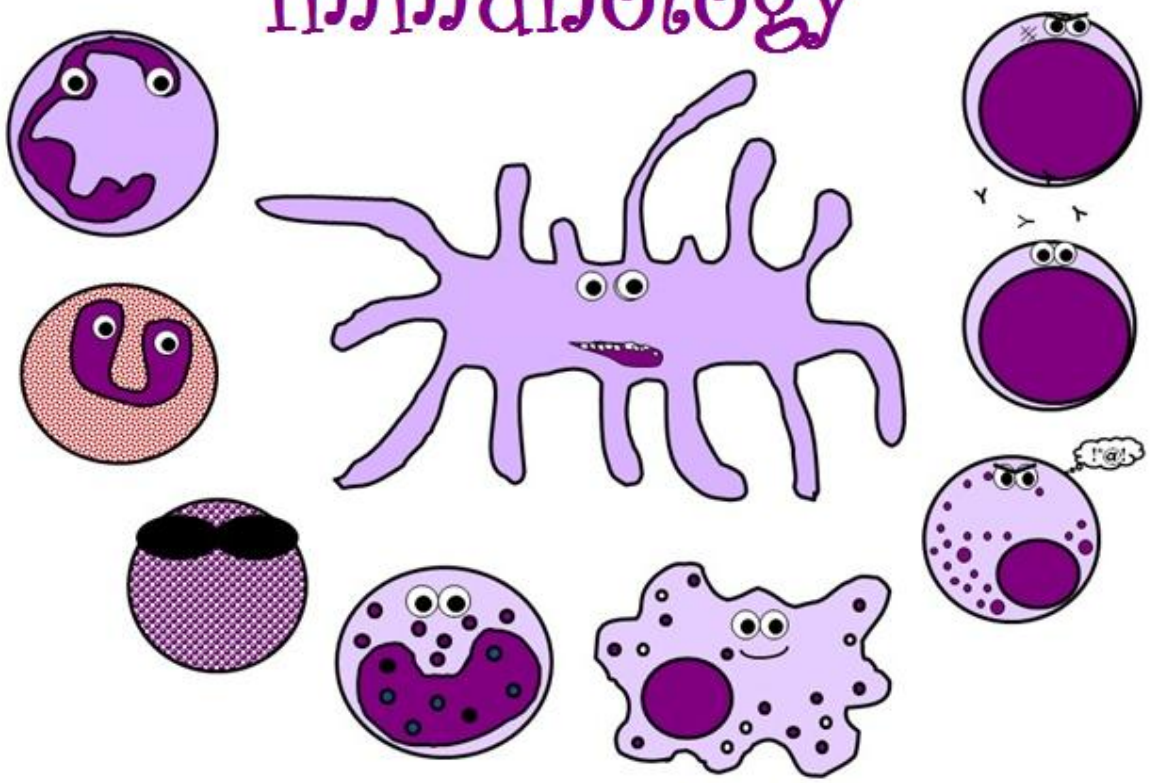
Immunology



Sheet Slides

Number: **6**

Subject: **Antigen presentation**

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Before we start ..

●This sheet was written according to the recording that belongs to section 1.

●Many explanations in this sheet are from evolutionary point of view. If you are creationist, you can skip it.

●The concept of Antigen Presentation is one of the most important topics in immunology for us as a doctors, so try to understand it 100% ..

I. Quick Revision

●Our immune system has two arms; ***innate*** and ***adaptive*** immunity:

- *Innate immune system (non- specific*); which is responsible for <u>fighting the foreign bodies instantly without being specific to any of them</u>, but being able to detect the pathogens that entering our bodies, and end the infection, or at least buying some time so that the adaptive system will be activated and will handle the infection.
- *Adaptive immune system:* B-Cells & T-Cells.

II. Antigen presentation

●Antigen presentation aims to <u>activate the adaptive immune response</u>, so it results from the interaction between two cells; an ***<u>antigen presenting cell</u>*** or ***APC*** (i.e *Macrophages, dendritec cell, or even B-lymphocytes*) and a ***<u>T-lymphocyte</u>***, which is the activated cell.

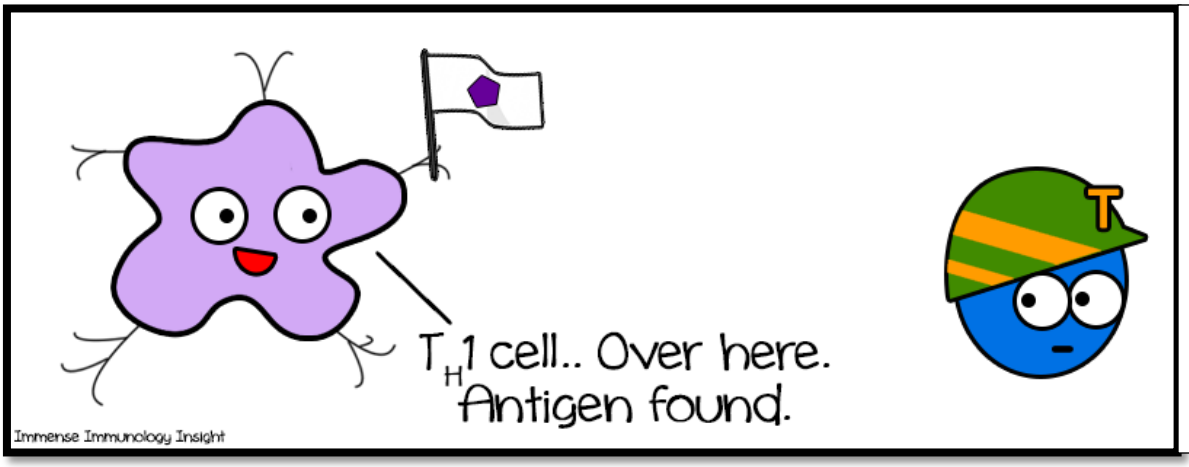

Figure 1: ***What's antigen presentation ?***

●What is needed to present antigen?

- We need the assistance of group of molecules, called ***MHC (Major histocompatibility Complex)*** or ***HLA (human lymphocyte antigen***.
- *MHC* is considered as the "whiteboard" of our immune system, that has the ability to present many molecules.
- As we will see at the very end of this lecture, such molecules are important in organs transplant, as it is used to determine the homology or ***compatibility*** between the donor and the recipient.

a) Structure of MHC molecules

●We have talked about the structure of *MHC* in the first lecture. Let's go back and review it:

●There are two types of MHCs:

- ***MHC class I:*** made of a long chain and small chain called beta2 chain micro-globulin
- ***MHC class II***: made of two peptides, an alpha chain and a beta chain.

●The interaction between *MHC* molecule an the antigen is similar to preparing a hotdog sandwich, If we compare *MHC* to a hotdog:

- MHC class I the peptide that sits in it is very short (only 9 amino acids), and it is very picky. The amino acids at the end are very specific. It is important to remember that the *MCH* class I presents its antigen to the cytotoxic T cells.
- MHC class II on the other hand has a longer peptide (20 amino acids), and the amino acids are more flexible on the sides and is seen by the T helper cell.

●Important note: ***beta 2 microglobulins*** are elevated in some cases of ***multiple myeloma***, that have a very bad prognosis.

b) antigen processing

●So by now, you know that *MHCs* present antigens, but what's this antigen?

- Antigens that are presented by *MHC* molecules are ***peptides***, which result from degradation of proteins.
- We have two pathways to degrade peptides; namely are ***Ubiquitin-Proteasome pathway*** and ***Lysosomal proteolysis*.**

1-Upiquten –protease pathway (see figure 2)

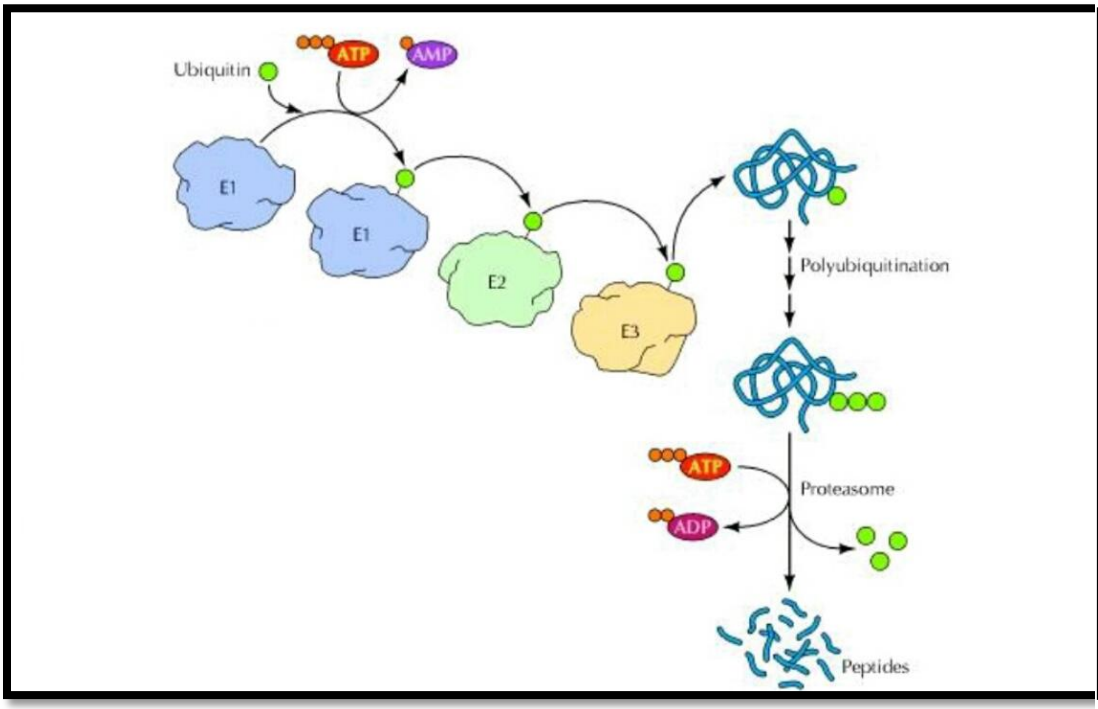

Figure 2: Upiquten – protease pathway

●*Ubiquitin* is a 76 amino acid polypeptide.

●It's highly conserved protein:

- There's no significant different in *ubiquitin* structure across individuals and even species.
- This means that this protein is highly important, thus conserved in all species during evolution.

●There's a series of enzymes (***E1, E2 and E3***) that activate *Ubiquitin* gradually:

- The last one (*E3*) is called ***Ubiquitin Ligase***. That ligates (attach) *Ubiquitin* a target protein.

●the process occurs millions of times (***Polyubiquitination***),

- *Polyubiquitination* signal will recruit proteasomes, to splice and degrade this protein into small fragments.

●Note: ***Ubiquitin-Proteasome*** pathway is important to present antigens on ***MHC1***.

2- ***Lysosomal proteolysis.*** (See figure 3)

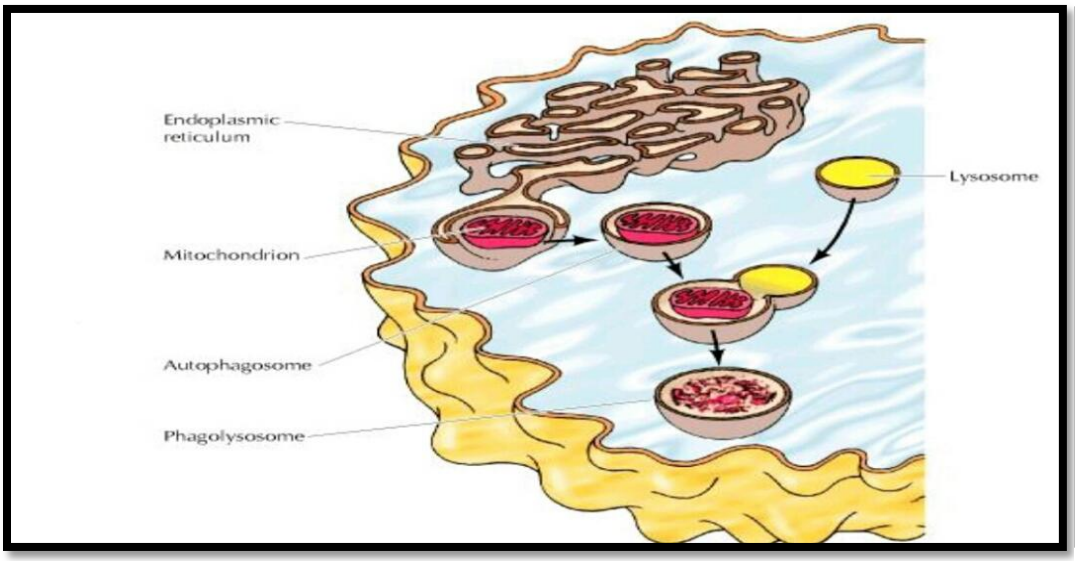

Figure 3: Lysosomal proteolysis

● As the name implies, it occurs in the *lysosomes*:

- Almost all our cells contain *lysosomes*, which have many pyrolytic enzymes and offer an acidic environment that is optimal for its enzymatic activity.

●A membrane bound vesicle ,which is called ***phagosome*** ,encloses certain proteins, this *phagosome* will then fuse with the lysosome, forming ***phagolysosmoe*** that degrade these proteins.

- *Phagosomes* can originate from external or internal material, if originated from internal material it is called ***autophagosome.***

●Note: ***Lysosomal proteolysis*** pathway is important to present antigens on ***MHC2***.

c) MHC Class I

●It is Expressed on all nucleated cells.

●Recall that ***Natural killer*** cells use *MHC I* to determine if it's dealing with normal cell or not. Due to the fact that some neoplastic and viral infected cells downregulate MHC I .

●Every human has three MHC I genes: ***HLA-A, HLA-B, HLA-C*** located on ***chromosome 6***. (i.e. We have a total of 6 MHC I genes.)

- These genes are highly polymorphic: example, we have at least 370 variant of *HLA-A* and 660 variant of *HLA-B*.
- This variation is very important, Since each variant has its one affinity toward Certain peptides . This important to give some of us selective advantages.
- So some of us can resist *TB*, others can resist *cancers*, etc..
- because of that also, we say that some disorders have genetic predisposition, as some of these variants may have high affinity to present self antigens as a foreign.
- This also important in organs transplant, as we try to match receptor and donor *MHCs* as much as possible.

●***Remember****: MHC I* are picky on end amino acids but flexible on central ones.

●Conclusion: *MHC I* molecule can bind to and present a large number of different peptides, each of which fits the particular amino acids present at the ends of its binding groove.

● But how does Antigen Presentation by *Class I MHC* occur ?

- *MHC I* is dependent on ***Ubiquitin-Proteasome*** pathway in antigen presentation, Since it presents internal peptides. And, as you know, the *cytotoxic T* cells need *MHC I* to recognize Neoplastic or virally infected cells that produce antigens from internal peptides.
- So the internal protein will be degraded, and a Generation of small peptide by the proteasome will occur.
- Then this peptide will be transported into the ER by ***TAP*** proteins that has preference To 8-15 A.A fragments. The idea here that MHC I can bind only short peptides, so the peptides transporter, TAP, will try to transport candidate peptides only.
- These peptides will meet *MHC I* in the ER, and binding will occur.
- *MHC I – peptide complex* reaches Golgi, that tag the complex with a certain signal inserting it into the membrane, in order to be recognized by *cytotoxic T* cells.

●**Important note**: In non immune cells, proteasomes cut the proteins randomly. But Proteasome cutting process is more customized in *APCs* compared to non-immune cells.

- In activated immune cells (ex: Macrophage activated by *IFN-γ*) some proteasomal components will be replaced by special proteins (*LMP2, LMP7, MECL-1*).
- These proteins cleave proteins after hydrophobic or basic amino acids, since *MHC1* doesn't present any antigen that doesn't have hydrophobic or basic amino acids on its sides.
- by this mechanism, *APCs* become more efficient in antigen presentation.

d) MHC class II

●Is expressed by immune cells ONLY.

●like *MHC I,* its highly polymorphic and coded by ***HLA-D*** region of *chromosome 6.*

●Unlike *MHC I*, the *MHC II groove* is open at both ends, so peptide can hang out of the groove, the critical peptides are not at the end of the groove like *MHC I*, but rather spaced along it.

● But how does Antigen Presentation by *Class II MHC* occur ?

- In *MHC class II* the antigen comes from outside the cell via ***Lysosomal proteolysis***, because we are presenting an external protein, to be recognized by a *T helper cell.*
- Unlike *MHC I,* The *MHCII* is bound to invariant peptide. Because ER is filled with internal peptides, and if we don't protect the binding groove it will bind some internal peptides and act as *MHC I.*
- So *MHC II* are synthesized and injected Into the *ER* where they bind to an ***Invariant chain.***
- Then, *Invariant chain* guides *MHC II* From *ER* to endosomes.
- Endosome fuses with phagosome and exogenous peptides are loaded on the open *MHC II* groove.

- And now we will have an *MHC II* molecule attached to an *APC*.. :D

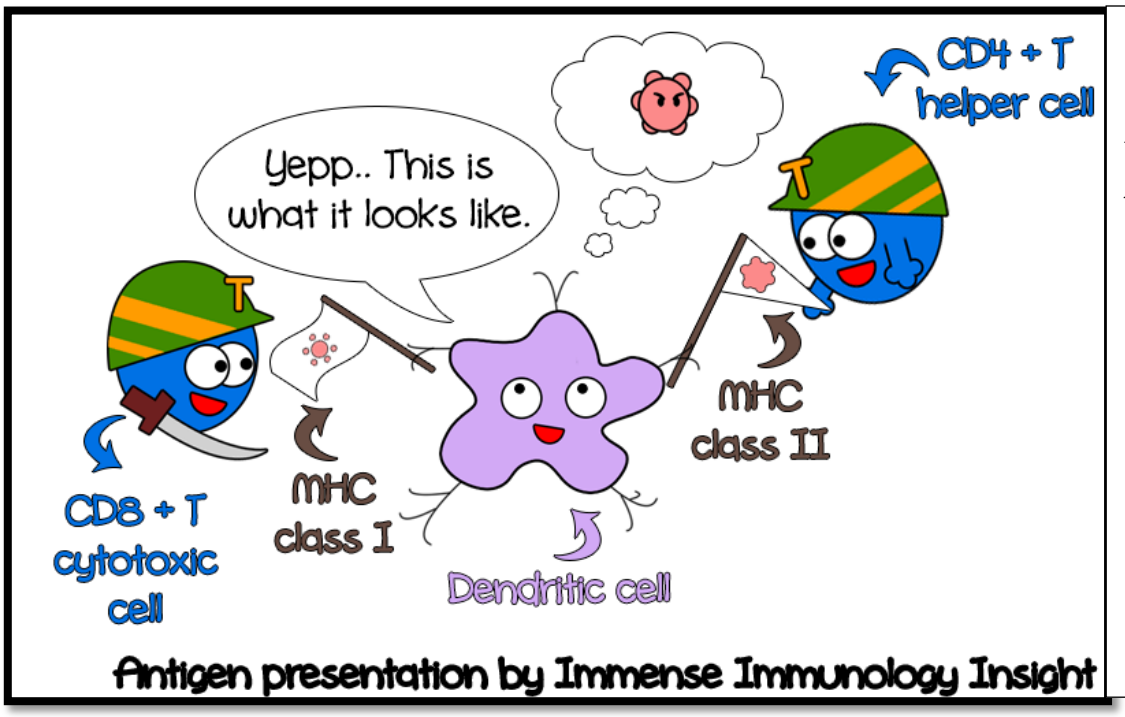

Figure 4: Summery of Antigen presentation

III. antigen Presenting Cells (APCs)

●Remember that In order to activate *T cells*, *APCs* have to produce two signals:

- The first signal is <u>binding of the Antigen that is presented in *APC* with a *T-Cell receptor*.</u>
- but that first signal is not enough. Your *APC* have to produce a costimulatory signal. Which is <u>*B7* with the receptor *CD28*.</u>
- It is like a safe it has a code and a key, for safety.

●There are 3 main types of *APCs*: *Dendritic Cells*, *Macrophages and B cells*.

a) *Dendritic Cells*

●*dendritic cells* are Starfish-like cells that can initiate the immune response by activating *virgin (Naïve) T cells*.

- As the name implies *dendritic cells* have *dendrites* (زوائد شجرية).
- Not very good APCs in resting state, <u>but get activated during an invasion</u>.

● *Dendritic cells* were discovered accidently. The story began when an immunologist was working on *WBCs* differentiation by addition of certain cytokines. In one of his experiments, he added a wrong combination of **cytokines**. This combination yielded a group of strange cells, which are dendritic cells !!

●There are two modes of activation of *dendritic cells:*

1- *TNF* secreted by *Macrophages* and *neutrophils* or chemicals secreted by attacked cells.
2- ***Toll-like Receptors*** (TLRs):
   - Toll like receptors are group of receptors in mammals that resembles the structure and function of Toll receptors, first discovered in fruit fly.
   - Toll like receptors belong to the family of ***PAMPS***, which are group of receptors that are able to recognize patterns on pathogens.
   - These patterns are essential structural components for the pathogens, so our immune system developed these receptors to take advantage from these structures, *EX:TLR-4: LPS (External) /TLR-7: ssRNA (Internal)HIV, influenza etc.. /TLR-9: dsDNA (Internal) bacteria, HSV!*

●Activation of *dendritic cells* :

- *Dendritic cells* are found mostly under surfaces, thus working as a sensors that detect any invasion.
- Before attack (resting state), *dendritic cells* have few *MHC* and *b7* molecules on their surfaces.
- Upon invasion, an activation signal (like *TNF* which is produced by *macrophages*) initiates *dendritic cells* recruitment.
- The first activation signal starts *MHC* and *B7* overexpression. At this time, *dendritic cells* start migrating into lymph nodes.
- These cells go to the lymph node in order to send the picture from the battle field where the pathogen is located to the lymph nodes where *virgin T* cells are located.

- So lymph node is the meeting point between dendritic cells and Naïve T lymphocytes. If these cells were scattered randomly allover the body, It will be impossible for antigen presentation to occur!
- Full activation is accomplished after 24 hours, by this time activated *dendritic cells* reach the *lymph node.*
- *Dendritic cells* activate *naïve T cells*, and the party starts ✌

b) Macrophages

●***Macrophages*** are tissue monocytes that have phagocytic activity, they play a central role in antigen presentation, by internalizing, processing and presenting foreign bodies.

●In order to present antigens properly, *Macrophages* have to be activated, there many activatory signals, like:

- Cytokines (IFN-γ)
- its complement receptor.
- FC receptors.
- Toll like receptors.

●Recall that ***macrophages*** exist in 3 states:

- ***Resting state*** : characterized by Low MHC II expression. they find dead tissue, cells, ingest them and kill them.
- ***Primed state :***the macrophage senses interferon gamma (IFN- γ) "which is a very potent activator of macrophages" up regulate MHC II expression.
- **Hyperactivated state:** 2nd signal of danger (IFN- γ) + LPS the macrophages will be highly phagocytic, more lysosomes, ROS, NO.

●Unlike *dendritic cells*, *macrophages* do not leave infected tissue to go to lymph nodes, How can they serve as APC???

- When dendritic cell activate *T cell*, activated *T Cells* will migrate to the infected tissue.
- In order to perform its function properly, T cells require persistent stimulation, otherwise they will be *neglected* (undergo apoptosis) or *inactivated*.

- The activation of *T cells* at the site of the infection (distant tissues) is accomplished by *Macrophages*.

c) B cells

●We know that *B cells* are important in the adaptive immunity as they are responsible for humoral immunity, we know also that B cells need some signals from T cells in order to be activated.

●But in some instances (ex: the first contact with a foreign antigen) *B cells* will work as an *APC*, and thus activating *T cells*.

- **Note**: *T cells* are important in the second exposure to the antigen. but in the first exposure, *B cells* are more important, as *T cells* will be activated in later stages of the first exposure.

●Now think about it, even if we wanted to activate *T cells* in the first exposure, why do we need B cells? We have many other *APCs*! What's unique about B *cells*?

- *B cells* are considered as antigen concentrators.
- Normal, *APC* can present an antigen only when there's a lot of this antigen around it!
- But in case we have few antigens in the tissue, it will be like looking for a needle in a haystack!
- Remember that *B cells* can bind antigens selectively as they carry antibodies on their surfaces.
- So using *B cells* as *APC* is just like using a Magnet to find a Needle in the Haystack :p .

IV. The logic of MHC presentation

●This is the most important concept in antigen presentation, if you understand it, you will understand the whole (حفلة) that's mentioned earlier. Here we are going to answer a very deep question. why bother with antigen presentation at all?? We will answer it in two parts:

a) Why bother with Class I presentation?

● It Focuses the attention of *killer T cells* on infected cells not on pathogens outside cells.

- We know that Anything outside cells can be dealt with by antibodies and phagocytes.
- At certain stages of organisms evolution, viruses prospered. They eradicated many species as they evolved the ability to enter the cells and hide from immune system.
- However, higher vertebrates adapted *MHC I* to present intercellular proteins, in order to catch viruses.
- Actually, some scientists say that adaptive immune system have evolved to fight cancers and viral infections.

● Without Class I system, any pathogenic Antigen stuck to a surface of an innocent cell could trigger T cell killing.

- This makes our immune system specific for pathogens only. Otherwise, if some antigens stuck to self cell, killer T cells will destroy our cells!

●This system allows display of pathogen proteins that are inside the cells which would normally never make it to the cell surface.

- Many antigens are hidden as they are internal peptides in the 3D conformational shape of the protein.
- MHC I requires proteins to be chopped into short pieces exposing hidden epitopes to killer T cells.

b) Why bother with class II presentation?

●Many pathogens do NOT infect human cells, infecting tissue and blood. MHC II system samples the outside environment and alerts T helper cells.

●MHC II restriction requires that APC and T helper cell agree there is danger. Adaptive response decision is NOT made by a SINGLE cell.

- Decision making in immune system requires more than one cell, due to the fact that immune response is a very dangerous process.

●**MHC II system requires antigens to be chopped to smaller pieces allowing more T helper Cells to recognize the antigen and mount a more efficient response.**

c) Why are MHC molecules so polymorphic??

●We have 6 genes in total that codes for MHC I molecules, why do we waste 6 genes of our genome to produce 6 isotypes of the same molecule?!

- <u>These variants gives some of us selective advantages to survive.</u>
- Suppose a pathogen mutates in a way where its peptides cannot bind a single MHC I molecule!
- If you have more MHC molecules, you will have a better chance of being able to present mutated antigen. (HIV patients with 6 MHC I genes live longer than those with fewer genes).
- If These variants were so beneficial, it will be naturally selected, and after few generations, most of the humans will be resistant to this pathogen.

Note: The concept of natural selection (not mentioned by the doctor):

●Darwin described four observations of nature: (1) <u>*Members of a population often vary in their traits*</u>, (2) <u>*Traits are inherited from parents to offspring*</u>, (3) All species are capable of producing more offspring than their environment can support, (4) <u>*Owing to lack of food or other resources (or a pathogen in our case), many of these offspring do not survive.*</u>

●From these observation, he drew two inferences: (1) <u>*Individuals whose inherited traits give them a higher probability of surviving and reproducing in a given environment tend to leave more offspring*</u>, (2) <u>*this will lead to the accumulation of favorable traits in the population over generation.*</u>

d) Why not more than 6 MHC I genes then??

● So now we understand that we need high variability in MHC molecules, so why do we have only 6 genes? Why not 1000 MHC genes? Why not to be "superhuman"?

- Excess MHC will lead to excessive presentation of self antigens, causing many autoimmune diseases.
- In order to achieve "good binding" between APC and T cells, we need thousands of binding between TCR and the same type of MHC. if we have many types of MHC, this will decrease the probability of binding the same MHC type, thus no immune response will occur.
- So our "superhuman" will die very early, and won't be super nor human :p

V. MHC proteins and organ transplants

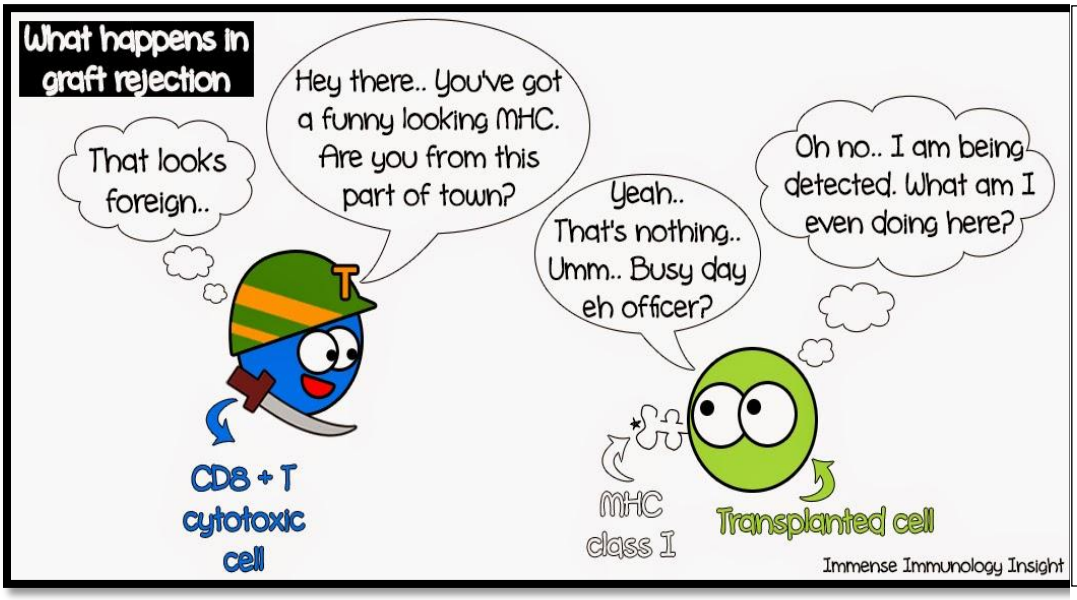

Figure 5: What happens in graft rejection ?

● In the 1930s, it was observed that tumor cells could only be transplanted from one mouse to the other when they are from the same inbred strain.

- they started to think that these mice have different *MHC* on their tumors and therefore evade immune system.

●Similar observations was seen in skin grafts between mice.

- If a skin graft with wrong combination is done, immune rejection will occur.
- Then, scientists discovered that *MHC* molecules used in Antigen presentation, are the very same molecules responsible For immediate rejection of transplanted organs.

●In order to transplant any organ or tissue, you have to do *MHC* matching test between donor and recipient.

- Sequencing of *HLA-A*, *HLA-B*, *HLA-C* and *HLA-D* is required.
- If there's low cross matching, you must cancel the operation.
- Otherwise, *killer T cells* attack foreign *MHC*, cells lining blood vessels of tissues dye cutting blood Supply to transplanted organ.

●To find a class I and II compatible person (non-relative): You need to scan 10,000,000 different people for a 50% chance!

- So even if your patient was fortunate enough to find a good donor with reasonable matching, weak immune reaction will occur. So you have to give him immunosuppressive drugs, in order to stop his immune cells from attacking the transplanted organs !

VI. Revision

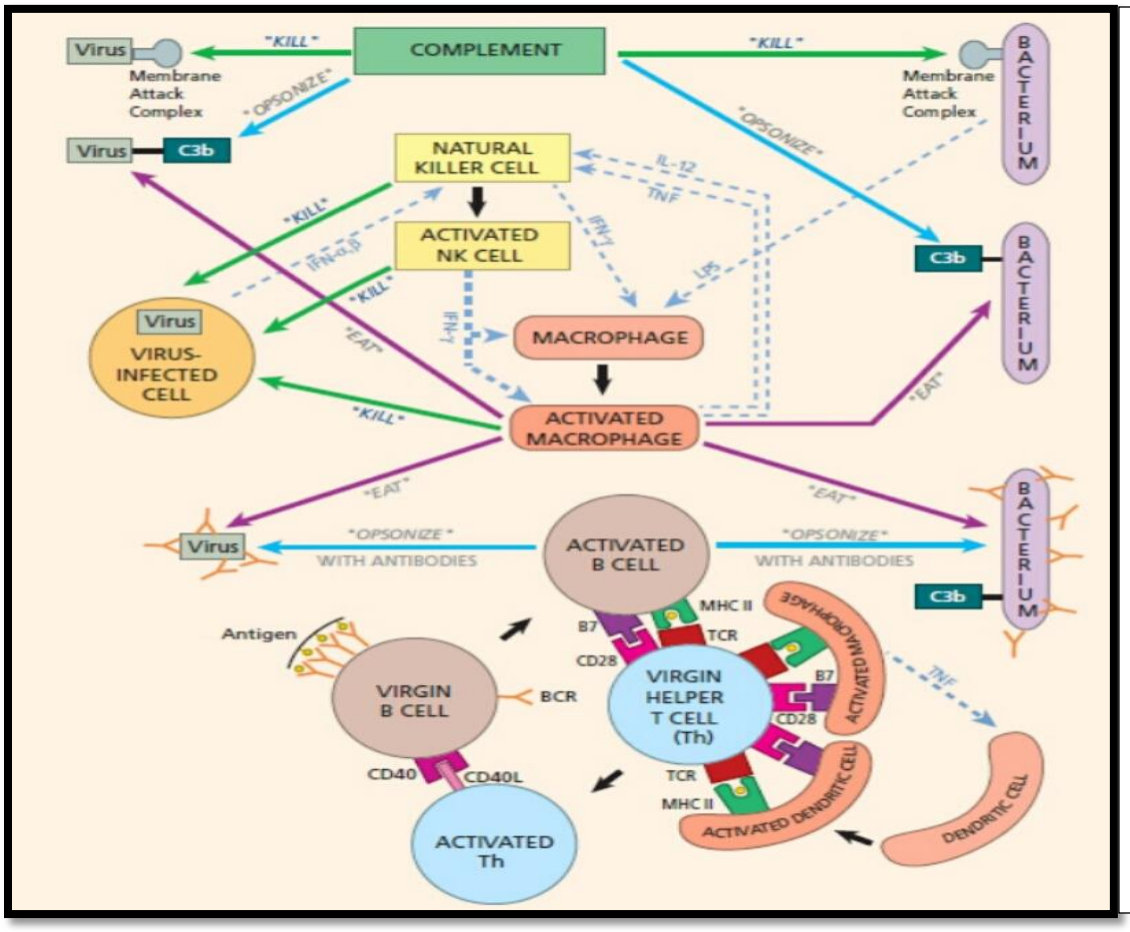

Figure 6: Summery of lectures 1, 2, 4 and 6.

●We talked about the upper parts of figure 6 in the previous lectures.

●In this lecture we have discussed the lower part:

- The activated *Macrophage*, presents viral antigens to T cell, thus activating it.
- This *macrophage* produced also *TNF* that activated dendritic cells.
- we can see that even *B cells* can present antigens and also activate *T helper cells.*

●And This concludes our discussion on antigen presentation

- *"*Character cannot be developed in ease and quiet. Only through experience of trial and suffering can the soul be strengthened, vision cleared, ambition inspired, and success achieved.*" – Helen Keller*

●Best wishes

●*Mohammad Qussay Al-Sabbagh*

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