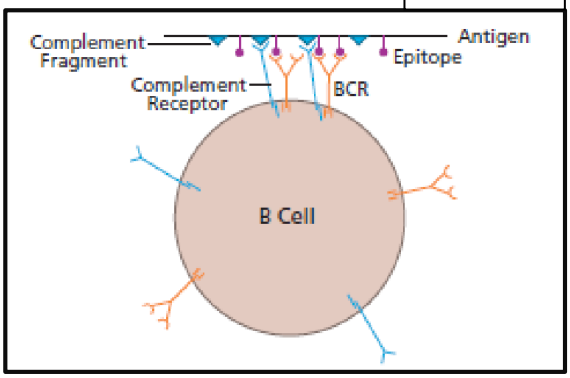
- The complement system –part of the innate immune system – interacts with the adaptive immune system and other cells of the immune system.

A quick reminder:

- The Complement system is capable of carrying out the function of "opsonization" to enhance the phagocytic function of macrophages and neutrophils.
- Macrophages *(and certain other cells)* have receptors for complement on their surfaces.

- B cells are also capable of seeing the complement. *(Figure (7))*
  - As mentioned above, the BCR binds to epitopes of the antigen.
  - If the bacteria (the antigen) has complement on its surface, the complement will bind to a complement receptor from the B cell.
  - The Complement – Receptor engagement tightens the BCR-Epitope binding and enhances the BCR signaling to fight the invading organism.

Figure (7)



<< Opsonization by complement system greatly amplifies BCR signaling>>

IV. How are B cells activated?

- We mentioned before that the immune system cannot be activated by one step (to avoid autoimmune reactions).
  In other words, even if a B cell encounters its antigen, certain proper conditions must be provided in order for the B cell to become activated.
  ➔ That's why we need <u>"The Two-Step Activation System":</u>

1) BCR engagement and clustering
This step –alone- is not sufficient for B cell activation. We need another signal.

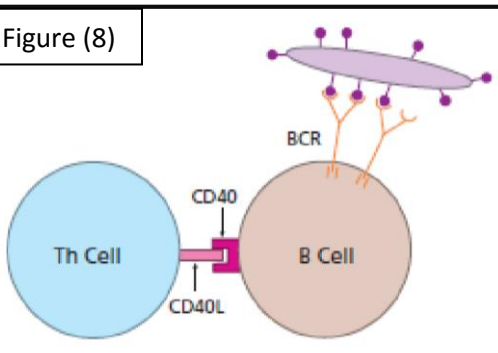
2) Co-stimulatory signal
This signal is usually –*not always*– provided by T cells.
Note: T helper cells play a key role in the activation of the immune system

The fact that engagement alone is not sufficient and that two signals are needed, constitutes a self-guard mechanism in the immune system to ensure that activation will occur only if it's favored.

<u>The co-stimulatory signal can be:</u>

a. <u>T – cell dependent</u>
As shown in figure (8), a molecule termed "CD40 Ligand (CD40L)" binds to its receptor (CD40) on the surface of the B cell.
➔ T helper cell will encourage the B cell (co-stimulate it) so that B cell activation can take place.

Figure (8)



b. T – cell independent
in this case, cytokines – like IFN-γ – play a key role in the co-stimulatory signal.

Cells that can produce IFN-γ include:

- NK cells
- T helper 1 cells
- Gamma delta T cells

Question: why aren't all co-stimulatory signals T – cell dependent?
(In other words, what is the purpose of T – cell independent activation?)

- T cells can only recognize peptide antigens (antigens of protein origin).
- Not all antigens entering the body are composed of peptides. For example, many bacteria and viruses have carbohydrate moieties or fat molecules as their antigens.
  - ➔ The T- cell independent activation pathway allows B cells to be activated even if the antigen was not of protein origin.
    (this is one way by which the immune system can deal with different types of antigens that enter the body)

Note: another benefit could be that if the T cells are defective, the B cells could still be activated through the T-cell independent pathway.

❖ B Cell Maturation

What happens when the naïve B cell encounters its antigen?
3 main steps or stages occur:

1. Class switching
   The first Immunoglobulin produced by a B cell is the IgM, but later on, the need of other isotypes of immunoglobulins will induce "class switching".
2. Somatic hyper-mutation
   After B cell formation and class switching, mutations in the genome occur to aid in the "fine-tuning" of the antibody that will be formed.
3. Career decision (plasma cell or B memory cell?)

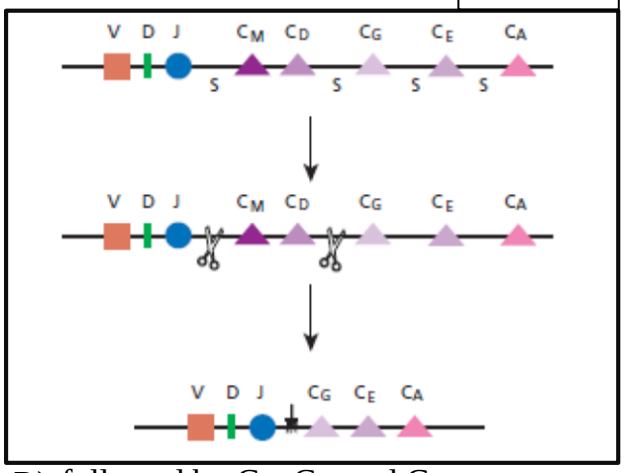
I. Class switching

- Class switching is changing the antibody class from IgM or IgD (that are present initially) to IgG, IgA, or IgE.
- Why?
  - Each antibody class is better than other classes in carrying out certain functions.
  - According to the conditions, the nature of the invading organism, and the nature of the required health state, class switching will take place to produce the proper Ig type.

Note: it's not possible to produce an antibody that is capable of carrying out all functions (opsonization, complement activation, and neutralization) effectively in a high degree. This is due to the fact that these functions run against each other, structurally.
For example, certain sequence in part of the structure of the Ig could be responsible for opsonization process, but if another sequence is present in this part, it might be more suitable for the Ig to carry out another function.

- The mechanism of class switching (at the level of the DNA):
  - We said before that the genes of the heavy chain include the V, D, J regions, followed by the constant region *–as shown in figure (9).*

Figure (9)



  - The constant region starts with C_M and C_D
    (C: for constant, M: for IgM, D: for IgD, signifying that these segments are present for the production of IgM and IgD), followed by C_G, C_E, and C_A.

  - As long as the region containing C_M and C_D segments is present, the B cell is an IgM-IgD producing B cell. (this B cell exposes IgM molecule on its surface).

The significance of IgD in immunology is still not clearly understood, and the degree of its expression is very low, that's why we're mostly concerned with IgM. However, as a general rule, IgD is usually present with IgM.

  - If a class switch from IgM to IgG is required:
    - Certain enzymes will cut the region containing C_M and C_D, and remove it.
    - The VDJ region will be fused with the other region that starts with C_G.
      → the genome formed is actually a signal to start producing IgG.
  - Further processes of cutting will take place if IgE or IgA is needed.

Important note: when we say V**D**J recombination, we are talking about the heavy chain. (the light chain lacks the D region. It only has V, J, and the constant region) ----*figure (10)*

(In the light chain genes, the recombination mechanism is similar, but without the D region.)

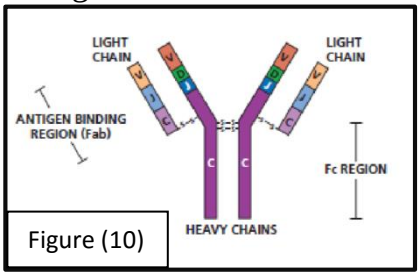
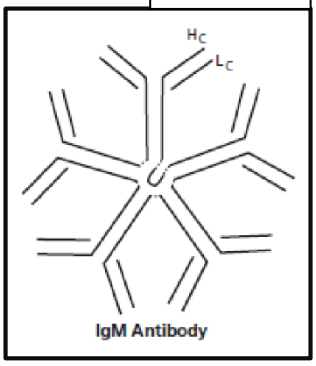


Figure (10)

Antibody Classes

(1). IgM class

Figure (11)



- IgM is a pentamer (five molecules associate with each other through their Fc regions to form a pentamer, *as shown in figure (11)*).
- Functions include; opsonization and activation of complement.

- The IgM pentamer is very efficient in triggering the classical complement pathway through bringing C1 molecules in close proximity.
  (in the previous lecture, we said that C1 molecules by which the classical pathway starts, need 2 sites to bind)
  ➔ The presence of so many sites (in the IgM pentamer) makes IgM a very potent activator of the complement system.
  This makes sense, because we said earlier that we need the complement system to act first (as part of the innate immune system), and the first antibody present is IgM → IgM must be a good complement activator.
- C1s binds to the Fc portion, gets activated, and subsequently activates C3 convertase (cleaves C3 into C3a and C3b) 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 does the IgM class (not the IgG) activate the complement first?
  Because IgM is the first antibody present, so it must be able to activate the complement system. *(to save time)*

- IgM is a better complement fixer than IgG, and it has a better neutralizing ability.
  - The better neutralizing ability is dictated by the bulky structure of the IgM pentamer that would prevent the virus –for example – from binding or entering the cell (it will hinder the movement and action of the virus).

(2). IgG class

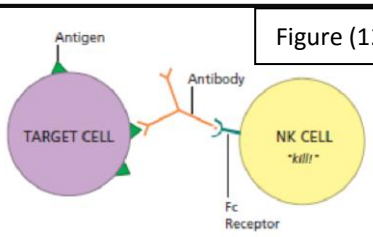
- Called Gamma Globulins
- Bad complement fixers
  There are subtypes, some are better than others in complement fixation, but in general, IgG class is not very effective in complement fixation.
  (remember that we said in previous lectures that at least, 2 IgGs are needed for activation of complement).
- Good virus inactivators.
- IgG can cross the placenta
  - ➔ Significance: it's the only class that can provide immunity from the mother to the fetus during pregnancy.

- Half-life: about 3 weeks (21 days)
  Which is relatively long, and this is consistent with its important function as being the only class that can cross the placenta and provide immunity to the fetus.

The half-life of IgM is 5 days only

- There are four subclasses of IgG: IgG1, IgG2, IgG3, and IgG4.
  - ➢ IgG 1 is a good opsoniser. That's why macrophages and neutrophils have receptors for IgG1-Fc (the Fc region of IgG1).
  - ➢ IgG 3 fixes complement better than other subclasses. That's why natural killer cells have receptors for IgG3-Fc
    - → The NK cell will get activated when binding occurs (*figure (12)*)
      This process is known as "Antibody-Dependent Cellular Cytotoxicity (ADCC)"

Figure (12)



Antibody-dependent cellular cytotoxicity (ADCC)

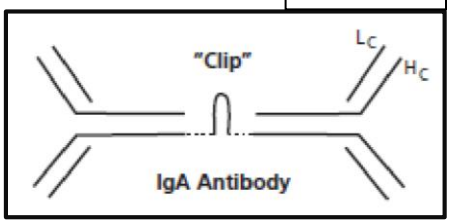
Note: when NK cells were discussed in the second lecture, we said that there are activating receptors and inhibitory receptors. However, this is not the only way by which a natural killer cell can work. Another way is the presence of a target cell with antigens on its surface:

- ➔ IgG will bind to the antigen, thus giving a signal to the NK cell through an Fc receptor found on the surface of the NK cell.
- ➔ Activation of the NK cell to kill the target cell.

The previous is another example on the cooperation between the innate immune system (through NK cell) and the adaptive immune system (through antibodies).

(3). IgA class

Figure (13)



- Mainly, found in secretions, and it is the main antibody class that guards the mucosal surfaces of the body.
- Two IgA monomers are associated together in a dimeric structure (with a "clip" or loop structure between them).
  - ➢ Its structure –mainly, the flexible loop – facilitates its transport to the intestines (from blood to the mucosal tissues) and its structure also makes it **resistant to acids and enzymes**. That's why it's very well suited to be in the digestive system.
  - ➢ The dimeric structure *(through working as a clamp)* helps clump bacteria together to be swept out with mucus or feces.
- IgA gets secreted into the milk of nursing mothers.
  This is one of the reasons why women are encouraged to breastfeed their babies.
  - ➔ IgA provides immunity to the newborn in his first few months.
    (the immune system in the newborn is not fully developed, and is incapable of synthesizing its own antibodies until the 6th month of age).
- IgA is a bad complement fixer.
  Being a bad complement fixer is actually a good thing, because we want calm immune responses in the intestine. If the IgA was a good complement fixer, severe inflammations in the intestines will easily occur (like celiac disease, inflammatory bowel disease, ..., etc.).

There are certain residues in the Fc portion that can bind C1q.

These residues are found in IgM and IgG, but not IgA. **That's why IgA is a bad complement fixer**

(4). IgE class

- The main purpose or function of the IgE class is to fight parasitic infections. (not for allergy. Allergy is considered one of its side effects or disadvantages).
- IgE antibodies are very well designed to bind to parasites.
- During a parasitic infection:
  - ➢ IgE is made, its Fab portion binds to the parasite.
  - ➢ The Fc portion of the IgE is very effective in binding to mast cells
    - ➔ Binding will cause the release of histamine and other cytokines like TNF (Tumor Necrosis Factor) and IL-3,4,5, that are very important for killing parasites.

Parasites have existed for millions of years. That's why our bodies must have mechanisms against them.

- 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.

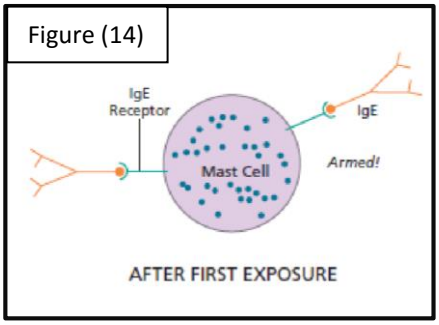
➢ If a person has allergy towards certain substance, the first exposure to this substance will not result in a severe allergic reaction. For example, if a person is allergic to peanuts, he will not develop an anaphylactic shock directly after the first time he eats peanuts. Why?

Because the mast cells are not primed (armed) yet.

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. *(figure (14))*

→ the mast cell is now armed.

Figure (14)



➢ Upon second exposure to the same allergen *(figure (15))*, 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.

Figure (15)

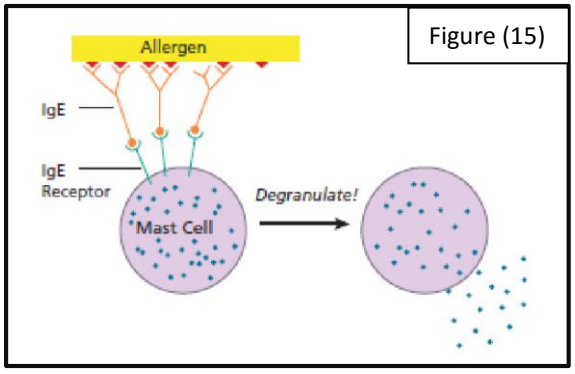


Table (1): Summary of Antibodies classes and functions

Table (2): functions and properties (represented in degrees) of different antibody classes.

(Dr. Issa did not focus on the details in table (2))

Additional piece of information:

(mentioned by Dr. Issa)

Nowadays, there are attempts to treat allergies with very low doses of the allergen over a long period of time (to cause desensitization and cause a switch in the B cells from IgE to IgG)

(1)

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

(2)

Function	IgM	IgD	IgG1	IgG2	IgG3	IgG4	IgA	IgE
Neutralization	+	–	+++	+++	+++	+++	+++	–
Opsonization	–	–	+++	*	++	+	+	–
Sensitization for killing by NK cells	–	–	++	–	++	–	–	–
Sensitization of mast cells	–	–	+	–	+	–	–	+++
Activation of complement system	+++	–	++	+	+++	–	+	–

Property	IgM	IgD	IgG1	IgG2	IgG3	IgG4	IgA	IgE
Transport across epithelium	+	–	–	–	–	–	+++ (dimer)	–
Transport across placenta	–	–	+++	+	++	++	–	–
Diffusion into extravascular sites	+/–	–	+++	+++	+++	+++	++ (monomer)	+
Mean serum level (mg/ml)	1.5	0.03	9	3	1	0.5	2.5	5×10^{-5}

What triggers class switch?

Cytokines produced by T helper cells

- Parasitic infections induce immune cells to produce IL-4 and IL-5 → these favor class switch to **IgE** that is needed to fight parasites.

- Entry of bacteria or viruses leads to the production of IFN-γ → INF-γ is a potent class switch signal to **IgG** that is ideal to fight bacteria and viruses.

- Common cold (certain viral infections) or intestinal infections lead to the production of TGF-β → TGF-β favors a switch to **IgA**.
  TGF- β = Tumor Growth Factor β

II. Somatic Hypermutation

- Mutation rate in our genome is low (approximately 1 : 100,000,000 base pair per replication cycle).
- In B cells: after their formation, selection of VDJ segments, and class switch, the VDJ region undergoes very high mutation rate (as high as 1 : 1000 b.p. per generation)
  - The rate of mutations increases <u>on purpose</u> so as to affect or change the antigen binding site slightly.
  - The somatic hypermutation affects the affinity of the Fab region of the B cell antibody.
    → the affinity of the antigen binding site will either increase, decrease, or stay the same.
- The antibodies that can bind with higher affinity, due to the mutation, give survival signal to the B cell (B cells with higher affinity are better stimulated by antigen and so proliferate more and take over)
  - In other words, the result of somatic hypermutaion is selecting B cells that are able to exert better binding to the antigen.

The antibody gets modified (to become better adapted to fight invaders) by two ways :

1) <u>Class switch</u>
   Change in the Fc region *(to be more accurate: in the constant region of the heavy chain)* to produce an antibody that is better in dealing with the situation of interest.
2) <u>Somatic hyper mutation</u>
   Change in the Fab region *(to be more accurate: in the VDJ region)*

III. Career choice

The fate of the cell will either be a plasma cell, or a memory B cell.

- **Plasma cell**
  - Goes to the spleen or bone marrow and starts secreting high amounts of antibodies (up to 2000 antibody/second)
  - As a consequence of its function, it's Short lived (few days).
- **Memory cell**
  - A cell that remembers the first exposure for many years.
  - Defends against subsequent exposure. If the antigen enters the body again after 2, 3 or 4 years for example, the memory B cell (in which class switch has already occurred during the first exposure) will deal with the antigen quickly and differentiate into plasma cells that will produce antibodies.
    <u>Note</u>: the presence of memory cells is one of the principles of vaccination.

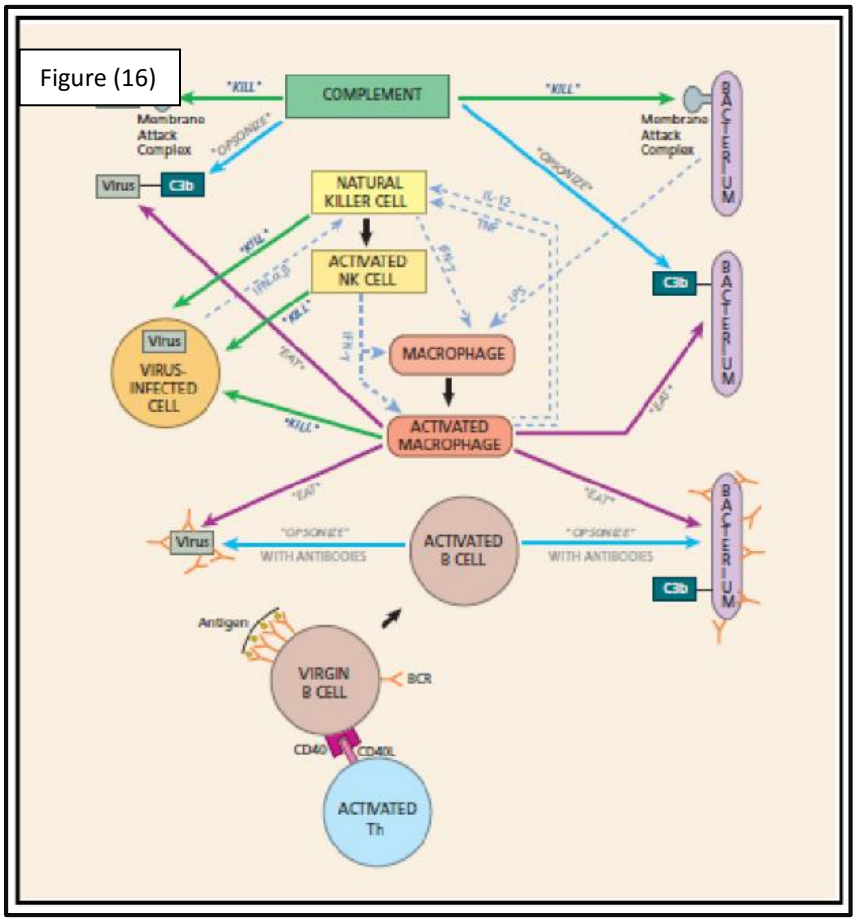
- The B memory cell needs T helper cell to develop (CD40L-CD40 interaction).

- **Note:** for class switch to occur, we need T helper cells. If T helper cells did not co-stimulate the B cell, the B cell will not be able to carry out the proper class switch, nor form B memory cells.

Figure (16)

This figure was discussed in the previous lectures, but now we add to it few points:
(the lower part of the figure)

- If a virgin B cell encounters its antigen (Recognition = the first signal), and then gets co-stimulated by the activated T helper cell (Co-stimulation via CD40-CD40L interaction = the second signal):
  - 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 in page 13)*.



I apologize for any mistake I may have made.

Wish you all best of luck **:D**