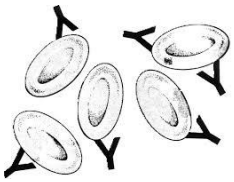
Few things to add to agglutination subject:

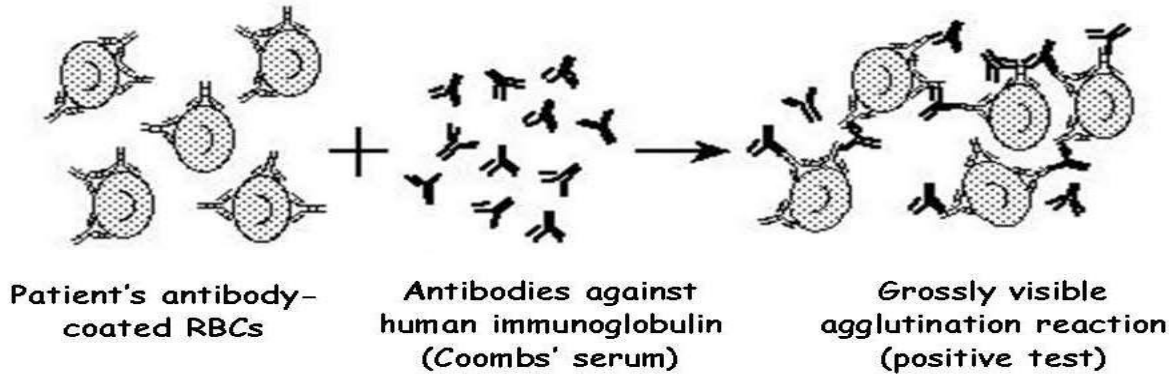
When you agglutinate red blood cells (hemagglutination) you cross link the antigens that are present on two adjacent red blood cells, and of course red blood cells have negative charge on their surface, which means that there is some sort of repulsion (zeta potential) and there is always a kind of gap or space between them, very small but although cannot be bridged by certain antibodies.
If the antibody is big it can bridge the gap. For example the IgM which is very extensive can join and bridge the gap easily, so it is excellent agglutinating agent. But IgG can be referred to as incomplete antibody because it cannot really bridge the gap between the two, so when you mix them together you get something like this picture.



So here we have IgG which is attached to the surface of the RBCs *but there is no agglutination*. We call these cells: sensitized cells, they are adhered to an antibody but no agglutination.

Know how can we produce agglutination? We use another antibody. And this another antibody is produced in animals against IgG [anti IgG] directed against the Fc fragment.
So it will join these RBCs together and can cross link the gap and get agglutination.

This is known as Coomb's test or Globin test or Anti-Globin test



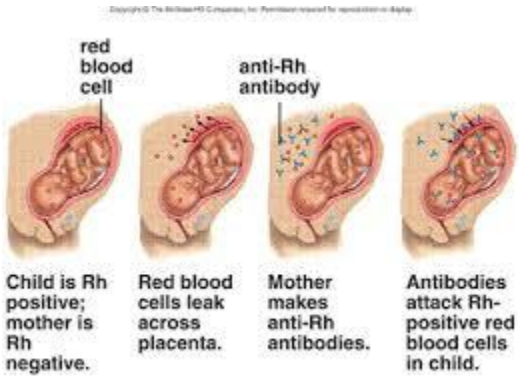
This is really important in blood banking, because very often you can have sensitized red blood cells but you can't detect the agglutination unless you add an anti IG to the mixture.

An example of this is *Rhesus Incompatibility (Rh disease):*

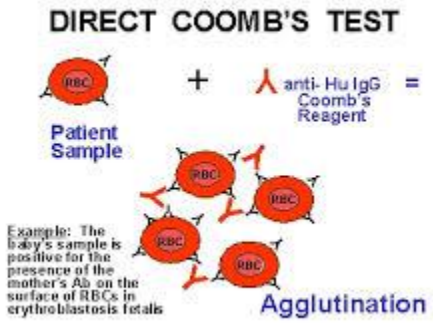
[Some people are Rh positive and some are Rh negative whether they have the D antigen on the surface of their cells or not].

This disease occurs during pregnancy when the mother has Rh negative blood, and her baby has Rh positive blood.
So if the mother is Rh negative and she has a pregnancy and the baby is Rh positive, when the baby's blot goes into the mother at child birth, the mother produces antibodies against the D antigens and these are anti D antibodies from IgG variety, so if the women gets pregnant again these antibodies cross

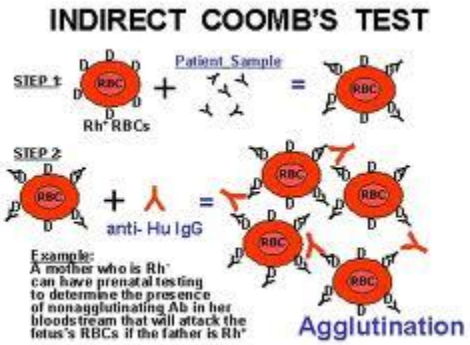
the placenta and attach to the red blood cells in the baby and destroy them, and the baby will have hemolytic anemia, jaundice, and other complications, and this is called *hemolytic disease of the newborn HDN*.



So if you have a baby born with jaundice you have to suspect that he has HDN, so you need to find out if he really has HDN or not. What do you do? You look for antibodies on his RBCs -sensitized RBCs-. How? By doing **direct coombs test:**
You take a sample from the baby and do coombs test; add anti IgG, if there is HDN you will get agglutination.



The **indirect coombs test**:
Usually done for detection of anti D *in the serum of the mother*.
We take a serum sample from the mother, and bring RBCs from anybody who is Rh positive, and mix it with the serum. If the mother's serum has antibodies it will sensitize these RBCs, then we add the anti IgG to the RBCs it will agglutinate.



So direct coombs test is looking for antibodies on RBCs of the baby. And the indirect is looking for antibodies in the serum of the mother.

Know we move to the MHC subject

MHC molecules are molecules that are present on cells and concerned with antigen presentation. They were first discovered on the surface of WBCs, so they called them *HLA – human leukocyte antigens.* But of coarse they present on all cells of the body.

Later on, they have discovered them in relation to transplantation. When you transplant an organ from animal to another, if these molecules are similar the graft will be accepted, if they were not similar the graft is rejected. So they called them *Major Histocompatibility Complex* (the tissues are compatible with each other or not).

Really, Allah Ta'ala didn't give us these antigens to make life difficult for transplant surgeons, but for resisting infection, so their main role is in immune response against infections from the environment. So these are the *immune response genes IRG* -MHC molecules genes which are present on the complex-

We have **three classes** of these molecules:
MHC class 1 and class 2 are concerned with the combating infection and are cell-bound.
Class 3 can take part in immune response but are not cell-bound.

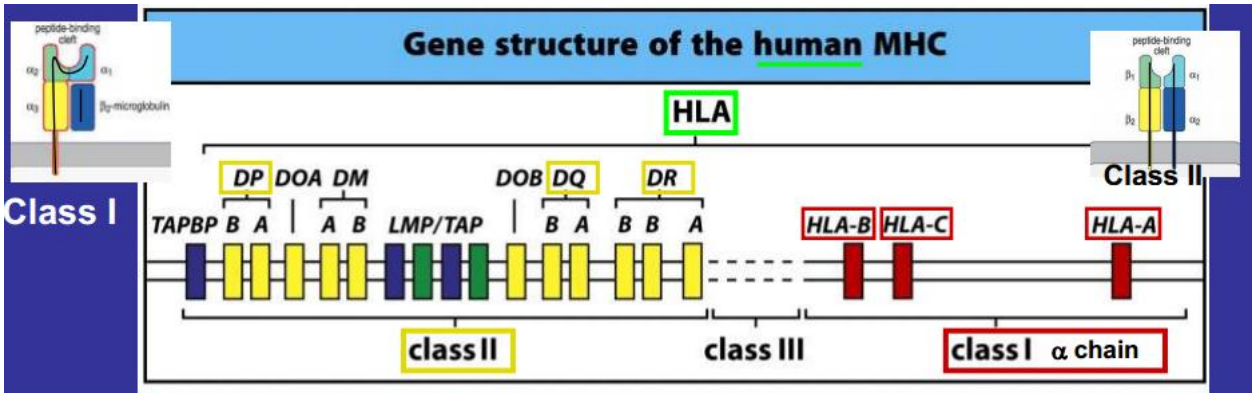
Where we find the genes of this system? *We find them on the short arm of chromosome 6*

MHC gene consists of three regions.
Class 1 region, class 2 region, and between them class 3 which codes for complement C4, C2, factor B and lymphotoxin which is a cytokine involved in immune response.
class three molecules are soluble and not cell bound.

These genes are inherited from the mother and father, and their expression is co-dominant. Which means that we will express both genes inherited from mother and father.

Class 1 molecules:

*When we come to class one region we find that there is three genes: HLA b, HLA c, HLA a.



*Class1 molecules are present on all nucleated cells in the body. So the exception is RBCs (they don't have nuclei so don't have class 1 MHC molecules.)

*Class 1 molecules are made of two polypeptide chains:

1. **alpha chain**: it is made of three domains; alpha 1 domain, alpha 2 domain and alpha 3 domain. Alpha 3 domain is a member of super IG family so these MHC molecules are members of super IG family.

*We have a transmembrane part and cytoplasmic part that anchors it to the cell.

*coded for by genes in the MHC on the short arm of <u>chromosome 6</u>

2. beta 2 microglobulin: Associated with the alpha chain we have another molecule know as beta 2 microglobulin.

*beta 2 microglobulin is coded for by a gene on <u>chromosome 15.</u>

*Notice that beta 2 microgloblulin is not covalently attached to alpha chain, and is not anchored to the membrane, it is just like a hook to maintain the structure of MHC class1 molecule.

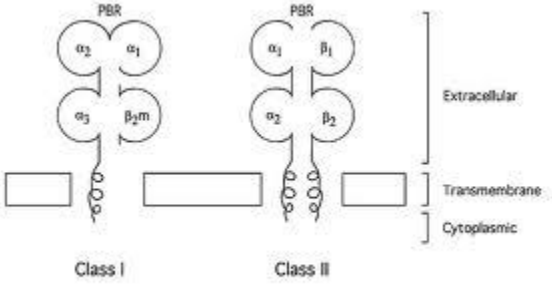


Figure 2. Schematic presentation of the structure of MHC class I and class II molecules. PBR = peptide-binding region. (Reprinted, with permission, from the Annual Review of Genetics, Vol. 32 ©1998 by Annual Reviews, www.annualreviews.org).

Class1 genes:

Here we have HLA a; the first discovered, then they discovered HLA b, then HLA c. These are the main types of MHC class 1 molecules in our body. This means that each of these genes produce alpha chain which is different from the other, **but they have similar beta 2 microglobulin.**

So the gene produces the alpha chain then it associates with beta chain microglobulin then it is displayed on the surface of the cell.

So how many types of MHC class 1 molecules can you have on your cells?

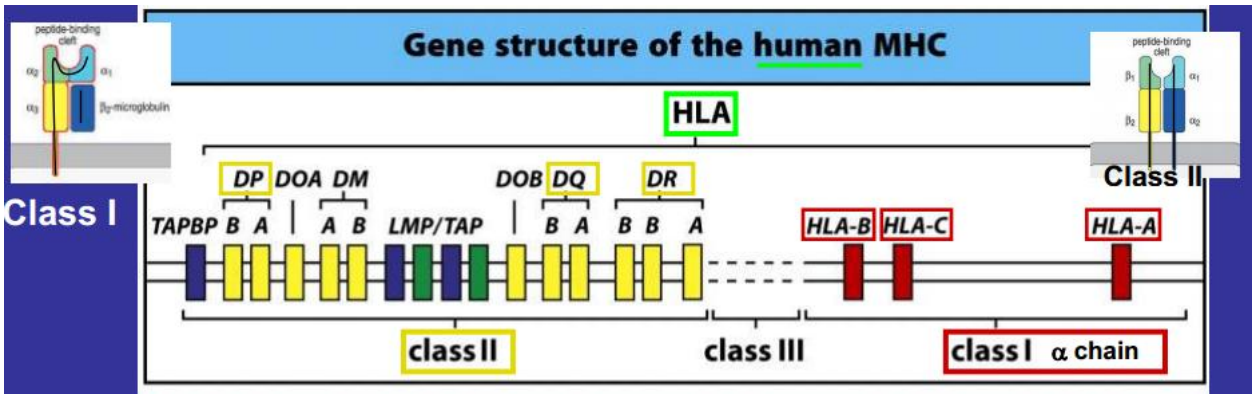
You can have 6; 2 As, 2 Bs, and 2Cs, one from the mother and one from the father (co-dominance).

Class 2 molecules:

*Made of two polypeptide chains:
Alpha chain and beta chain, and each has two domains (alpha1, alpha2, beta1, beta2).
Notice again that these alpha2 and beta2 domains are members of super immunoglobulin family.

Here ***both chains*** *are coded for by genes on* <u>*chromosome 6.*</u>

*Class 2 region as a whole was labeled as the D region; because at the beginning they discovered HLA a, b, c and called them class 1, later on they discovered more of these molecules and collectively by cell interaction they called them D region.
Then they found that there is three different gene loci in D region, they called them : DP, DQ, DR.



In each locus we have genes, for example in Db we have genes: A1, A2, B1, B2
A gene should code for alpha chain, and *B gene* should code for beta chain.

Usually there are only one A functional gene. For example: in the DB region we have 2 A genes, but only one of these is functional coding for one alpha chain.

The B genes also most of them are not functional. So in DR which is the most varied we have 3 functional B genes, but in DP and DQ we have one functional B genes.

So in **DP**: we have one functional A gene produces alpha chain and one functional B gene produces beta chain, and they will combine to produce MHC class 2 molecule.

DQ: the same as Dp.

DR: have 3 functioning B genes, and codes for at least 2 kinds of MHC molecules.

So 1 molecule from DP, 1 from DQ, 2 from Dr, so we have four different MHC class 2 molecules expressed on the cells. And don't forget that there is co-dominance, so there are 8 MHC class 2 molecules on the cell.

Sometimes you can have up to 18 molecules on cells, because it is believed that alpha chain from the father can associate with beta chain from the mother and vice versa, which increases the chances of having different MHC class 2 molecules.

So class 1 we always have 6 different molecules, but class 2 molecules we can have between 16 to 18.

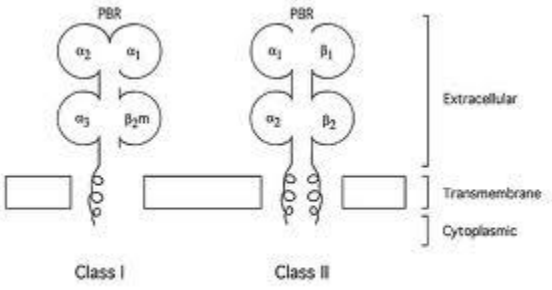
*These HLA a, b and c are classical genes.
*There are other non-classical HLA genes, they are really not known, but among them, the function of HLA g and HLA e is known and the presence of these on cells can prevent the killing of cells by NK cells; which kill cells that don't express MHC class 1 molecules.

For example: when we talk about the placenta when it overlies the uterus in pregnancy some cells there don't have MHC class 1 molecules because this is the way the fetus is accepted and not rejected by cytotoxic T cells. But NK cells can come and destroy the barrier between the placenta and the uterus so these cells -the trophoblastic cells- express HLA g and HLA e and this will stop the NK cells from attacking.

*Where we find MHC class 2 molecules? We find them on the antigen presenting cells; macrophages, dendrytic cells and B cells.
((if you want to consider that these MHC class1 and MHC class2 molecules are ways for presentation of antigen, then all cells of your body are antigen presenting cells)) but these cells that have MHC class2 antigen on their surface are the professional antigen presenting cells.

*What do they present? They present peptides.

In class 2 molecule, there is a groove between the alpha 1 and beta 1 domains, and this is the peptide binding groove.
In class 1 molecule also there is a groove between alpha1 and alpha2 domains of the alpha chain. Beta has nothing to do with it.



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The peptide actually should have some sort of correspondence to the groove.

*We have variation within MHC class molecules. But this variation is not as those as antibodies and TCRs , here the variation is actually a polymorphism.
For example we have HLA a, b, and c
Each one is different from the other by having different amino acids In them.
And indeed within the a, b, and c we have different alleles, for example : HLA a there are 151 alleles , HLA b 301 alleles, HLA c 83 alleles.
These alleles are distributed within the population, so as one individual can have 1 or 2 alleles, how?

If your mother and father are not related you will get for example A23 from you father and from your mother A16. But if there is consanguinity like in animals if you mate brother to sister, after generations you will get identical HLA antigens.

For example:
HLA a1, a3 / HLA b27, b52 / HLA c10, c9 so here this person have 6 HLA antigens and 6 different alleles.
Sometimes you can have only 5 alleles like if you have HLA a1 , a1 .

*Which is better: to have more alleles or less alleles?
The groove for each peptide is different, so every groove of every allele should be different, so the more alleles you have the better the chance to fit the peptides from pathogens.

But we don't need to have too much diversity like the Igs because TCR recognizes the antigen in conjunction with MHC molecule, so it is impossible if there are too many variations.

And that's why we have too many alleles: why some people get disease like small pox which killed many people and others survive? They must have MHC class molecules that are better in displaying the peptides for the immune system.
So this variation in alleles spread all over the population, whenever a pathogen comes it can affect some of us but can't affect us all.

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***Endogenous antigens are produced within the cells, they can be our own proteins or viral proteins, and exogenous antigens are from extracellular organisms that have been phagocytosed and broken down in the phagolysosome.
Endogenous antigens are displayed as peptides on MHC class1 molecules, and exogenous antigens are displayed as peptides on the groove of MHC class2 molecules.