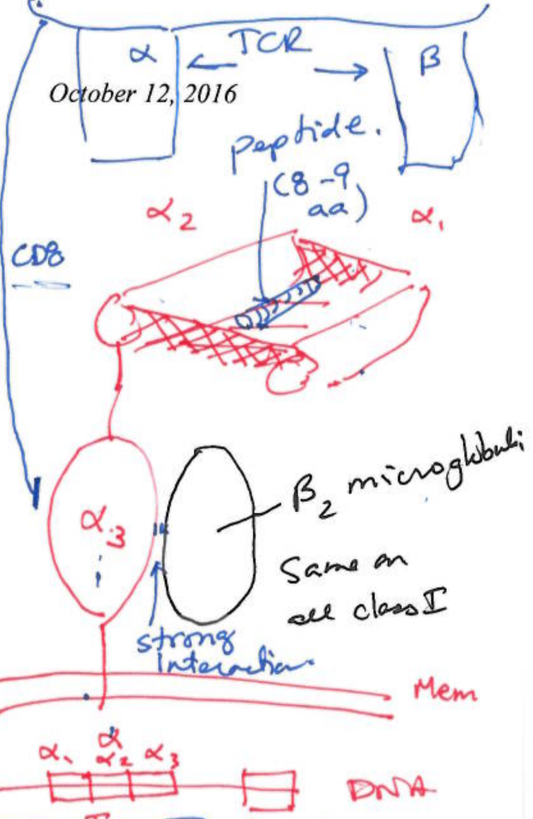
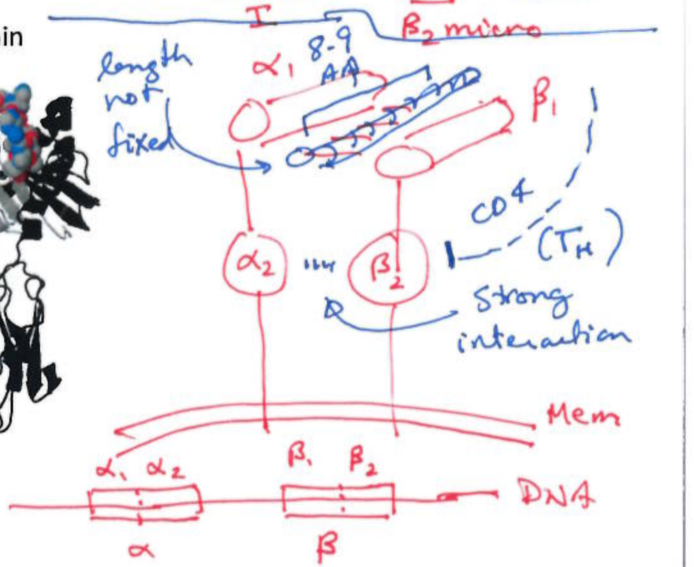
Lecture 12 - MHC Structure and Genetics.

MHC I



- Larger chain: α1α2 α3 domains (α-refers to a chain label, not to an α-helix)
- α1-α2 domain forms an 8-stranded β-sheet that serves as a platform for peptide binding.
- Edges of the peptide binding site are defined by long α-helices, one from α1 and one from α2.
- α3 domain is an immunoglobulin fold and is the attachment point to the membrane.
- α3 is paired with β_2 microglobulin, which also has a typical Ig fold.
- β_2 microglobulin is essential for stability and peptide binding and is the same on all class I MHC.
- CD8 on T_C cells binds to the α3 domain.
- Binds peptides 8-9 residues long, some degree of specificity.

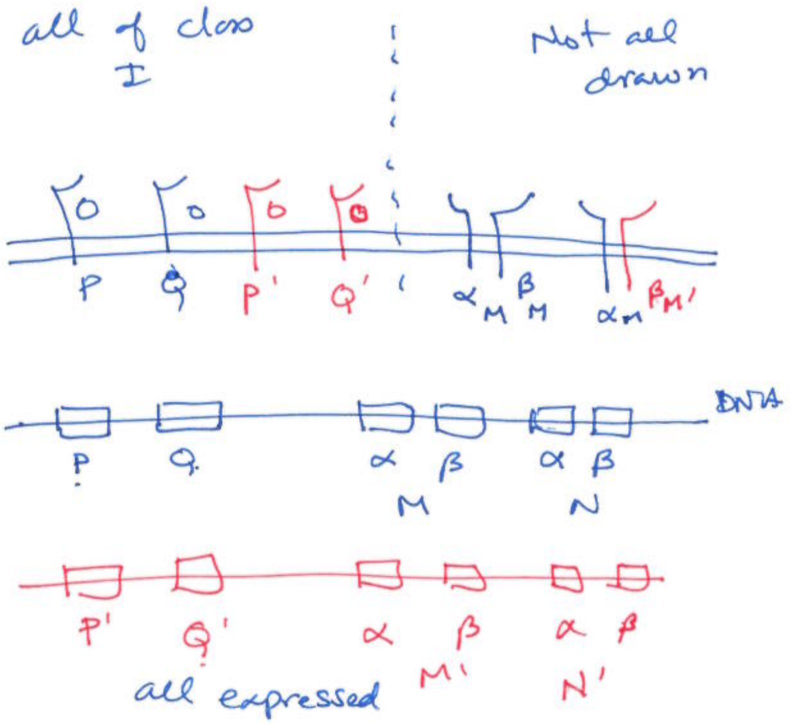
MHC II:



- Almost identically sized α and β chains (α and β are chain labels, not secondary structures).
- Each chain is divided into two domains, e.g. α1α2, β1β2
- α1 and β1 form the peptide binding domain
- α2 and β2 are immunoglobulin domains that pair with each other.
- α2 and β2 are the point of membrane attachment.
- CD4 on T_H cells binds to β2 domain.
- Can bind peptides 8 or longer, some degree of specificity.

Genetics of MHC (major histocompatibility, mice) & HLA (human leukocyte antigen)

- Class I MHC are expressed on all nucleated cells, including antigen presenting cells.
- Class II MHC are expressed only on <u>*professional*</u> antigen presenting cells (macrophage, dendritic cell, B-cell)
- MHC are not subject to allelic exclusion, both maternal and paternal MHC are expressed on cells.
- There are a number of homologous MHC genes, *all* of which can be expressed (co-dominant).
- The locus is highly polymorphic, with *many different alleles* in a typical population.
- Each individual in a population has a *unique* and invariant collection of MHC alleles/molecules.
- The MHC molecules are the major determinant of immunological "self" and cause the majority of the problems in solid organ transplantation.

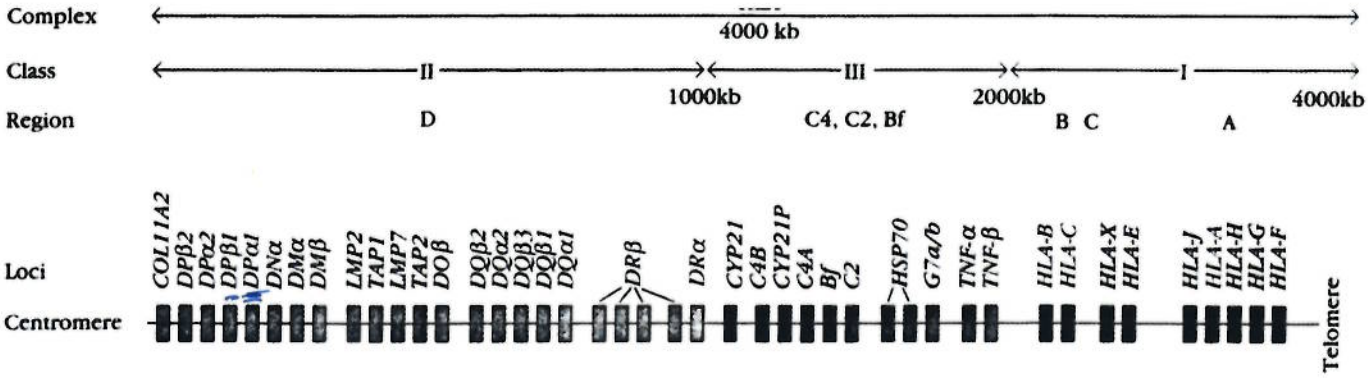


Mice: Major MHC (Major Histocompatibility Complex) Genes - General Structure:

Complex	**H-2**				
MHC Class	I	II		I	
Region	K	IA	IE	D	
Gene Products	H-2K	H-2 IA $\alpha\beta$	H-2 IE $\alpha\beta_1\,\beta_2$	H-2D	H-2L

Humans: In humans, the term HLA (Human leukocyte antigen) is used - General Gene Structure:

Complex:	**HLA**					
MHC Class	II			I		
Gene Products	HLA-DP $\alpha_{1,2}\beta_{1,2}$	HLA-DQ $\alpha_{1,2}\beta_{1,2,3}$	HLA-DR $\alpha\beta_{1,2,3,4}$	HLA-B	HLA-C	HLA-A

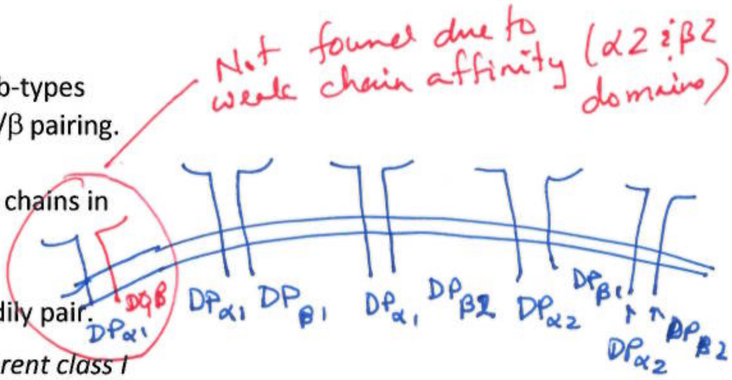


Human Class I Region:

- Although there are 9 "class I" genes, only HLA-A, HLA-B, and HLA-C are "classical" class I MHC, presenting antigens to $CD8^+$ T_C cells.

Human Class II Region:

- DP, DQ, DR refer to "classical" class II MHC. These sub-types differ significantly in homology & ability to pair via α/β pairing.
- Within a subclass, there can be multiple chains.
- Variable number of chains are possible, e.g. four DR_β chains in some individuals.
- Any α and β chains within a sub-class can pair.
- α and β chains from different sub-classes do **not** readily pair.



You do not need to remember the exact order of the different class I and class II genes, just the expression and pairing rules.

Polymorphism of Class I and Class II MHC

Any given individual has a fixed and relatively small set of MHC molecules. However, there are 506 HLA-A, 872 HLA-B, and ~274 HLA-C documented alleles in the human population. As an example, hypothetical sequences for different alleles of HLA-B and HLA-C are shown below:

Gene	Sequence	Allele Number
HLA-B	Ala-Gly-Thr-Ser-Phe-Val-Leu-Ser-Val-Val-Leu-Arg-Phe-Lys-	1
	Ala-Gly-Thr-**Leu**-Phe-Val-Leu-Ser-Val-Val-**Ile**-Arg-Phe-Lys-	2
	.	
	Ala-Gly-Thr-Ser-**Tyr**-Val-Leu-Ser-Val-Val-Leu-**Glu**-Phe-Lys-	871
	Val-Gly-Thr-Ser-Phe-Val-Leu-Ser-Val-Val-Leu-Arg-**Val**-Lys-	872
HLA-C	Phe-Arg-Val-Leu-Phe-Val-Ser-Ala-Gly-Met-Arg-Ser-Val-Thr-	1
	Phe-Arg-**Ala**-Leu-Phe-Val-Ser-Ala-Gly-Met-Arg-Ser-Val-Thr-	2
	Tyr-Arg-Val-Leu-Phe-Val-Ser-Ala-Gly-Met-**Glu**-Ser-Val-Thr-	3
	.	
	Phe-Arg-Val-Leu-Phe-Val-**Tyr**-Ala-Gly-Met-Arg-Ser-**Ser**-Thr-	273
	Phe-**Met**-Val-Leu-Phe-Val-Ser-Ala-Gly-Met-Arg-Ser-**Ser**-Thr-	274

There are about 506 x 872 x 274 = 1.22×10^8 different class I MHC sequences in the human population.

As for class II MHC, a large number of alleles are also found. In addition, there is some variability in the number of α/β chains. Overall, the diversity of class II MHC is estimated to be 10^8 different protein combinations. This gives an overall diversity is on the order of 10^{16}.

The chances of two individuals on the planet having *exactly* the same HLA alleles for all genes is practically zero, unless the individuals are very closely related. Consequently, it is more likely that an individual will have different alleles of MHC because they would have inherited different alleles from their parents.

The location of these allelic differences within an MHC molecule is shown below (Figure from Kuby, 2nd Edition):

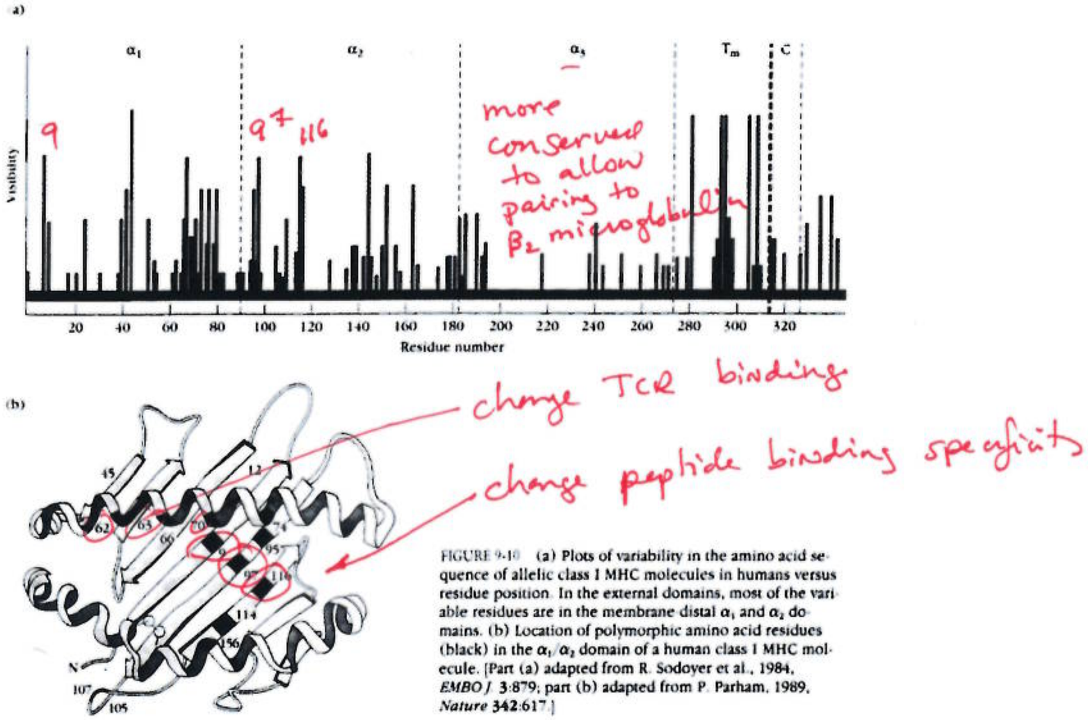


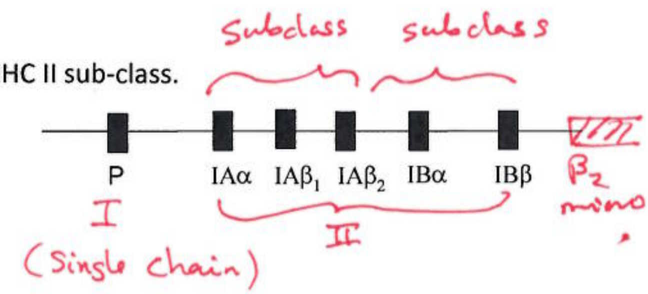
FIGURE 9-10 (a) Plots of variability in the amino acid sequence of allelic class I MHC molecules in humans versus residue position. In the external domains, most of the variable residues are in the membrane-distal α_1 and α_2 domains. (b) Location of polymorphic amino acid residues (black) in the α_1/α_2 domain of a human class I MHC molecule. [Part (a) adapted from R. Sodoyer et al., 1984, *EMBO J.* 3:879; part (b) adapted from P. Parham, 1989, *Nature* 342:617.]

What region of the MHC are these allelic differences found? What would you expect them to affect?

Expression of MHC Genes: *Just follow the rules:*

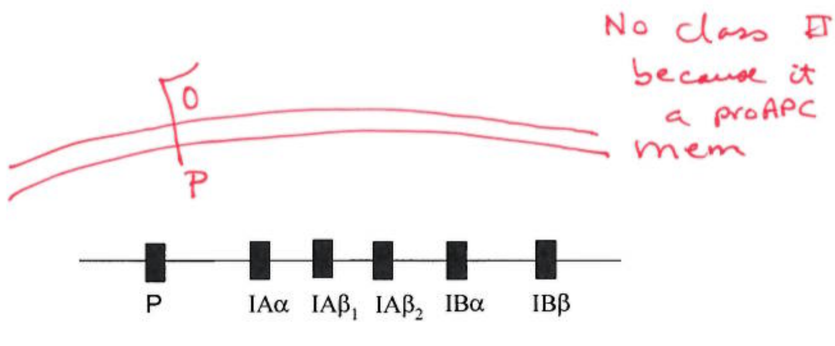
- co-dominant expression
- Different alleles are different proteins.
- Pairing only occurs between α and β chains within a MHC II sub-class.

Example: The MHC locus is shown on the right.

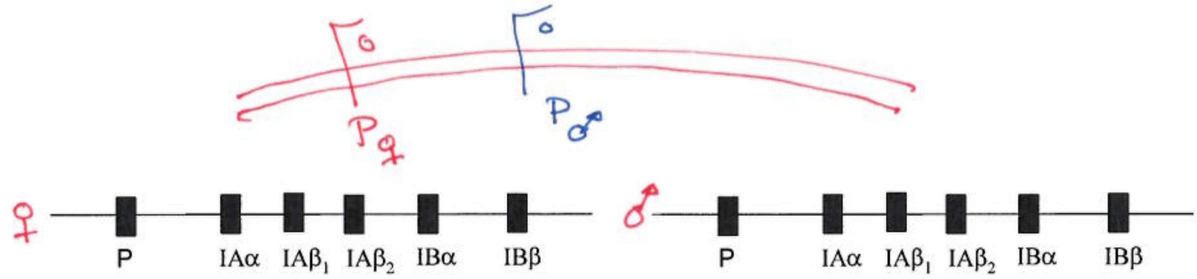


i. Which genes encode class I MHC and which encode class II MHC? Why?

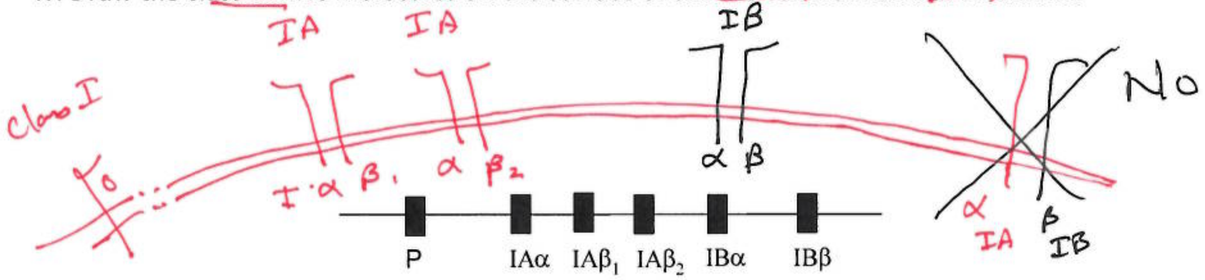
ii. Draw the class I MHC molecules on the surface of a liver cell from an *inbred* animal (same alleles on each chromosome).



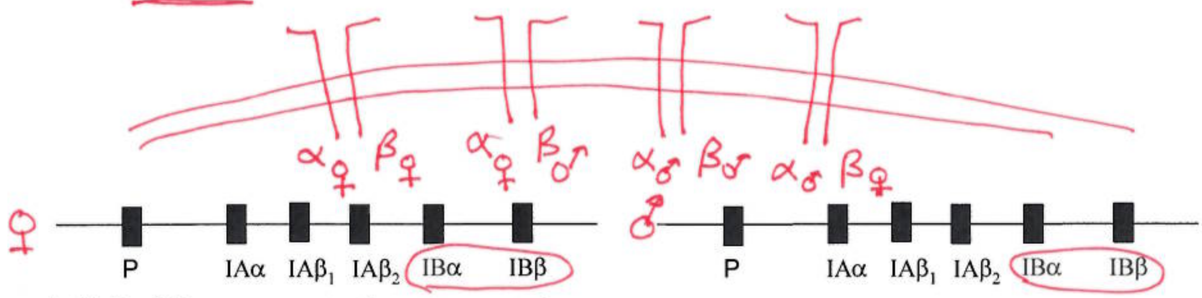
iii. Draw the class I MHC molecules on the surface of a liver cell from an *outbred* animal (different alleles on each chromosome).



iv. Draw the class II MHC molecules on the surface of an APC cell from an *inbred* animal.



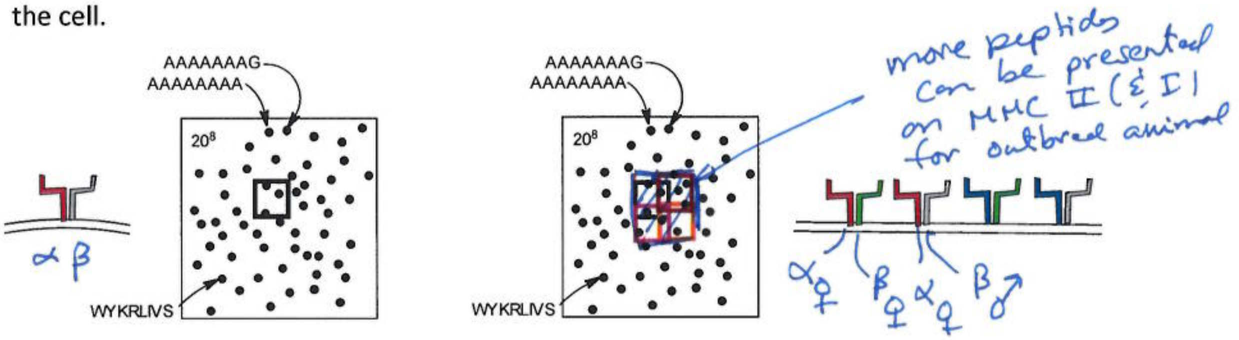
v. Draw the IB type II MHC molecules on the surface of an APC cell from an *outbred* animal.



Effect of Allelic differences on Antigen Presentation:

The following diagrams represent the number of peptide sequences you would find in an 8 residue peptide. Each dot represents a particular sequence. Dots that are near each other are similar in sequence.

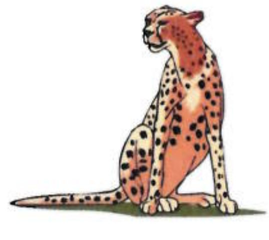
- The square in the left diagram represents the peptides that are presented on an in-bred organism with a single class II MHC.
- The squares on the right diagram represent the peptides that are presented on the same MHC II of an out-bred organism. Because it is outbred there would be four different MHC II on the surface of the cell.



Key point: Allelic differences increase the number of different MHC molecules on the surface of the cell, increasing the number of possible peptides that can be presented.

Allelic variation in different Species

Species	Polymorphic loci
Cheetah	0%
Lion	12%
Domestic cat	21%
Mouse	20%
Human	32%

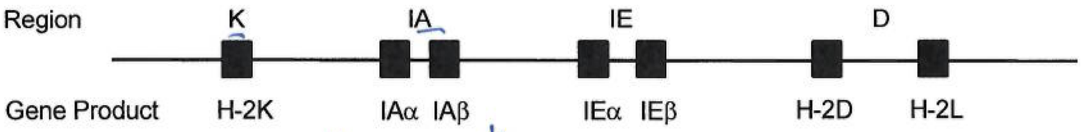


MHC Haplotypes: There are so many different alleles of MHC that a short hand notation has developed to describe the particular set of alleles that are found in a region of one chromosome. This is referred to as a ***haplotype***. In general, the haplotype refers to the genetic composition of one chromosome.

For example, the haplotypes of various **inbred** mouse strains containing different alleles of the various MHC genes is given in the following table. Note that these strains, because they are inbred, have the same alleles on each chromosome. An outbred stain would have a different haplotype associated with each chromosome. In this table the number refer to specific alleles, *not* a particular amino acid in the MHC protein. There are generally multiple amino acid changes when comparing different alleles.

		Allele at each MHC Locus (alleles represented by arb. #)						
Mouse Strain	**Haplotype**	**K**	**IA_{α}**	**IA_{β}**	**IE_{α}**	**IE_{β}**	**D**	**L**
		MHC I	**MHC II**				**MHC I**	
CBA	*k*	3	54	22	97	11	13	80
C3H	*k*	3	54	22	97	11	13	80
C57BL	*b*	12	3	74	20	18	5	65
C90		12	3	74	20	32	5	65

$H\text{-}2^k$



$H\text{-}2K^k$ (K^k) IA^k IA_{α}^k IA_{β}^k
#54 #22

An individual class I gene is named according to the haplotype of the entire locus. The K chain in a mouse of *k* haplotype would be called K^k (k of k) and the other two class I genes would be D^k and L^k. This implies that allele #3 is found on the class I K molecule, allele #13 on the class I D molecule, and allele #80 on the class I L molecule. Unfortunately, the same letters are used to refer to genes as well as haplotypes.

- An individual class II MHC allele is also named according to the haplotype, for example in a mouse of k haplotype the class II IA molecule would be IA^k (IA of k). Each chain of this allele would be called $IA\alpha^k$ and $IA\beta^k$. This implies that allele #54 would be found on the α-chain of sub-type IA.
- If two strains of different haplotype are crossed, then the F_1 progeny will have both haplotypes. For example, if CBA is crossed with C57BL, the resultant haplotype is $H\text{-}2^{k/b}$ and alleles from *both* parents will be expressed on the surface of cell.
- If two strains differ at only one MHC gene locus they are **congenic strains.** In the above table C90 is congenic to C57BL at the IE_{β} gene, all other MHC genes would be the same.
- Strains with the same MHC haplotype can (and probably do) differ at many other loci besides MHC.

Using the simplified diagram above, please answer the following questions:

i) A $H\text{-}2^k$ inbred mouse would express how many different MHC class I molecules? 3 $H\text{-}2K^k$, $H\text{-}2D^k$, $H\text{-}2L^k$

ii) A $H\text{-}2^b$ inbred mouse would express how many different MHC class I molecules? 3 $H\text{-}2K^b$, $H\text{-}2D^b$, $H\text{-}2L^b$

iii) A $H\text{-}2^{k/b}$ mouse, from a $k \times b$ cross, would express how many different class I molecules? 6