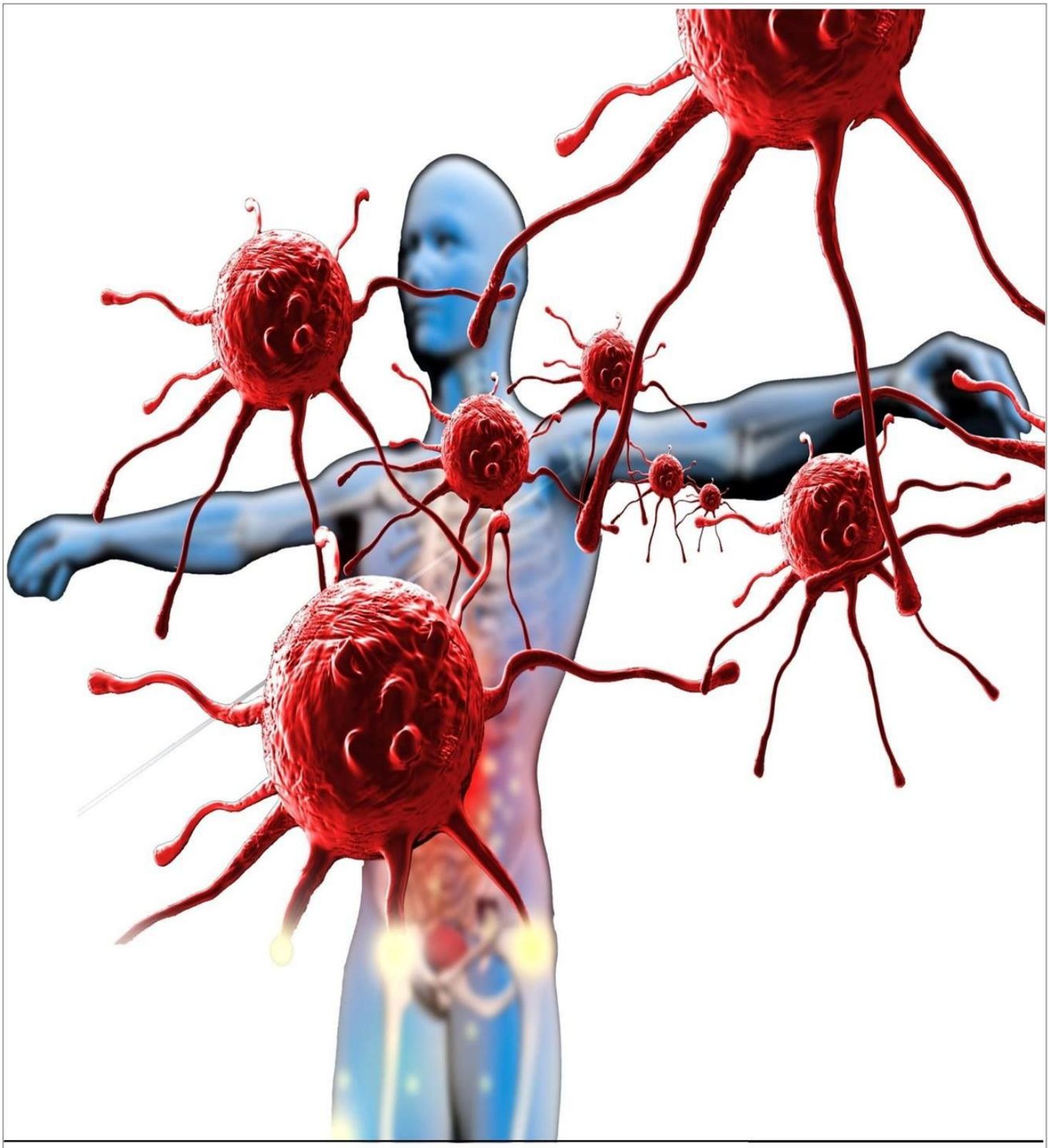


IMMUNOLOGY

Done By: Loay zaghloul

9

Dr. Hassan Abu Al-Ragheb

By Mohammed Nawasieh

MHC CLASS I & II

If you don't have time to go through the whole sheet you can simply check the last page table, in addition to the 2 processing pathways and Tissue typing and transplantation.

What are MHCs?

It stands for **Major Histocompatibility Complexes**, which are molecules found on the surface of cells, they were first discovered when tissue/organ transplant was first introduced, if these molecules were similar then the graft would be accepted, otherwise it would be rejected. But really this is not the function of these molecules (to make it hard for surgeons to do transplantations), they serve a different function, in the immune response, when they were first discovered in humans, scientists called their genes HLA (Human leukocyte antigen) as they were found on white blood cells.

The genes which encodes these molecules are found on the short arm of **chromosome 6**, the HLA region contains 3 sub regions: class I --> which encodes for class I molecules, Class II --> which encodes for class II and in between them class 3 --> which encodes for class III molecules (these are somehow different as they are soluble, which are part of the complement system like C4 and Factor B etc.)

MHC class I

Structure (check structure picture later)

- As you can see it has 2 parts, an α part which contains 3 **domains** called α1, 2 and 3. And also it has a tail which anchors it to the cell membrane which attaches to α3, in between **α1 and 2** we find a small groove which serves as the binding site for the antigens, usually **8-9 amino acids** can fit in this groove.
- In normal conditions the groove is filled with a self antigen, but if the cell is a cancerous or virally-infected cell, foreign antigens would be presented inside this groove.

- Now this complex of foreign antigen and MHC class 1 molecule are recognized by the **cytotoxic T-cell**, which has on its surface a CD8 molecule which bind on its receptor which is found on the α3 domain of the MHC molecule, and this binding induces the activation of the cytotoxic T-cell (in addition to binding the antigen on the TCR).
- The 2nd part is a β2-microglobulin which in fact is not encoded on chromosome 6 but on chromosome 15, and it doesn't play a part in recognition or presenting of the antigen it's also not covalently bound to α subunit nor anchored to the plasma membrane but it is found there as a rigid part necessary for **maintenance** of the conformation of MHC class I.

Gene Locus:

We notice that in Class I we have 3 genes which encode for 3 separate α chains(HLA-A, HLA-B & HLA-C), any of these α subunits associated with a B2-microglobulin will give us a unique MHC Class I (keep in mind the B2-microglobulin is a constant subunit with 1 gene), so from each parent we get **one** chromosome 6, and since the inheritance of these genes follows the co-dominance method, we will have a maximum of **6 different types MHC class I molecules** how? 2 HLA-A (1 from father and 1 from mother) 2 HLA-B (1 from father 1 from mother) and so on.

This is the case if the offspring inherited different alleles from the mother and father, However, in some cases especially in endogamy (زواج الاقارب), the offspring would have a duplicate gene for example 2 HLA-A1 or 2 HLA-B4 etc.(so we will have less than 6 different types of MHC class I molecules) and here the ability to bind to foreign peptides to present them is impaired, so the more polymorphism we have the better we are to bind and present a larger number of foreign molecules.

We will talk about polymorphism of the population later.

As we know MHC class 1 molecules are only found in nucleated cells, so **RBCs** which lack a nucleus doesn't have these molecules, platelets on the other hand, even though they are non-nucleated they have MHC class 1 molecules on their surface as they developed from the nucleated **megakaryocytes**.

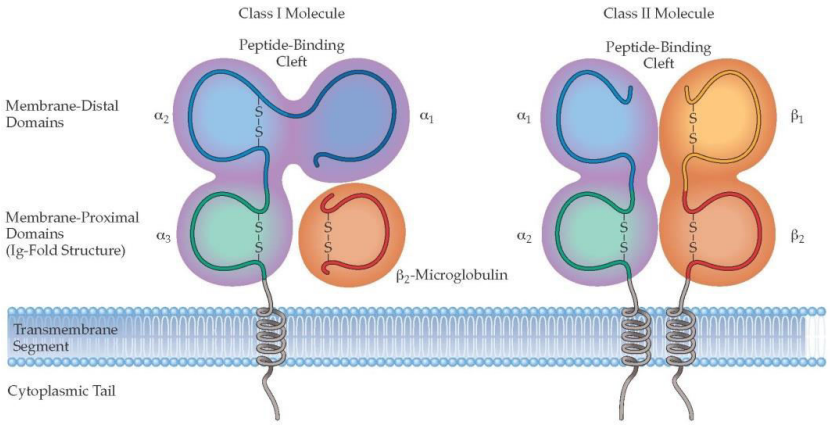
Trophoblast of the placenta is an exception because although it's nucleated it is devoid of MHC class 1 molecules.

There are HLA-X, J and H etc. which are called **pseudogenes** because their products are similar to HLA-A,B and C but they cannot bind antigens, other genes are called **non-classical genes** HLA-E HLA-F HLA-G they are similar in structure but they aren't widely distributed and they serve a different function we'll talk about later.

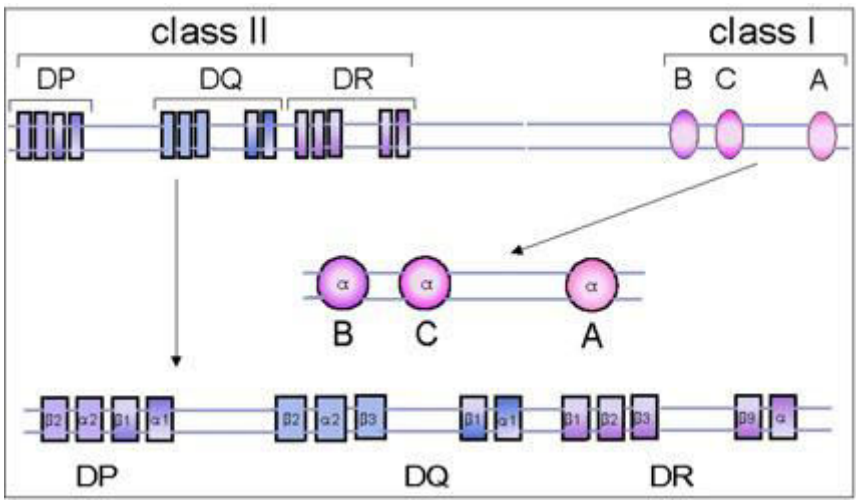
The peptide and this particular part of the MHC molecule are what are actually recognized by t-cells. MHC1 molecules are recognized by cytotoxic t-cells which have CD8 molecules on their surface. The α3 domain on the MHC1 molecule actually has a receptor for CD8 which allows for the good adhesion between the two cells. The α3 domain is a β pleated structure; this means that MHC1 molecule is a member of the super-immunoglobulin family.

A *student* asked about the number of MHC molecules on a given cell and the doctor answered that a cell may have thousands of HLA molecules however they should be of 3-6 different types. They cannot be less than 3 types.

MHC class 2



MHC2 molecules are made of two polypeptide chains, α and β. Both of them are anchored to the plasma membrane of the cell on which they are residing. There is also a peptide binding groove that is a bit **bigger** than that of the MHC1; this means that it can bind bigger 20-25 amino acid long peptides compared to the 7-9 long amino acid peptides the MHC1 molecule can bind.



- Since we have 2 polypeptide chains we must have 2 genes that code for them. In MHC1 molecules, chromosome 6's short arm has the gene for the α chain, and the β2 microglobulin is constant and is expressed somewhere else. However in the case of MHC2, the genes coding for both α and β are present in the same complex which makes things a little more difficult.

- The Class 2 region was originally called the D-region (The A, B, C regions are for MHC1). At first they thought that this region coded only for 1 molecule but upon further studies it was concluded that the D region consists of 3 separate entities. The subdivisions are DP, DQ and DR.

Each subdivision ideally should have a gene that codes for α, and another that codes for β but if we look closely at the gene arrangement we can see that:

DP: there are A1, A2, B1, B2 genes. However only 2 are really functional (A1 and B1) and these code for the chains.

DQ: although we have 2 A genes and 3 Bs, only A1 and B1 are functional and one type of MHC molecule can be formed.

DR: there is only 1 A gene that codes for α and several B genes of which 2 or 3 are functional; so B1, B2 and B3 each code for a different type of β chain. In theory 3 types of MHC molecules can come out of the DR region.

Note: the α chain coded by one subdivision cannot join the β chain coded by another. This is why DP forms one type MHC2; DQ also forms 1 type, and DR forms 3. These all join up to make **5 types of MHC2 molecules**.

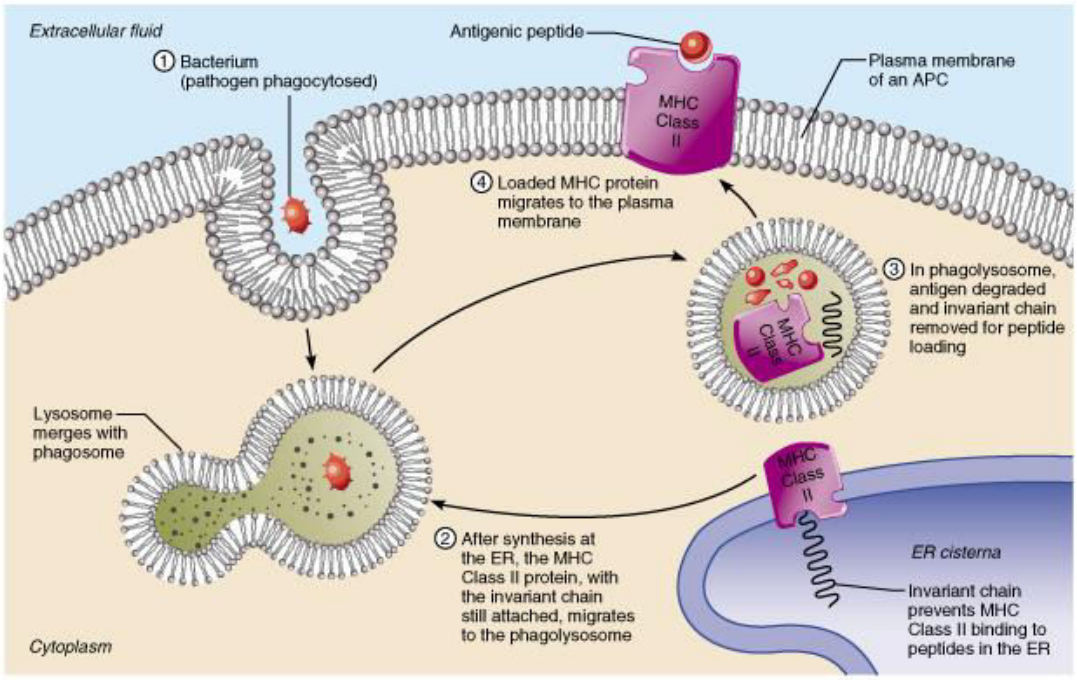
- The inheritance of these genes is co-dominant. The mother can have 5 different types of MHC molecules and the father can have 5 different types as well which calls for more diversity. Also the β chain from the mother can join the α chain from the father forming yet another type of MHC2 in the offspring. People usually have 18-20 different kinds of MHC2 molecules. The variation of class II MHC is more than that of class 1 because:
  - The chains are coded by different genes in DP, DQ and DR regions
  - The genes are expressed by the father and the mother and they can also be swapped in between.
- MHC class II as we know, are present on **APCs** which are macrophages, Dendritic cells and B-lymphocytes. The more variety of MHCs class II we have the more we are able to present more antigens. They present the antigen to the TCR of CD4 T-helper cell.
- On the β 2 domain of the MHC class II we have a receptor for CD4.
- Here again keep in mind that the MHC molecule can never be intact without the presence of the peptide antigen, in normal physiological conditions we will have a self-antigen but in cases of infection or abnormal cell structure we will have a foreign antigen inside the groove.

Note: Allelic polymorphism of class I is also present in class II which are the variation in the population (HLA-A1, HLA-B9, DR4, DP7 etc. so my 18 MHC class II are different from your 18 MHC class II molecules).

For an antigen to bind to the groove of an MHC molecule there should be compatibility similar to that between the antigen and its relative antibody, this compatibility is actually the product of the non-covalent electrostatic/hydrophobic interactions between the antigen and the groove. So not any antigen would fit in any MHC molecule therefore the more MHC molecules we have the more likely we are able to bind antigens in the groove for presentation.

Now we discuss the 2 antigen processing pathways: (check the pictures before reading)

1-Exogenous antigen processing pathway:



(b)

*MHC class II presents exogenous antigens.

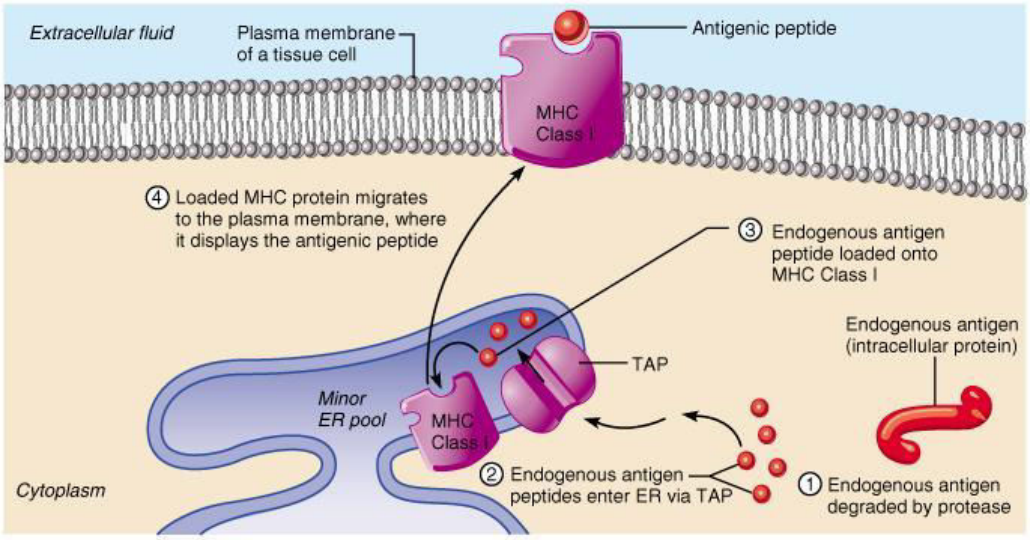
Macrophages phagocytose the bacteria and then inside the macrophage the bacteria is degraded by lysosomes which results in small bacterial peptides around 20-22 amino acids in length.

During the process of translation and transcription of the α and β chains of the MHC class II molecule after activation of class II gene, we have the arrangement of the α and β chain together, however the molecule isn't stable on its own, there must be an antigen in the groove to stabilize the structure, but at that time we have no antigens present as no bacterial peptides are present, so how do we solve this? We have a small molecule called the **invariant chain** which sits in the groove of the MHC II before the exogenous antigen takes its place. So what happens exactly is that the MHC II molecules are found inside endosomes with the invariant chain inside its groove, then fusion between these endosomes and vesicles which contain bits and pieces of the bacteria, following the fusion there will be a swap by kicking the invariant chain by a molecule called **DM** which is encoded on the class II gene loci called **HLA-DM** and then the bacterial peptide will take its place, which is then followed by the export of this MHC class II molecule to the surface.

Recap: DM removes CLIP (or Class II-associated invariant chain peptide which is the part of the invariant chain that binds MHC class II groove and remains there until the MHC receptor is fully assembled. The purpose of CLIP

is to prevent the binding of self-peptide fragments prior to MHC II localization within the endo/lysosome.) After that the bacterial peptide replaces the invariant chain and then the whole MHC molecule with the antigen will be displaced to the surface membrane.

2-Endogenous Antigen processing:



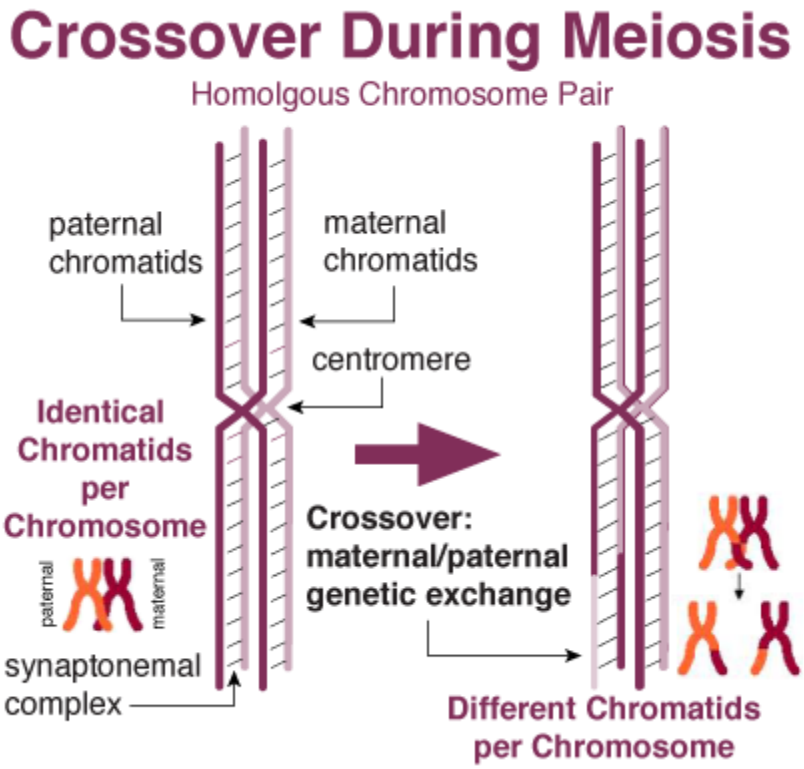
(a)

1. First, there is this organelle called **proteasome** whose function is to breakdown proteins produced inside the cell, these peptides can be either normal cell proteins, proteins produced by a virally infected cell or a cancer cell.
2. Now these peptides are transported via special molecules called **TAP 1 & 2 (Transporter associated with antigen processing)** to the α and β subunits of the MHC class I molecule and stick those peptides inside the groove of the MHC, after that this final assembled MHC molecule is exported to the cell membrane after being packaged inside endosomes.

Note: self antigens can be present on MHC class II molecules, as APCs can phagocytose normal dead cells, and process self antigens into the MHC II molecule. This happens under normal conditions, but in case of bacterial infection we will find bacterial antigens on MHC II molecules. Also foreign antigens can be present on MHC class I molecules, as virally-infected cells or mutated cells can express foreign peptides which gets broken down by proteasome and are presented on MHC I molecules. So it isn't a rule that MHC class II is for foreign antigens and MHC class I is for self antigens. (Understand the difference between exogenous/endogenous and self/foreign).

Endogenous → produced inside the cell, Exogenous → produced outside the cell (the MHC molecule containing cell).

Tissue Typing and Transplantation:



Chromosomal Crossover

Homologous recombination is the process by which two chromosomes, paired up during prophase 1 of meiosis, exchange some distal portion of their DNA.

Crossover occurs when two chromosomes, normally two homologous instances of the same chromosome, break and then reconnect but to the different end piece.

If they break at the same place or locus in the sequence of base pairs the result is an exchange of genes, called genetic recombination.

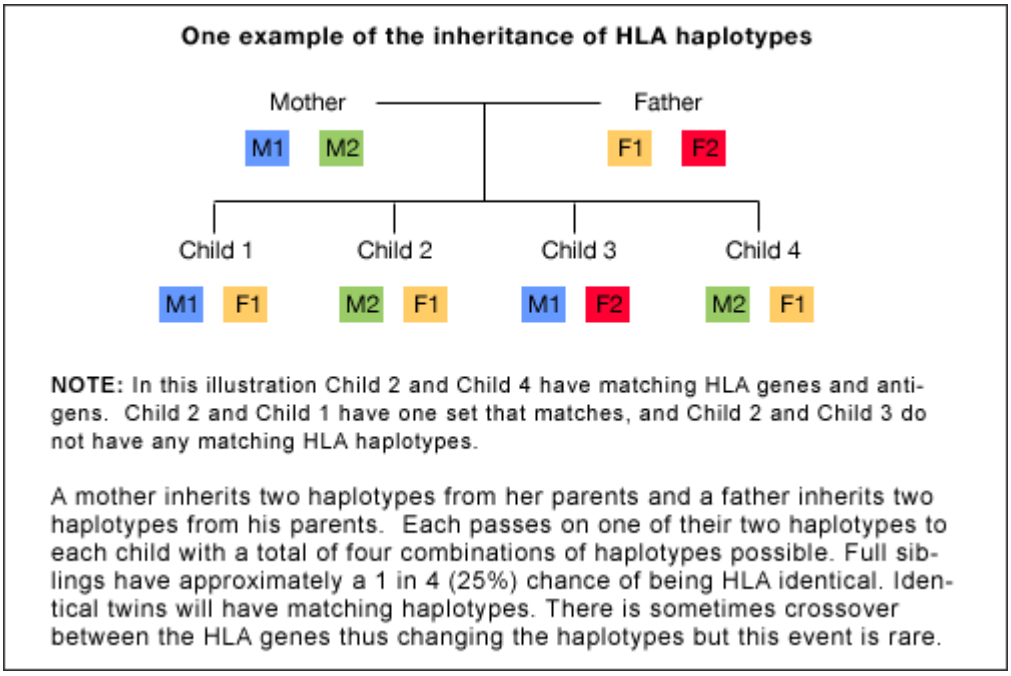
This outcome is the normal way for crossover to occur.

Now, because the genes responsible for coding for MHC I, II, and III molecules are so close to one another, and are extremely short, they are passed down to the offspring from the mother as a package (i.e. inherited as one gene) called a haplotype, and as a haplotype from the father as well, without the occurrence of chromosomal crossover (crossover is unlikely to happen in the MHC genes, however, it can take place).

[The offspring has two haplotypes, a maternal and a paternal one.]

A haplotype is a set of DNA variations, or polymorphisms, that tend to be inherited together. A haplotype can refe to a combination of alleles or to a set of single nucleotide polymorphisms (SNPs) found on the same chromosome.

The haplotypes are inherited in a dominant Mendelian inheritance pattern and are NOT sex-linked.



In comparison to you:

What is the likelihood of your sibling having identical haplotypes?

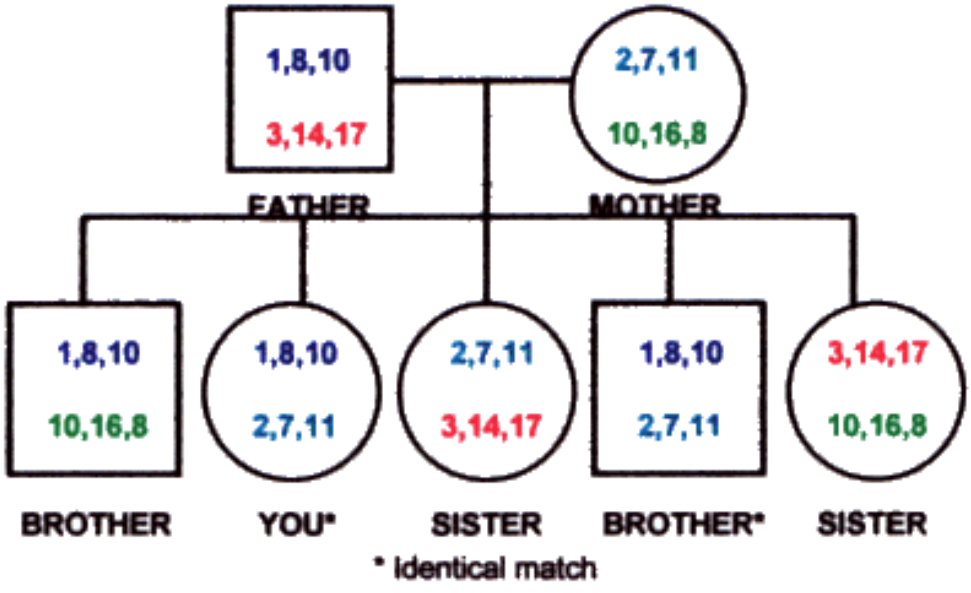
1 in 4.

What is the likelihood of your sibling having completely different haplotypes?

1 in 4.

What is the likelihood of your sibling having one identical haplotype?

1 in 2.



You and your sibling are a match, kidney transplantation is performed. Will the kidney survive?

Yes, it will. However, after ten or more years, the kidney will undergo tissue rejection due to Minor Histocompatibility Complex molecules, which are just normal body proteins that show small differences due to the different genes present in the two of you.

Disease Association:

Certain HLA molecules are associated with some autoimmune diseases.

For example, HLA-B27 gene is associated with ankylosing spondylitis. [All those who have ankylosing spondylitis have HLA-B27 gene, however, not all those who have HLA-B27 gene have ankylosing spondylitis.]

Some scientists believe that there is a gene located near to the HLA-B27 gene that is passed down with it to the offspring, and that in fact, this gene is the gene responsible for ankylosing spondylitis.

This was just an example; this topic will be further explained in the next lecture.

Molecule	MHC I	MHC II
Structure	1 α & 1 β, with the α having 3 domains and doing all the presentation while the β is there to maintain the shape of the molecule.	1 α & 1 β both having 2 domains both anchoring the cell membrane and both doing the presentation.
Groove and peptide	Small and hosts peptides of 7-9 amino acids in length.	Large and hosts peptides of 20-25 amino acids in length.
Genes loci	α on short arm of chromosome 6 class I with 3 separate genes (HLA-A, HLA-B and HLA-C) while the β is found on β2-microglobulin gene	α & β on short arm of chromosome 6 class II with 3 separate genes (DP, DR & DQ).

	found on chromosome 15.	
Max # of MHC molecules	6.	18-20.
Present antigen to	Cytotoxic CD8 T-cell.	CD4 T-helper cell.
Cluster of differentiation (CD)receptor location	α3 domain.	β2 domain.
Processing pathway	Endogenous	Exogenous

Special thanks go to everyone who helped me with this sheet.

Remember remember, all men are created equal. Some work harder in pre-season.

This sheet is dedicated to Abu Zghayar, ".", el 5afash, 3arman, sultz and smadi.

Forza Napoli.