Expression of HLA Genes and Regulation of HLA Expression

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Key Concepts in Major Histocompatibility Complex (MHC)

1. Ag presentation to TCR is mediated by Two classes of MHC molecules.
   - Class-I MHC => peptides from cytosolic (intracellular) proteins => CD8 T cells
   - Class-II MHC => peptides from extracellular (exogenous) proteins from phagocytosis => CD4 T cells
   - Class-III MHC => components of the complement system molecules involved in inflammation
2. In humans, the MHC is also called as the **HLA (Human Leukocyte Antigen).**
3. Located in the short arm of chromosome 6 (part of MHC).
4. MHC genes are the most polymorphic genes present in the genome and co-dominantly expressed in each individual.
5. MHC molecules express on the cellular surfaces of only in presence of Ag-peptides.
   Class-I => all nucleated cells
   Class-II => APCs (DC, Macrophages & B cells)

Expression of HLA Genes

- The MHC is referred to as the H-2 complex in mice and as the HLA complex in humans. In both species, the MHC is organized into a number of regions encoding class I (pink), class II (blue), and class III (green) gene products.

- The class I and II gene products shown in this figure are considered to be the classical MHC molecules. The class III gene products include other immune function-related compounds such as the complement proteins (C′) and tumor necrosis factors (TNF- and Lymphotoxin).

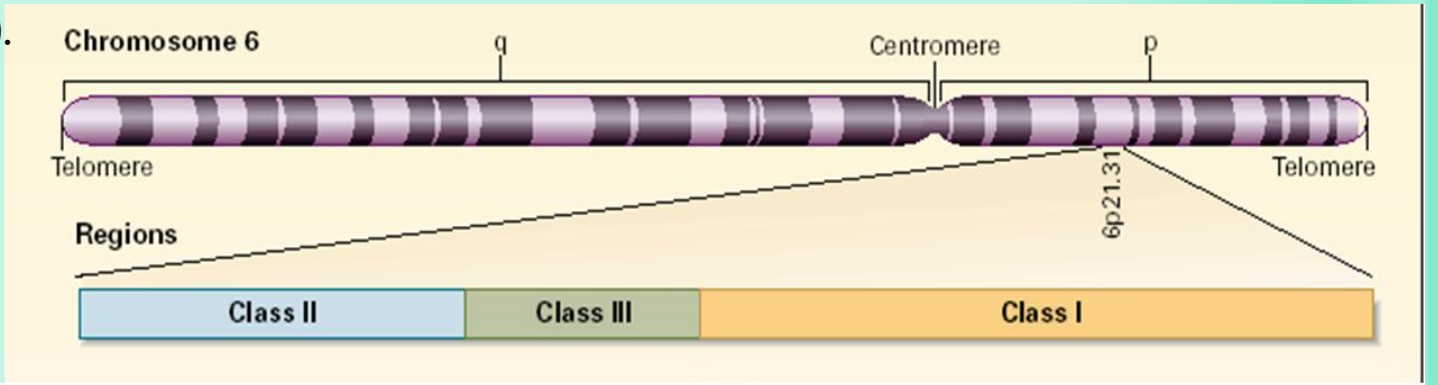
Human HLA complex

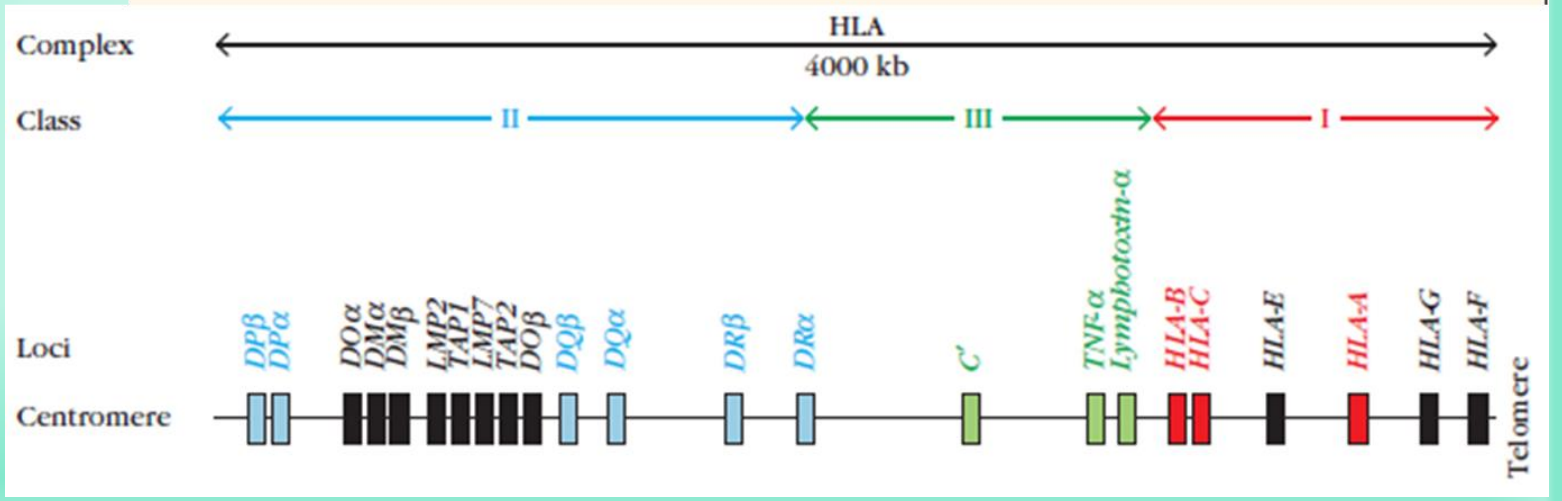
Source:Kuby

Complex	HLA								
MHC class	II			III		I			
Region	DP	DQ	DR	C4, C2, BF		B	C	A	
Gene products	DP αβ	DQ αβ	DR αβ	C′ proteins	TNF-α Lymphotoxin-α	HLA-B	HLA-C	HLA-A	

- The MHC class I genes are colored red, MHC class II genes are colored blue, and genes in MHC III are colored green. Classical class I genes are labeled in red, classical class II genes are labeled in blue, and the **nonclassical MHC genes** are labeled in black.
- The concept of classical and nonclassical does not apply to class III (functions of only some of their proteins are known).

Gene	Encoded protein
LMP2, LMP7	Proteasome-like subunits
TAP1, TAP2	Peptide-transporter subunits
TNF-α, Lymphotoxin-α	Tumor necrosis factor α and lymphotoxin β

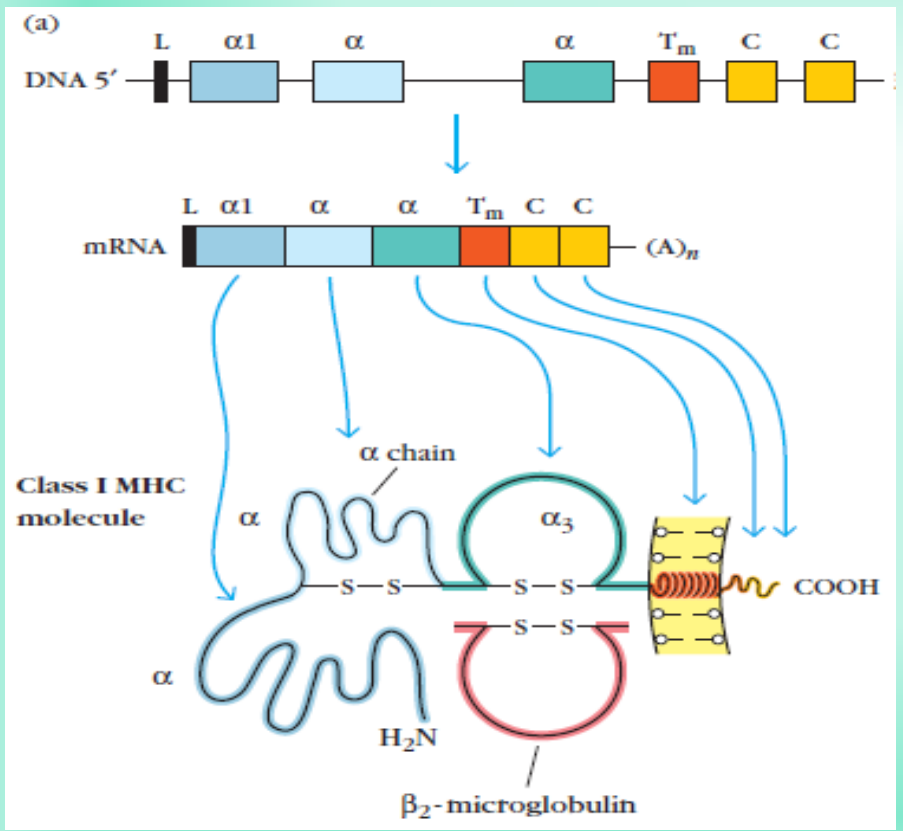


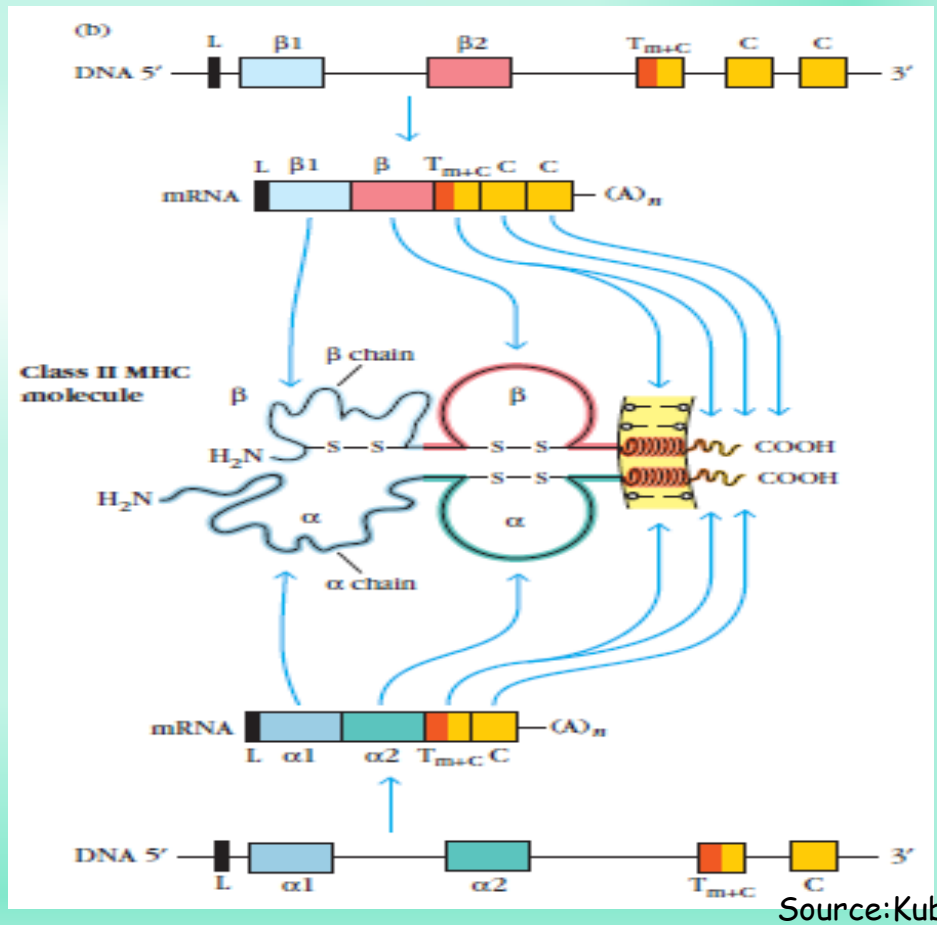


Simplified map of the human MHC loci

Source:Kuby

- There is strong correspondence between exons and the domains in the gene products of MHC molecules. Note that the mRNA transcripts are spliced to remove the intron sequences. Each exon, with the exception of the leader (L) exon, encodes a separate domain of an MHC molecule. The leader peptide is removed in a post-translational reaction before the molecule is expressed on the cell surface.
- The gene encoding 2-microglobulin is located on a different chromosome in human. Tm = transmembrane; C =cytoplasmic.



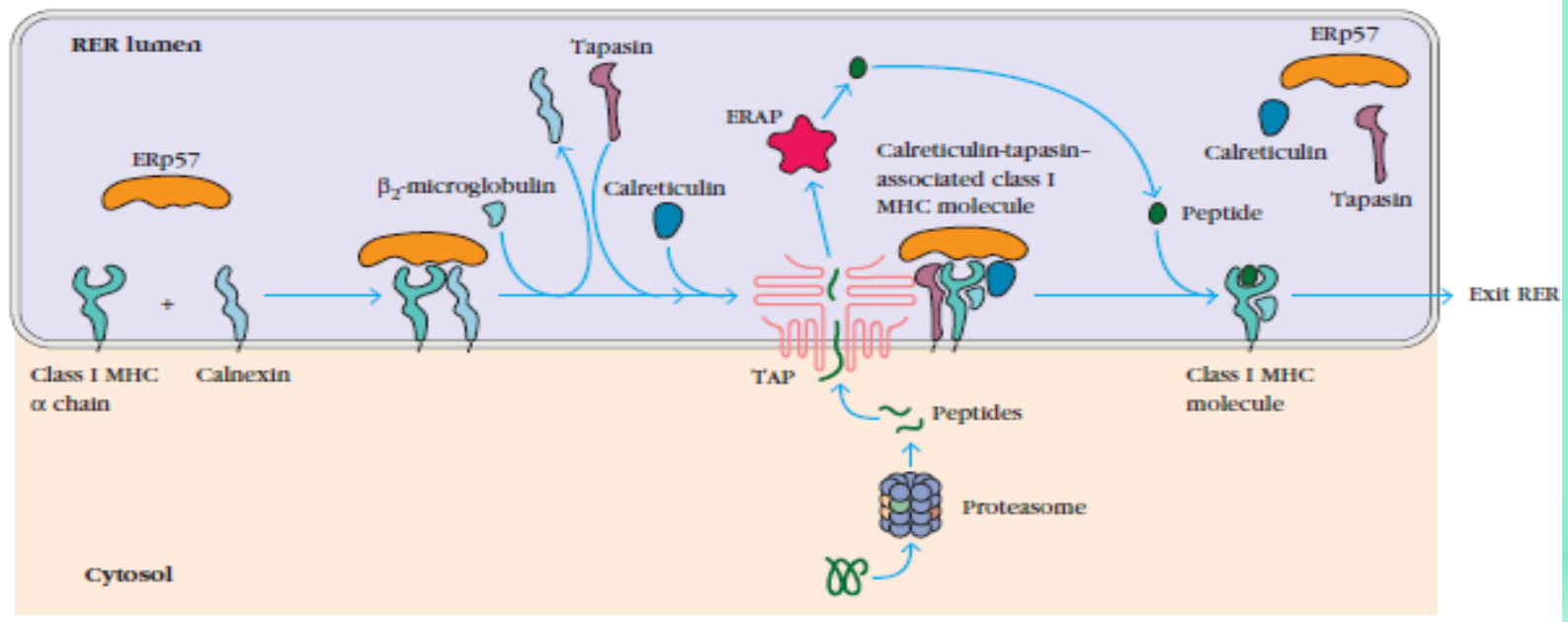


Source:Kuby

Schematic diagram of (a) class I and (b) class II MHC genes, mRNA transcripts, and protein molecules

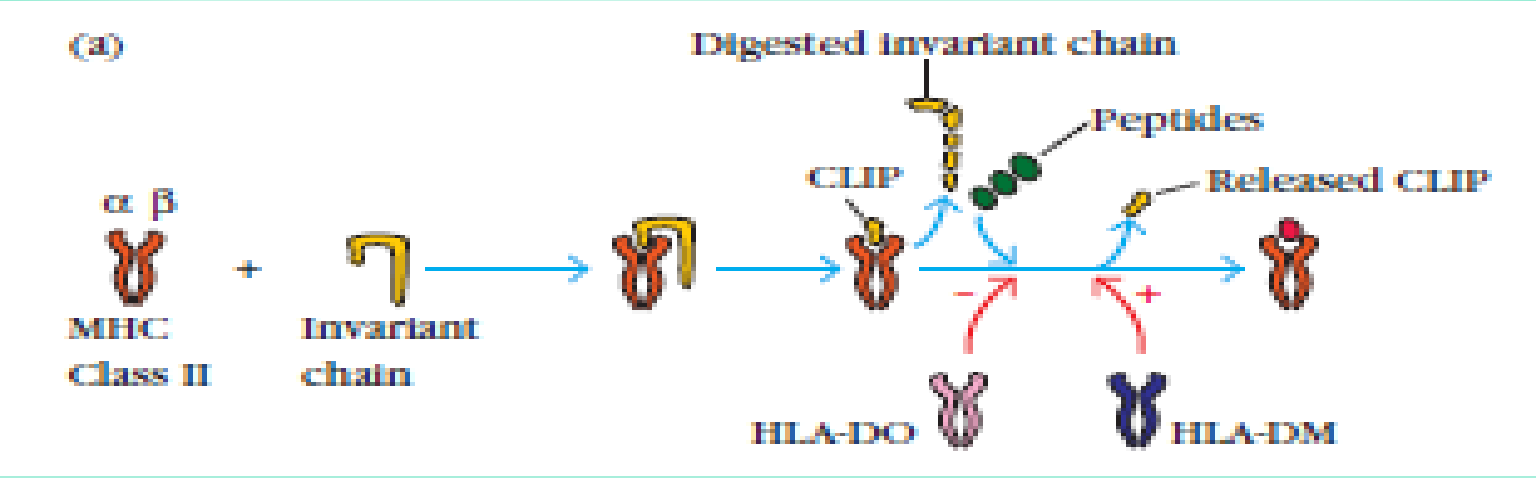
Assembly and stabilization of class I MHC molecules

- Within the rough endoplasmic reticulum (RER) membrane, a newly synthesized class I chain associates with calnexin, a molecular chaperone, and ERp57 until 2-microglobulin binds to the chain. The binding of 2-microglobulin releases calnexin and allows binding to calreticulin and to tapasin, which is associated with the peptide transporter TAP. This association promotes binding of an antigenic peptide. Antigens in the ER can be further processed via exopeptidases such as ERAP1, producing fragments ideally suited for binding to class I. Peptide association stabilizes the class I molecule-peptide complex, allowing it to be transported from the RER to the plasma membrane.



Assembly of class II MHC molecules.

- Within the rough endoplasmic reticulum, a newly synthesized class II MHC molecule binds an invariant chain. The bound invariant chain prevents premature binding of peptides to the class II molecule and helps to direct the complex to endocytic compartments containing peptides derived from exogenous antigens. Digestion of the invariant chain leaves CLIP, a small fragment remaining in the binding groove of the class II MHC molecule. HLA-DM, a nonclassical MHC class II molecule present within the MIIC compartment, mediates exchange of antigenic peptides for CLIP. The nonclassical class II molecule HLA-DO may act as a negative regulator of class II antigen processing by binding to HLA-DM and inhibiting its role in the dissociation of CLIP from class II molecules.



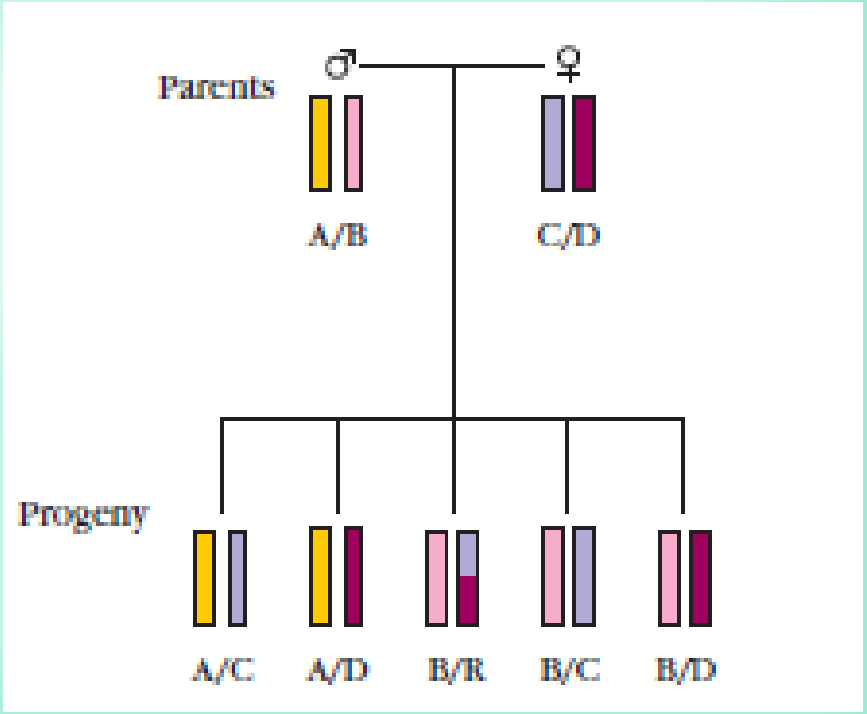
Source:Kuby

- Comparison of three dimensional structures showing the binding groove of HLA class II molecules (1α, 1β) containing different antigenic peptides or CLIP. The red lines show HLA-DR4 complexed with collagen II peptide, yellow lines are HLA-DR1 with influenza hemagglutinin peptide, and dark blue lines are HLA-DR3 associated with CLIP. (N indicates the amino-terminus and C the carboxyl-terminus of the peptides.)
- No major differences in the structures of the class II molecules or in the conformation of the bound peptides are seen. This comparison shows that CLIP binds the class II molecule in a manner identical to that of antigenic peptides.

Source:Kuby

Allelic forms of MHC Genes are Inherited in Linked Groups called Haplotypes

- The genes that reside within the MHC region are highly polymorphic; that is, many alternative forms of each gene, or alleles, exist within the population. The individual genes of the MHC loci (class I, II, and III) lie so close together that their inheritance is linked.
- This set of linked alleles is referred to as a haplotype.
- An individual inherits one haplotype from the mother and one haplotype from the father, or two sets of alleles.



Source:Kuby

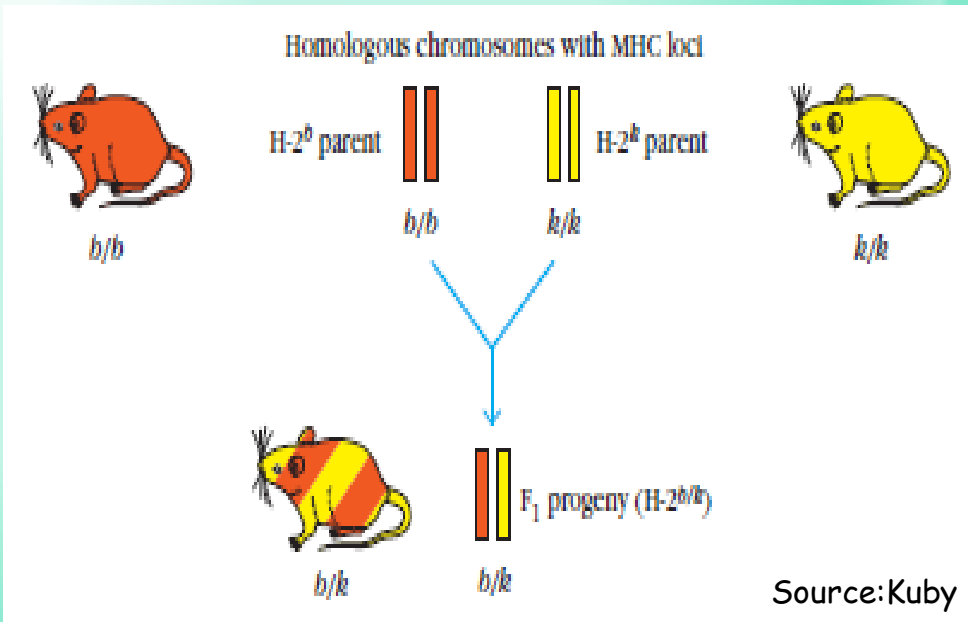
Inheritance of HLA haplotypes in a typical human family

Note: a new haplotype, R (recombination), can arise from rare recombination of a parental haplotype (maternal shown here).

Expression of MHC alleles is co-dominant

- The genes within the MHC locus exhibit a codominant form of expression, meaning that both maternal and paternal gene products (from both haplotypes) are expressed at the same time and in the same cells.
- Two mice from inbred strains possessing different MHC haplotypes are mated, the F1 generation inherits both parental haplotypes and will express all these MHC alleles.
- Such an F1 generation expresses the MHC proteins of both parental strains on its cells, it is said to be histocompatible with both parental strains.

- Off spring are able to accept grafts from either parental source, each of which expresses MHC alleles viewed as "self ".
- Neither of the inbred parental strains can accept a graft from its F1 off spring because half of the MHC molecules (those coming from the other parent) will be viewed as "nonself" (grafts rejection).



Mating of inbred souse strain with different MHC haplotypes in mouse

- In an outbred population such as humans, each individual is generally heterozygous at each locus. The human HLA complex is highly polymorphic, and multiple alleles of each class I and class II gene exist.
- However, the human MHC genes are closely linked and usually inherited as a haplotype. When the father and mother have different haplotypes, there is a one-in-four chance that siblings will inherit the same paternal and maternal haplotypes and therefore will be histocompatible (i.e., genetically identical at their MHC loci) with each other; none of the off spring will be fully histocompatible with the parents.
- The rate of recombination contributes significantly to the diversity of the loci in human populations (even if, crossover is low within the HLA complex).
- Genetic recombination can generate new allelic combinations, or haplotypes and the high number of intervening generations since the appearance of humans as a species has allowed extensive recombination

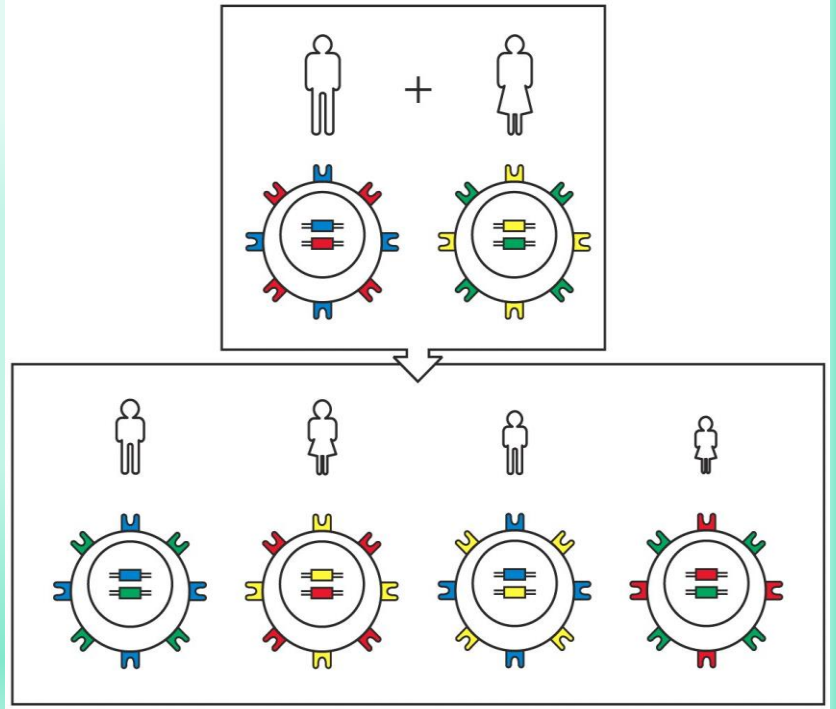


Figure 5-14 Immunobiology, 6/e. (© Garland Science 2005)

Gene conversion creates new alleles in MHC genes

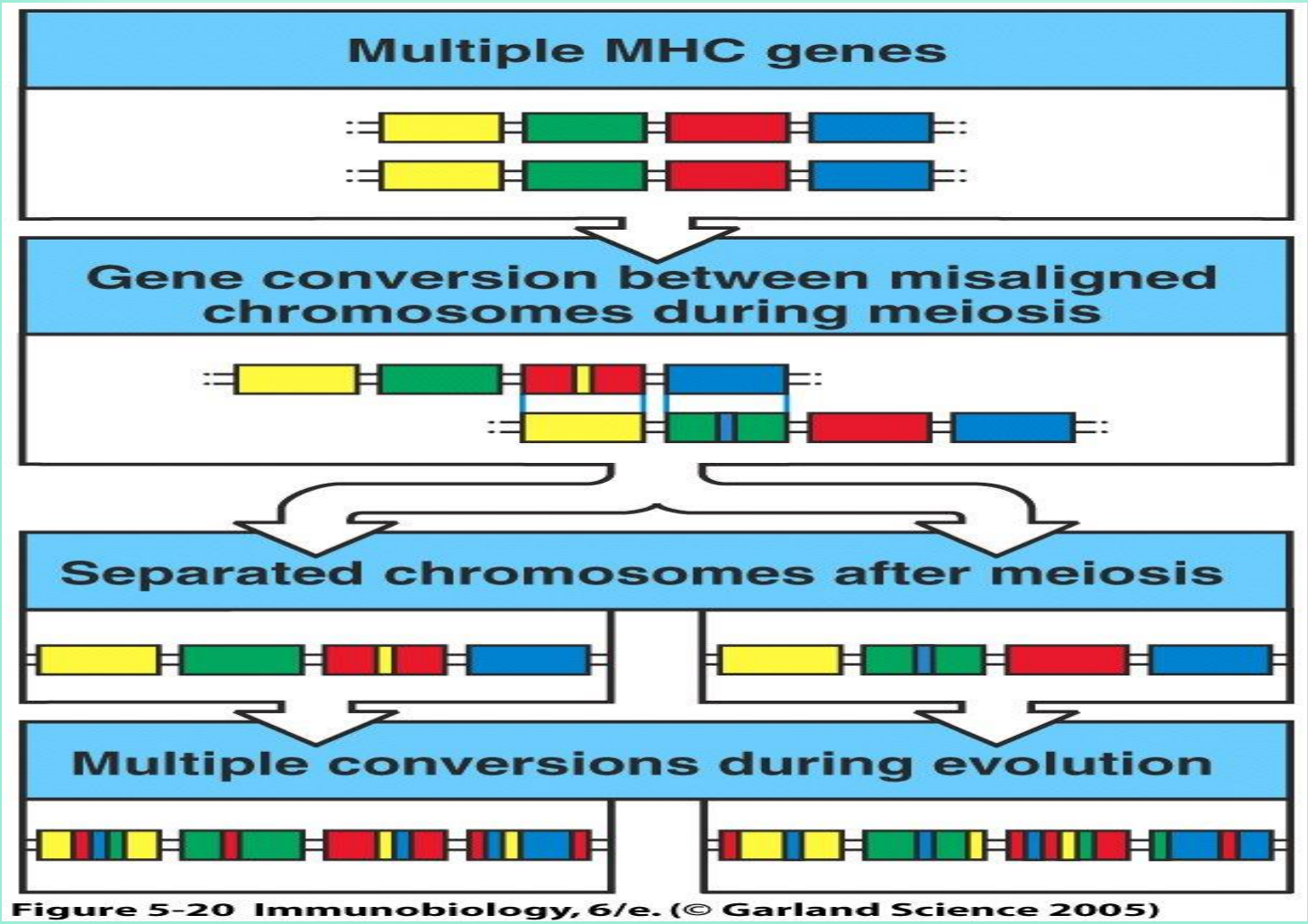


Figure 5-20 Immunobiology, 6/e. (© Garland Science 2005)

Copying sequences from one MHC gene to another

Gene recombination creates new alleles in MHC genes

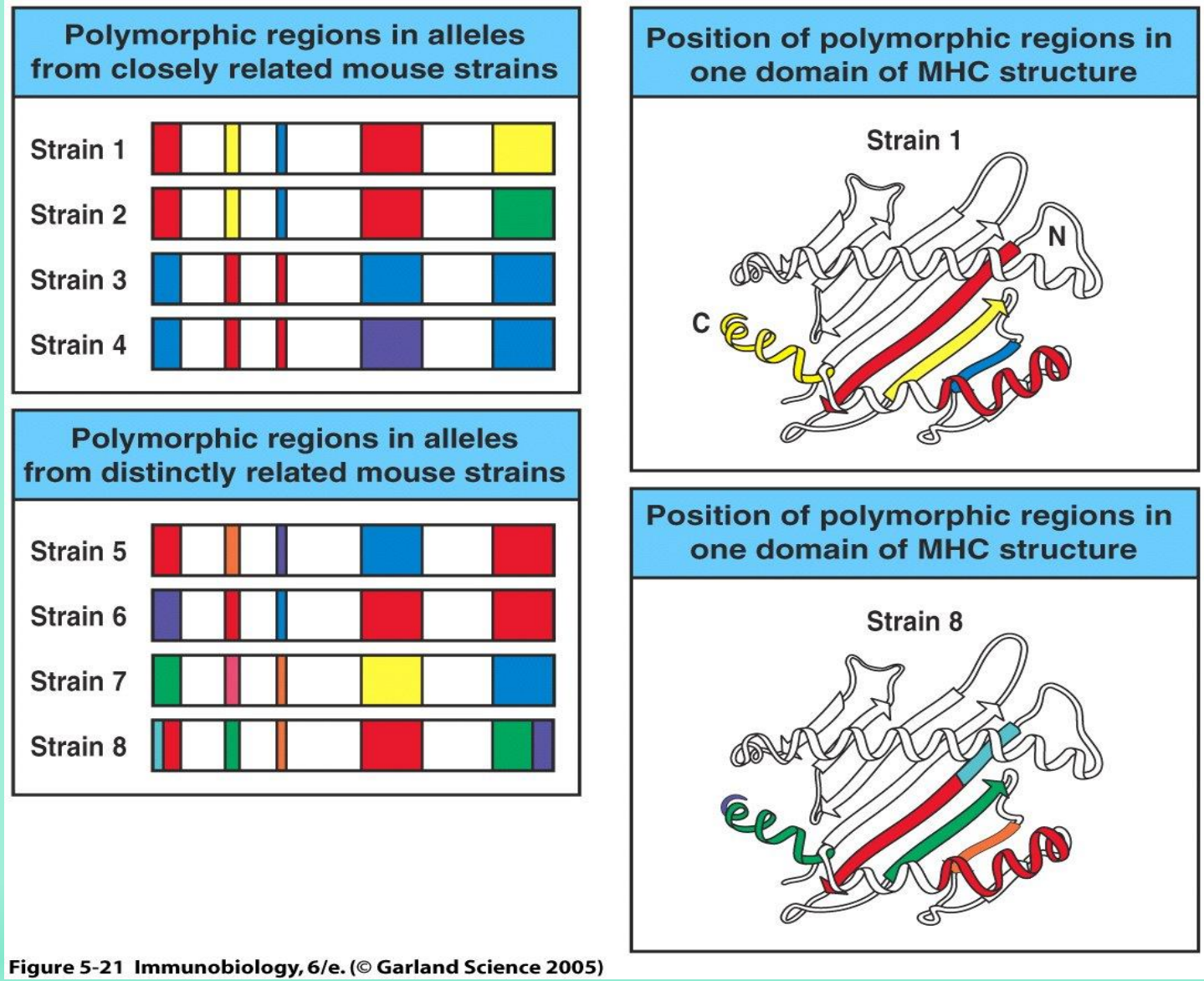


Figure 5-21 Immunobiology, 6/e. (© Garland Science 2005)

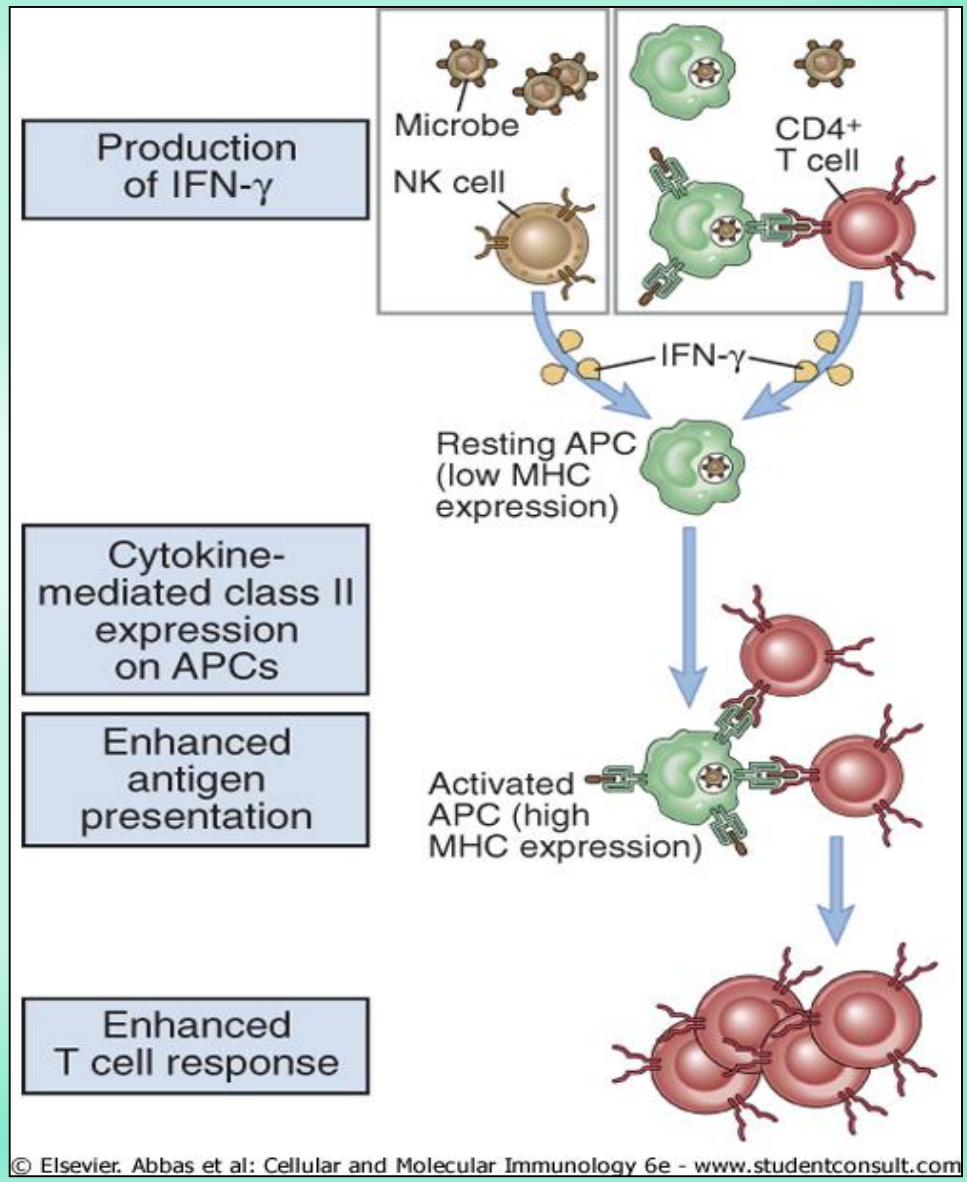
MHC expression on cells-I

Tissue	MHC class I	MHC class II
Lymphoid tissues		
T cells	+ + +	+ *
B cells	+ + +	+ + +
Macrophages	+ + +	+ +
Other antigen-presenting cells (eg Langerhans' cells)	+ + +	+ + +
Epithelial cells of the thymus	+	+ + +
Other nucleated cells		
Neutrophils	+ + +	–
Hepatocytes	+	–
Kidney	+	–
Brain	+	– †
Non-nucleated cells		
Red blood cells	–	–

Figure 3-19 Immunobiology, 6/e. (© Garland Science 2005)

MHC expression on cells-II

- Expression of MHC molecules is increased by cytokines produced during innate & adaptive immune cells, e.g. IFN



Features of Peptide-MHC interactions

- 1. MHC molecules show a broad spectrum for peptide binding, in contrast to the fine specificity of Ag recognition by Ab.
- 2. Peptide-MHC interactions are non-covalent and mediated by residues both in the peptides and in the clefts of MHC molecules.
- 3. Each MHC molecule binds one peptide at a time but can bind many "different peptides".
- 4. MHC molecules DO NOT discriminate between "Foreign Peptides" & "Self Peptides".

¤ MHC expression throughout the body plays a key role in maintaining homeostasis and health even when no foreign antigen is present.

Importance of Peptide-MHC interactions

- To display self class I to demonstrate that the cell is healthy.
- To display foreign peptide in class I to show that the cell is infected and to engage with T_C cells.
- To display a self-peptide in class I and II to test developing T cells for autoreactivity (primary lymphoid organs).
- To display a self-peptide in class I and II to maintain tolerance to self-proteins (secondary lymphoid organs).
- To display a foreign peptide in class II to show the body is infected and activate TH cells.

Regulation of HLA Expression

- MHC class I is constitutively expressed by most cells in the body, whereas class II molecules are of central importance to the adaptive immune system.

- To fulfil their functions, MHC class I & II molecules must be expressed according to a precise cell-type-specific and quantitatively modulated pattern.

- Certain instances specific changes in MHC expression may prove advantageous-

  - MHC class I production can be disrupted or depressed by some pathogens.

  - MHC class II expression on APCs can modulate by microenvironment surrounding an APC, usually enhancing the expression of these molecules.

The mechanisms driving these changes in expression are

- Genetic regulatory components
  - CIITA, RFX- Class II transactivators.
  - If defects causes bare lymphocyte syndrome.
- Viral Interference
  - Viral infection interfere with MHC class I expression.
  - Decreased level of components needed for peptides transport or MHC class I assembly.
  - Decreased transcription.
- Cytokine Mediated Signaling
  - Early stages of infection- IFN-α and TNF-α.
  - Later stages of infection- INF-γ α.

Genetic Regulatory Components

- Intracellular invaders or cytokines, can induce a signal transduction cascade that leads to changes in MHC gene expression.
- Both class I and class II MHC genes are flanked by 5′ promoter sequences that bind sequence-specific transcription factors.
- The promoter motifs and the transcription factors that bind to these motifs have been identified for a number of MHC genes, with examples of regulation mediated by both positive and negative elements.
- For example, a class II MHC transcriptional activator called CIITA (also known as class II, major histocompatibility complex, transactivator) and another transcription factor called RFX, have both been shown to activate the promoter of class II MHC genes.
- Defects in these transcription factors cause one form of bare lymphocyte syndrome.
- Patients with this disorder lack class II MHC molecules on their cells and suffer from severe immunodeficiency, highlighting the central role of class II molecules in T-cell maturation and activation.

Viral Interference

- Negative regulation of MHC comes from viruses that interfere with MHC class I expression and thus avoid easy detection by $CD8^+$T cells.
- These viruses include human cytomegalovirus (CMV), HBV, and adenovirus 12.
- In some cases, reduced expression of class I MHC molecules is due to decreased levels of a component needed for peptide transport or MHC class I assembly rather than decreased transcription.
  - Example, in the case of cytomegalovirus infection, a viral protein binds to 2-microglobulin, preventing assembly of class I MHC molecules and their transport to the plasma membrane.
  - Adenovirus 12 infection causes a pronounced decrease in transcription of the transporter genes (TAP1 and TAP2). TAP gene products play an important role in peptide transport from the cytoplasm into the rough endoplasmic reticulum.
  - Blocking of TAP gene expression inhibits peptide transport; as a result, class I MHC molecules cannot assemble with 2-microglobulin or be transported to the cell membrane.
- Decreased expression of class I MHC molecules is likely to help viruses evade the immune response (become targets for CTL-mediated destruction).

Cytokine-Mediated Signaling

- The expression of MHC molecules on cells is externally regulated by various cytokines such as IF (α, β, and γ) and TNF-α and Lymphotoxin- α.
- In early stage phagocytic cells produce these MHC regulating cytokines. In particular, IFN-α (produced by a cell following viral or bacterial infection) and TNF-α (secreted by APCs after activation) are frequently the first cytokines to kick off an MHC class I up-regulation event.
- In the later stages, IFN-γ, secreted by activated T_H cells as well as other cell types, also contributes to increased MHC expression.
- Binding of these cytokines to their respective receptors cascades the activation of transcription factors (genes encoding the class I α chain, β2-macroglobulin) and alter expression patterns.
- Class II transcriptional activator (CIITA), thereby indirectly increasing expression of class II MHC molecules on a variety of cells, including non-APCs (e.g., skin keratinocytes, intestinal epithelial cells, vascular endothelium, placental cells, and pancreatic beta cells).
- Expression of class II molecules by B cells is down-regulated by IFN- γ.
- Corticosteroids and prostaglandins can also decrease expression of class II MHC molecules.

Class II MHC Alleles Play a Critical Role in Immune Responsiveness

- The alleles in MHC haplotype determine which fragment of peptide to be presented as antigen to TH cells.
- Two explanations have been proposed to account for this variability in immune responsiveness observed among different haplotypes.
  - Determinant-selection model
    - ✓ Different class II MHC molecules differ in their ability to bind particular processed Ag.
    - ✓ Some peptides may be more crucial to eliminate the pathogen than others.
  - Holes-in-the-repertoire model,
    - ✓ T cells bearing receptors that recognize certain foreign Ags which happen to closely resemble self antigens may be eliminated during T cells development, leaving the organism without the cells/receptors for future responses to foreign molecules.

References

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THANK YOU!