INTRODUCTION TO MEDICAL

IMMUNOLOGY

SLIDE SHEET

NUMBER	7
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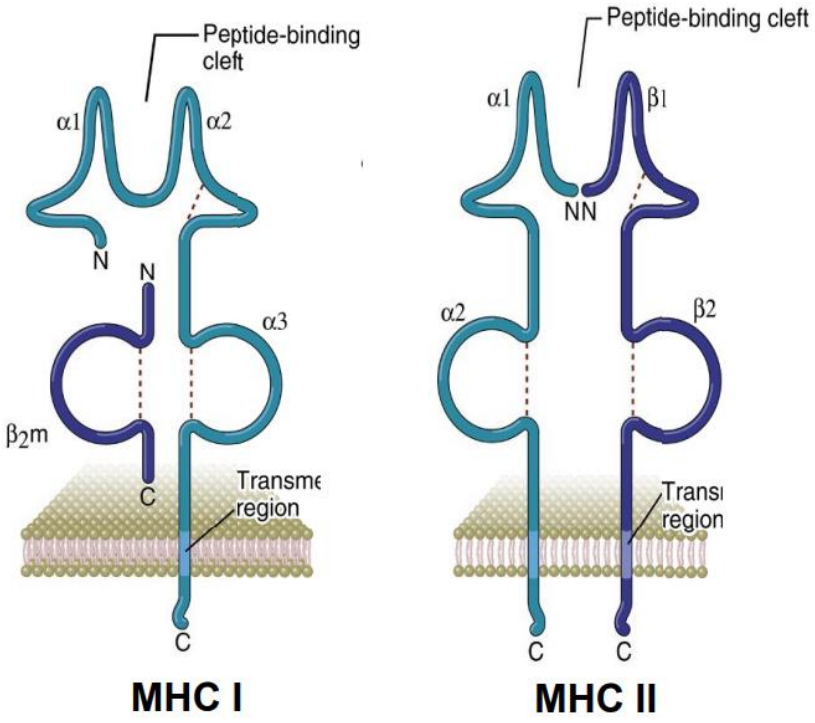
Antigen Presentation

When we talk about antigen presentation, we're concerned about two main cells which are antigen presenting cell (APC) and T cell for which APC shows the antigen.

To make this antigen presentation we need tools or a board to write on so that cells can see the antigen, the black board is called the **Major Histocompatibility Molecules (MHC)** or **Human Leukocyte Antigen (HLA).** (They are the same)

Structure of MHC molecules



- **MHC1**: (used to present antigens for cytotoxic T cells)

Consist of: -

1) alpha chain: forms a small peptide binding cleft where we load the antigen (picky on the size (smaller size) because it's made from one chain), and it's the variable chain

2) beta 2 microglobulin chain which is the conserved chain (all MHC1 molecules have almost the same beta 2 microglobulin)

- **MHC2**: (used to present antigens for helper T cells)

Consist of alpha and beta chains that attach to each other and form the binding cleft (with open ends between them making them more flexible with the size of the peptide bound between them (not picky on the size and can accommodate larger peptides)).

*Variability is present in both alpha and beta, but mainly more in beta than in alpha

Now these molecules are were some peptides are bound to be presented to other cells, and a peptide that binds need to have a
1) docking site to dock it itself in the MHC molecule
2) exposed site to contact with the T cell receptor
*not all peptides are presented

Antigen processing

We form these small peptides from larger peptides in two pathways:

1) Ubiquitin proteosome pathway

- Used for internal proteins

- The proteosome is a complex in the cell that breaks down the peptides that enters it after being signaled by certain signals (ubiquitylation)

- Ubiquitylation is attaching ubiquitin (6-7 amino acid peptide) on the peptides to be sent for the proteasome complex; first an enzyme E1 binds ubiquittin using 1 molecule of ATP, then gets transferred from E1 to E2 to E3 which is the ubiquitin ligase that ligate the ubiquitin to the polypeptide that we want to get rid of

- Poly- ubiquitination is needed for a peptide to be sent to the proteasome; one is not enough.

2) Lysosomal proteolysis

- Used mostly for external antigens (can be for internal)

- An endosome binds to the lysosome (containing proteases) forming a phagolysosome and peptides are broken into smaller pieces ready to be presented on the cell surface.

MHC class 1

- Present on all nucleated cells
  *many immune cells get benefit from it, such as cytotoxic T cell that recognizes MHC1 to start killing, and NK cell that starts killing in the absence of MHC1 molecule

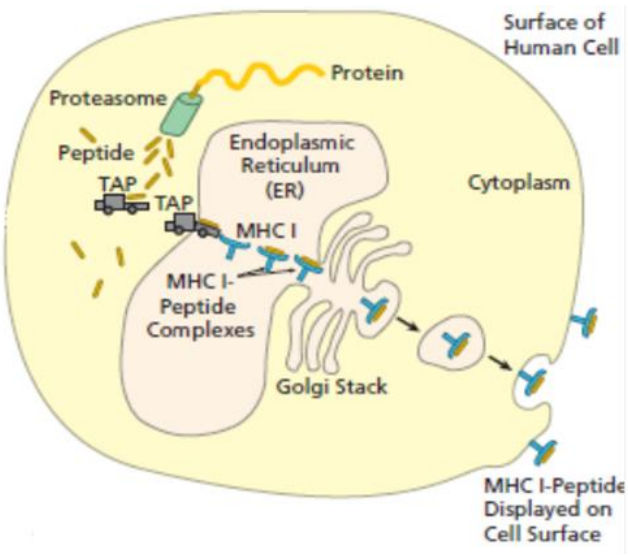
- Every human has three MHC I genes: HLA-A, HLA-B, HLA-C located on chromosome 6. These genes are very polymorphic, for example HLA-A has 370 variants, HLA-B has 660 variants; we have a total of 3 MHC1 genes (if paternal and maternal are the same) MINIMUM and 6 MHC1 genes MAXIMUM (if all were different)!!

- MHC1 are picky on end amino acids but flexible on the central ones, and by that they can bind to a large number of peptides each with a certain amino acid sequence that can fit its binding groove and present it to cytotoxic T cell

- The peptide of MHC1 is intracellular, because cytotoxic T cell kills virally infected cells and cancer cells (the antigen is inside the cell)

- Since it's intracellular, the protein (after ubiquitylation) goes to the proteosome and gets broken down into fragments, then a protein called **TAP** (transporter associated with antigen processing) transports the peptide from the cytosol to the ER

- In the ER, the MHC1 molecule is already there, so that the imported peptide can bind to the MHC1 and goes to golgi to be sent for surface expression so that the cytotoxic cell can recognize it

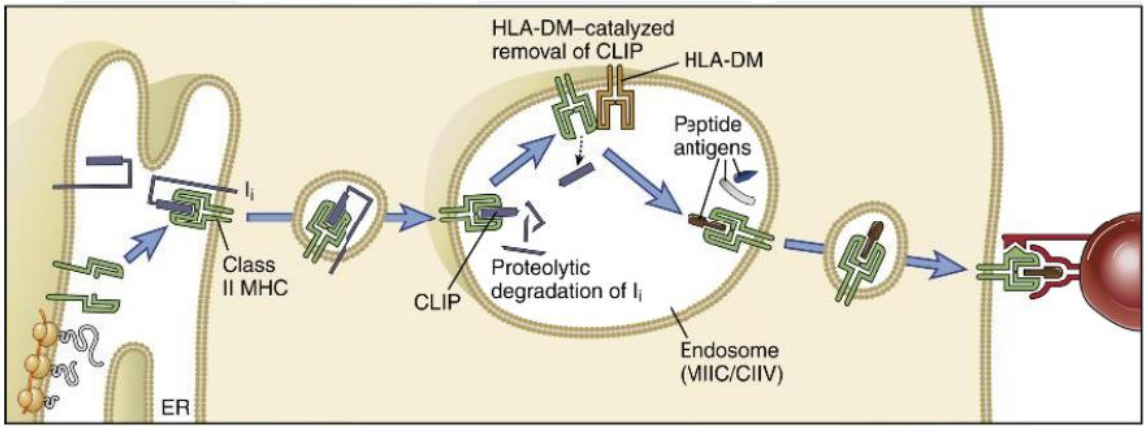


Notice from the figure above that the protein is intracellular → degraded by proteosome → transported by TAP to the ER where MHC1 is already present → loaded on MHC1 → to golgi → to the surface (now the antigen is presented on the surface)

recent researches discovered that the proteosomes of antigen presenting cells actually are more specific than proteosomes found in other non-immune cells, which makes sense, because random cutting shouldn't occur and most of the peptides won't bind to MHC molecule, while proteosomes of immune cells are upon activation by cytokine like interferon gamma, interferon gamma will activate macrophages and macrophages will activate many enzymes like LMP2, LMP7, MECL1, and these will force the proteosomes to start the specific cutting of peptide after the hydrophobic or basic amino acids, and those specific peptides will fit the MHC molecules more than random peptides.

MHC class 2

- Expressed by immune cells ONLY
- Coded by HLA-D region on chromosome 6 (HLA-DQ, HLA-DP, HLA-DR)
  * in the transplantation reports we are more concerned about HLA-DR
  A relationship exists between some HLA antigens and susceptibility to certain diseases (in celiac disease patients have DQ2 or DQ8 that increases the susceptibility of the disease)
- MHC genes are the most polymorphic mammalian genes; unlike the polymorphism in VDJ recombination in B cells that occur due to the recombination effect, MHC polymorphism occurs by having all these genes physically present on the chromosome.
  The genes base pairs that code for MHC molecules in our bodies equals almost the whole genome of E.coli, and we will further talk about the benefit of having this huge amount of variability in MHC genes
- The peptide of MHC2 is external; it enters the cell in a phagosome
- The MHC2 molecules gets synthesized and blocked by the invariant chain in the ER.
  Why? because if it wasn't blocked, any intracellular peptide can bind to it, just like MHC1
- MHC2 with the invariant chain get out of the ER to endosomes, the endosome fuses with the phagosome that contains the external peptide, the invariant chain gets cleaved by proteolytic enzymes leaving a CLIP on MHC2 which is still blocking MHC2, CLIP is removed by HLA-DM (from HLA class 3), the MHC2 molecule becomes available so that the external peptide can bind to it, then it can be presented to the surface



Notice from the figure above that MHC2 is bound to the invariant chain in the ER → it's transported to phagosome containing the extracellular protein → the invariant chain is cleaved leaving a CLIP → CLIP is removed by HLA-DM → peptide binds to MHC2 and gets transported to cell surface

Antigen presenting cells (APCs)

The main concept of antigen presentation is that we have an antigen presented by APC binding to a T cell receptor which gives the first signal, but we still need a second signal provided by a co-stimulatory molecule (B7 as an example) on the surface of APC that binds to CD28 T cell receptor(immunoglobulins super family). Just remember the safe key and code!

The three main types of APCs:

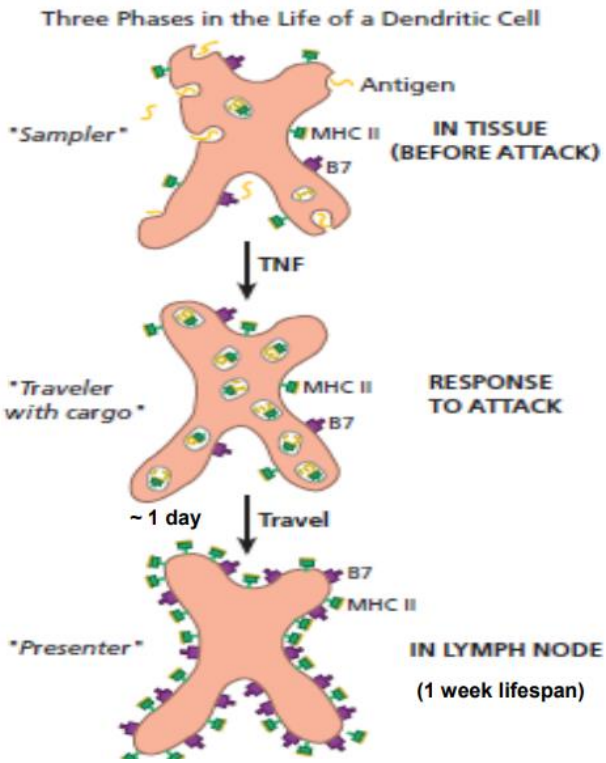
1) DENDRITIC CELLS

They are a starfish like cells that can initiate an immune response and called "professional APCs"

Actually, they were discovered by accident in a lab that worked on macrophages, by mistakenly not giving some of the cytokines needed for maturation of macrophages and noticing starfish like cells, which were called dendritic cells.

Two modes of activation of the dendritic cell:
1) activation by a cytokine, like TNF that is secreted by macrophages (a very potent activator) or chemicals secreted by attacked cells
2) by direct contact to the receptors, like toll-like receptors (TLR) (first discovered in fruit fly) like those found on macrophages, different types of tolls are able to recognize PAMPS (pathogen associated molecular patterns)

TLR4 (external) can recognize the LPS
TLR7 (internal) can recognize ssRNA (like in HIV virus, influenza virus…)
TLR9 (internal) can recognize dsDNA (like in herpes simplex virus, or in bacteria)



At resting: dendritic cells found in tissues and sampling and looking for antigens, having low amounts of B7 and MHC molecules on the surface

During attack: TNF from the macrophages will activate the dendritic cells, the dendritic cells will start synthesizing B7 and MHC2 molecules while migrating to lymph nodes, this process will almost take a day, when they reach the lymph nodes there will be a very high expression on B7 and MHC2 molecules and the cells will be very good in antigen presentation and will start looking for the T cells, and they live for around a week.

Dendritic 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 Naïve T cells are located.

2) MACROPHAGES

The first APC during infection

They also have TLRs; they phagocytose→ phagosome→lysosome→killing in different mechanisms (like NADPH oxidase, nitric oxide)

Macrophages go through 3 stages:

1) Resting state: low MHC2 molecules and low co-stimulatory molecules

2) Primed state: when it sees the first signal (like cytokines), becoming a good APC and a good killer and up-regulates MHC2 expression

3) Hyper-activated state: when it sees the second signal (like LPS), becoming an excellent APC and highly phagocytic

Unlike dendritic cells, macrophages don't leave the infected tissue to go to lymph nodes; how can they serve as APCs without going to the lymph nodes?

When dendritic cells activate T cell, activated T Cells will migrate to the infected tissue, and it should be re-stimulated by an APC (which is the macrophage), otherwise the T cells will die.

3) B CELLS

They need a longer time to be activated because they are adaptive immune system, so they will be the last APCs, but in subsequent infections or at late stages of an infection the B cells can act as APCs; how? The B cell has a B cell receptor (BCR) and the BCR can bind to antigens which are peptides, then the antigen will be internalized and gets broken down by the lysosomal pathway, they also have MHC2 in ER blocked by invariant chain, the MHC2 will bind with the phagosome and gets presented on the cell surface.

So the advantage of B cells is the concentrate antigens because it has a lot of BCRs on its surface, and if the antigen percentage in the blood was low, the B cell (becoming 100-10000 folds better as an APC than macrophages and dendritic cells) can collect these antigens and present them.

The logic of antigen presentation

Why bother with MHC1 system?

- It focuses the attention of killer T cells on the virally infected cells or cancer cells not on the pathogens outside the cell, because we are dealing with intracellular issues, and my solution is to expose these antigens to the surface.
- If antigens were presented without Class I system, any pathogenic Ag stuck to a surface of an innocent cell could trigger T cell killing. So we're making sure to internalize the antigen, processing it, and making sure it is pathogenic, then we present it on cell surface.
- This system allows display of pathogen proteins that are inside the cells which would normally never make it to the cell surface.
- MHC I requires proteins to be chopped into short pieces exposing hidden epitopes to killer T cells.
  Imagine the peptide as a robe, if the robe was very long and curled most of its internal parts will not be exposed, but after cutting and breaking down this long robe we will be able to expose these internal peptides, so the biggest number of T cells can recognize them

Why bother with MHC2 system?

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

- MHC2 restriction requires that APC and T helper cell agree there is danger. Adaptive response decision is NOT made by a SINGLE cell.
  This is important, why? When we do this system there should be 2 requirements, both T cell and APC must agree that there's a problem before any immune response, to avoid any unwanted damage (only NK cell can start an immediate immune response)

- MHC2 system requires antigens to be chopped to smaller pieces allowing more T helper Cells to recognize the antigen and mount a more efficient response.

Why are MHC molecules so polymorphic??

Humans are always susceptible for pathogens that can change the peptide structure, so if there wasn't enough variation in MHC in humans that can recognize the new peptides to present them; that will be a disaster!!
That's why there was an evolutionary pressure on MHC genes to be highly variable so that we can have an enough variation in the population and overcome any changes that happen on the level of the pathogen of variability.

As a prove, people who have the whole 6 copies will have higher MHC variability and can resist HIV infection and cope with it more than people with less number of copies, because HIV virus tend to make a very high mutation rate

MHC proteins and organ transplants

In the 1930s, started the first attempts of experiments on mice; there were strains of the mice, and they start inbreeding until they all become syngeneic (become genetically identical), they observed that tumor cells could only be transplanted from one mouse to the other when they are from the same inbred strain. Then they started to think of MHC role in rejection of tissues

Similar observations were seen in skin grafts between mice to treat burns in the World War 1, so if a skin graft with wrong combination is done, immune rejection will occur. Then, scientists discovered that MHC molecules are responsible For immediate rejection of transplanted organs, and if there was incompatibility, killer T cells attack foreign MHC, and cells lining blood vessels die, cutting blood Supply to transplanted organ.

In order to transplant any organ or tissue, you have to do MHC matching test between donor and recipient, and the most important test is HLA typing, that's why when we want to do an organ transplant we look for the sister, brother, mother and the father, WHY? To have a higher chance for MHC match. Some people store the fetal stem cells by freezing to have a 100% match in the future.

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

"true confidence has no room for jealousy and envy"

BEST WISHES