CHAPTER 8

- **Major Histocompatibility Complex (MHC)**
- **What is MHC?**
  - **HLA**
  - **H-2**
  - **Minor histocompatibility antigens**
  - **Peter Gorer & George Sneell (1940)**

Significance of the MHC

- role in immune response
- role in organ transplantation
- role in predisposition to disease

- MHC molecules were initially discovered during studies aimed at understanding the molecules responsible for rejection of transplanted tissues.

- Hence the name "Major Histocompatibility Complex" (MHC).

- The term "Major Histocompatibility Complex" actually refers to a region of the genome that encodes a number of genes (hence Complex) that play an important (hence Major) role in tissue transplantation (hence Histocompatibility).

- The term "MHC molecule" or "MHC antigen" refers to a molecule encoded by a gene within this region.

Mouse H-2 complex — Chromosome 17

Complex	H-2						
MHC class	I	II		III		I	
Region	K	IA	IE	S		D	L
Gene products	H-2K	IA αβ	IE αβ	C′ proteins	TNF-α TNF-β	H-2D	H-2L

Human HLA complex — Chromosome 6

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 TNF-β	HLA-B	HLA-C	HLA-A

In humans:

Class I = A, B and C (also called HLA-A, HLA-B and HLA-C)
- Ag (peptide) presentation to CD8+ cells

Class II = DP, DQ and DR (also called HLA-DP, HLA-DQ and HLA-DR)
- Ag (peptide) presentation to CD4+ cells

Class III = Complement proteins, Tumor necrosis factor (TNFs)-α, β

In the Mouse:

Class I = K, D and L molecules (also called H-2D, H-2K and H-2L)

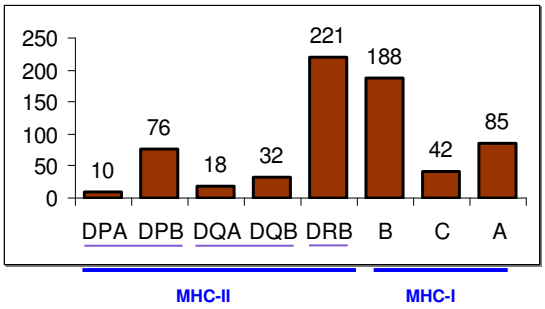
Class II = A and E (also called I-A and I-E)

Class III = Complement proteins, Tumor necrosis factor (TNFs)-α, β

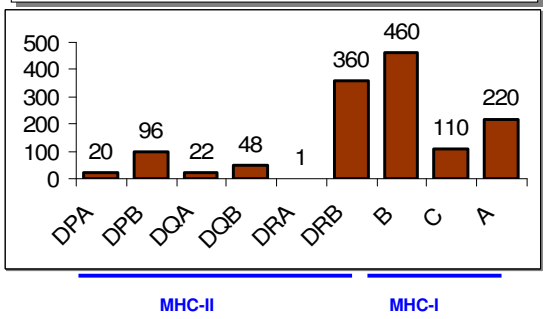
MHC- Polimorphism

- **MHC loci are highly polymorphic – presence of many alternative forms of the gene or alleles in the population**
- Inherited from mother and father
- New haplotypes are generated by recombination

Polymorphism of MHC antigens (based on phenotype)



Polymorphism of MHC genes (based on DNA sequence/ PCR)



MHC polymorphism

The loci that encode class I and class II MHC molecules are the most polymorphic known in higher vertebrates.

Within any species, there are many different alleles for each class I and class II MHC molecule.

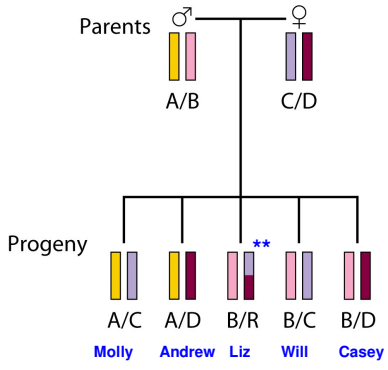
Humans:
HLA Class-I genes: A (240), B (470), C (110) alleles (1.2×10^7)
HLA Class-II genes:
DP= DPB1 (96) alleles
DQ= DQA1 (22), DQB1 (49) alleles
DR= DRB1 (304), DRB1 (1), DRB1 (35), DRB1 (11), DRB1 (15) alleles
1.8×10^{11} different Class II combinations, and

(1.2×10^7) x (1.8×10^{11}) = 2.25×10^{18} different combinations of Class I and Class II possible combinations

MHC- Polimorphism

- MHC loci are highly **polymorphic** – presence of many alternative forms of the gene or allele in the population
- **Inherited from mother and father**
- New haplotypes are generated by recombination

(c) Inheritance of HLA haplotypes in a typical human family



MHC- Polimorphism

- MHC loci are highly **polymorphic** – presence of many alternative forms of the gene or allele in the population
- Inherited from mother and father
- **New haplotypes are generated by <u>recombination</u>**

(d) A new haplotype (R) arises from recombination of maternal haplotypes

		HLA Alleles					
		A	B	C	DR	DQ	DP
Haplotypes	A	1	7	w3	2	1	1
	B	2	8	w2	3	2	2
	C	3	44	w4	4	1	3
	D	11	35	w1	7	3	4
→	R	3	44	w4	7	3	4

Terminology:

- **Haplotype**: set of alleles present in each parental chromosome (two sets).
- **Inbred mouse strains**: same set of alleles (homozygous) at each locus (K, IA, IE, L, D).
- Inbred strains are **SYNGENIC** = identical at <u>all</u> genetic loci
- Inbred strains have been bred by brother-sister mating for > 20 generations
- **Outbred mouse strains**: different set of alleles at each locus ~ like humans.
- **Congenic strains** = genetically identical except at <u>a single</u> loci

Mouse Strains

- **Thus, the strain C57BL/6 was designated H-2^b haplotype and said to possess the 'b' allele at each MHC locus.**

  Thus, it is: H-2b = K^b, D^b, L^b, I-A^b, I-E^b

- **Another strain, CBA/2 was found to possess different alleles than C57BL/10 and was arbitrarily designated as having the k haplotype (I.e. H-2^k).**

  Thus, it is: H-2k = K^k, D^k, L^k, I-A^k, I-E^k

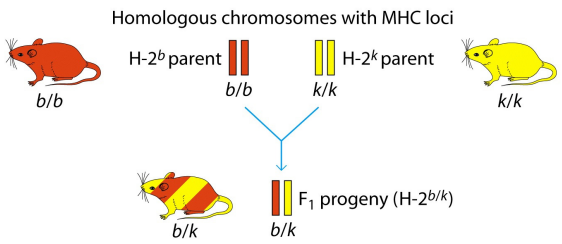
MOUSE HAPLOTYPES – INBRED STRAINS

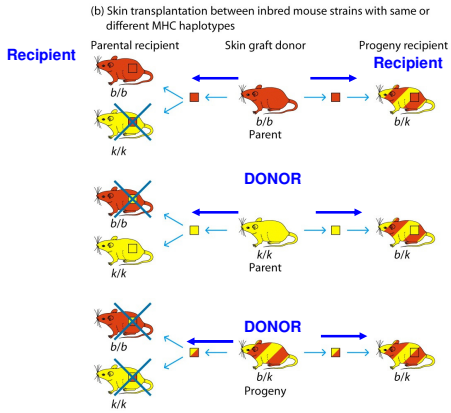
TABLE 7-1 H-2 Haplotypes of some mouse strains

Prototype strain	Other strains with the same haplotype	Haplotype	H-2 ALLELES K	IA	IE	S	D
CBA	AKR, C3H, B10.BR, C57BR	*k*	*k*	*k*	*k*	*k*	*k*
DBA/2	BALB/c, NZB, SEA, YBR	*d*	*d*	*d*	*d*	*d*	*d*
C57BL/10 (B10)	C57BL/6, C57L, C3H.SW, LP, 129	*b*	*b*	*b*	*b*	*b*	*b*
A	A/He, A/Sn, A/Wy, B10.A	*a*	*k*	*k*	*k*	*d*	*d*
A.SW	B10.S, SJL	*s*	*s*	*s*	*s*	*s*	*s*
A.TL		*t1*	*s*	*k*	*k*	*k*	*d*
DBA/1	STOLI, B10.Q, BDP	*q*	*q*	*q*	*q*	*q*	*q*

INHERITANCE OF MHC HAPLOTYPES

(a) Mating of inbred mouse strains with different MHC haplotypes





Summary:

There are three broad classes of MHC molecules:

Class I MHC:
- bind and present internally-derived peptide antigens to CD8+ cytotoxic T cells
- expressed on virtually all nucleated cells

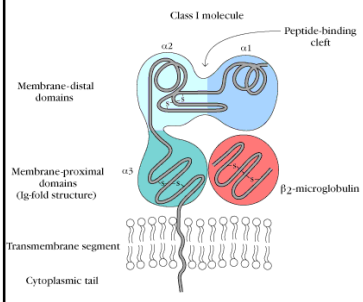
Class II MHC:
- present externally-derived peptides to CD4+ helper T cells
- expressed only on antigen-presenting cells (APC)

Class III MHC: any other molecule encoded within the MHC - many types

The MHC of humans is also referred to as the **HLA complex**.

The MHC of mice is also referred to as the **H-2 complex**.

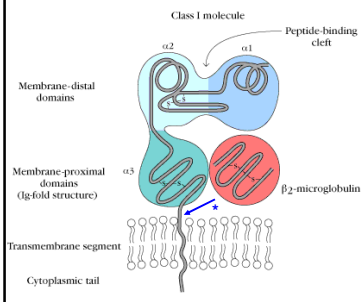
MHC-I Molecule



Kuby Figure 8-3

- Heterodimers
- Two noncovalently bound chains
- Alpha chain
  - encoded in the MHC
  - transmembrane
  - 3 domains (1, 2, 3)
- β2-microglobulin
  - not encoded in MHC
  - not transmembrane
  - 1 domain
- Class I MHC cannot be expressed without β2-microglobulin

MHC-I Molecule



Kuby Figure 8-3

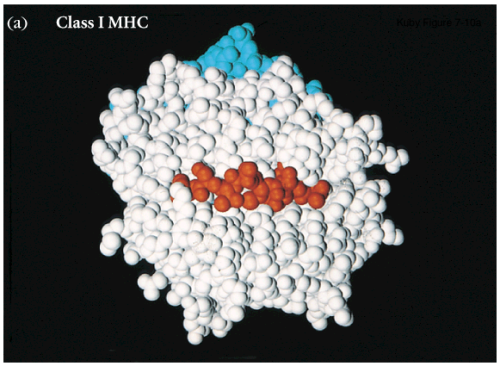
– α1 and α2 domains form the peptide-binding cleft

– α3 domain performs a structural role but has no direct role in peptide binding

- CD8 binds to the α3 domain

- Both, MHC-1 and β2-microglobulin belong to the Ig superfamily

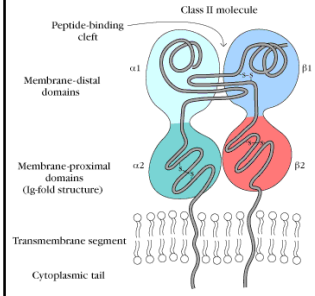
- * Papain cleavage



– β2- microglobulin shown in blue; antigen shown in red

- closed peptide-binding cleft - binds peptides 8-10 amino acids in length

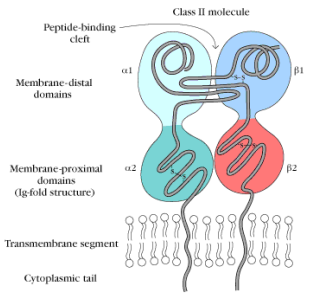
MHC-II Molecule



Kuby Figure 8-5

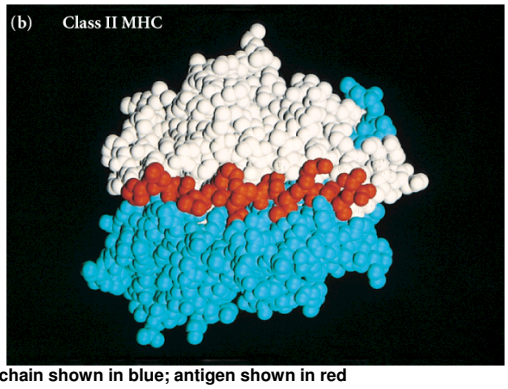
- Heterodimers (different molecules)
- Two noncovalently bound chains
- alpha chain and beta chains
  - both encoded in the MHC
  - both transmembrane
- both chains are necessary for expression of class II MHC

MHC-II Molecule



- α1 and β1 domains form the peptide-binding cleft
- α2 and β2 domains perform a structural role but have no direct role in peptide binding
- the β2 domain is bound by CD4

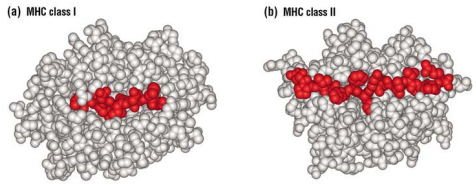
Kuby Figure 8-5



– β-chain shown in blue; antigen shown in red

- open peptide-binding cleft - binds peptides 13-18 amino acids in length

From **Immunity: The Immune Response in Infectious and Inflammatory Disease** by DeFranco, Locksley and Robertson



Class I genes - classical and non-classical

HUMAN	HLA GENE	MICB	MICA	B	C	E	A	G	F
	GENE PRODUCT	MICB	MICA	HLA-B	HLA-C	HLA-E	HLA-A	HLA-G	HLA-F

MOUSE	H-2 GENE	TAPASIN	K	D	L	Q	T	M
	GENE PRODUCT	TAPASIN	H-2K	H-2D	H-2L	Q	T	H-2M

Figure 4.13

Class II genes

HUMAN	HLA GENE	TAPASIN	DPB	DPA	DOA	DMA	DMB	LMP2	TAP1	LMP7	TAP2	DOB	DQB	DQA	DRB	DRA
	GENE PRODUCT	TAPASIN	DPβ	DPα	DOα	DMα	DMβ	PROTEASOME COMPLEX		PEPTIDE TRANSPORTER		DOβ	DQβ	DQα	DRβ	DRα
			HLA-DP		HLA-DO	HLA-DM						HLA-DO	HLA-DQ		HLA-DR	

MOUSE	H-2 GENE	Oα	Mα	Mb2	Mb1	LMP2	TAP2	LMP7	TAP1	Ob	Ab	Aα	Eb	Eα
	GENE PRODUCT	Oα	DMα	DMβ2	DMβ1	PROTEASOME COMPLEX		PEPTIDE TRANSPORTER		β	Aβ	Aα	Eβ	Eα
		H-2O	H-2DM							H-2O	H-2A		H-2E	

Figure 4.14

Class III genes

1. Complement components (C2, C4)
2. Tumor necrosis factor (alpha, beta)
3. Heat shock proteins

HUMAN	CYP21B	C4B	CYP21A	C4A	BF	C2	HSP70B	HSP70A	HSP70L	LTB	TNF	LTA

MOUSE	CYP21A1	C4	CYP21A2	Sfp	BF	C2	HSP70-1	HSP70-3	Hsc70t	LTB	TNF	LTA

Figure 4.15

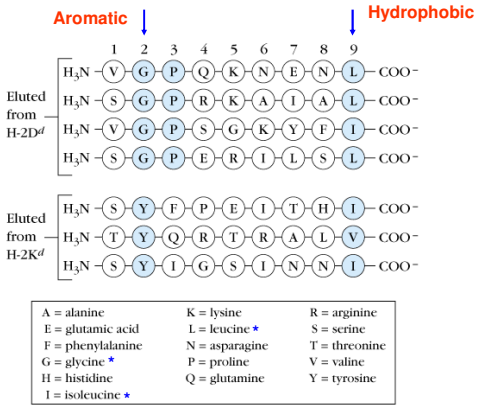
Peptide-MHC Interaction

- **Peptide binding by MHC molecules is not as specific as antigen binding by antibodies or T cell receptors.**
- **Any particular MHC molecule will bind a large range of peptides - but not all peptides.**
- **A given MHC molecule will bind peptides that have certain amino acids at key positions in the peptide (anchor residues).**
- **Each MHC molecule binds a unique set of peptides. Keep in mind that each allelic variant also binds a unique set of peptides!!**

MHC-Peptide Interaction

MHC-I:

- Each unique molecule (A, B or C) binds a unique set of peptides
- Single nucleated cell express 10^5 of each class I molecule
- As few as 100 peptide-MHC complexes are enough to target a cell for killing by CD8+
- Requirements:
  1) 8-10aa length,
  2) key amino acids at positions 2 and 9



Peptide-binding grooves for class I and class II MHC are structurally similar

- Both have a peptide-binding groove
- Close-ended groove for class I MHC requires an 8-10 amino acid-length peptide to bind
- Open-ended groove for Class II MHC lets it bind a peptide 13-18 amino acids long, not all of which lie in the groove
- Anchor site rules apply to both classes in particular Class I MHC (P2 and P9)

Aspects of MHC

1. Recognition by T cells requires cell-cell contact.
2. Peptides from cytosol associate with class I MHC and is recognized by Tc cells.
3. Peptides from endocytic vesicles associate with class II MHC and is recognized by Th cells.

Aspects of MHC (continued)

3. Although there is a high degree of polymorphism for a species, an individual has maximum of six different class I MHC products and eight class II MHC products.
4. A peptide must associate with a given MHC of that individual, otherwise no immune response can occur. That is one level of control.

Aspects of MHC (continued)

4. Mature T cells must have a T cell receptor that recognizes the peptide associated with MHC. This is the second level of control.
5. Each MHC molecule has only one binding site. The different peptides a given MHC molecule can bind all bind to the same site, but only one at a time.

Aspects of MHC (continued)

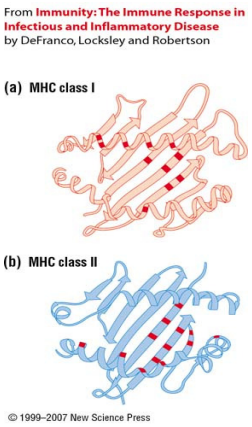
6. MHC polymorphism is determined only in the germline. There are no recombination mechanisms for generating diversity.
7. Because each MHC molecule can bind many different peptides, binding is termed **degenerate**.
8. Cytokines (especially interferon-γ) increase level of expression of MHC.

Aspects of MHC (continued)

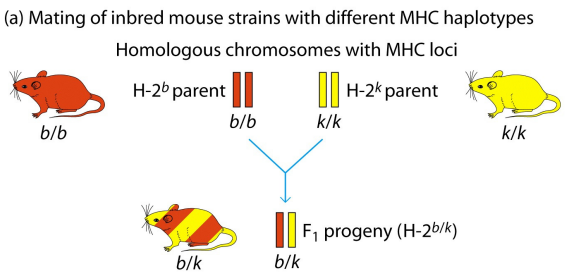
9. Alleles for MHC genes are **co-dominant**. Each MHC gene product is expressed on the cell surface of an individual nucleated cell.
10. Why the high degree of polymorphism?

Survival of species!

Where is polymorphism located in the molecule?



INHERITANCE OF MHC HAPLOTYPES



Crossing Inbred Strains

H-2b = K^b, D^b, L^b, I-A^b, I-E^b

X

H-2k = K^k, D^k, L^k, I-A^k, I-E^k

What would be the MHC complex of a liver cell in the F1?

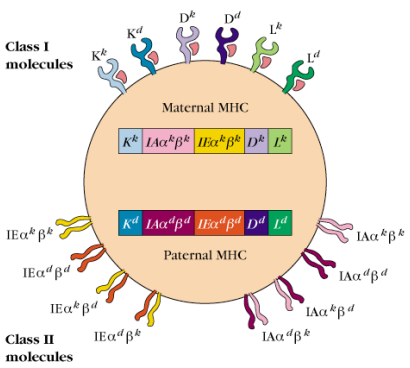
In a macrophage?

- 6 MHC-I molecules:

K^k K^b, D^k D^b, L^k L^b

- 8 MHC-II molecules:

$IA\alpha^k\beta^k$, $IA\alpha^b\beta^b$, $IA\alpha^k\beta^b$, $IA\alpha^b\beta^k$,
$IE\alpha^k\beta^k$, $IE\alpha^b\beta^b$, $IE\alpha^k\beta^b$, $IE\alpha^b\beta^k$,



Regulation of MHC Expression

- 1) Cytokines:
  - IFN-α, β, and γ - ↑ Class-I expression.
  - IFN- γ - ↑ Class-II expression in MO and DC
  - IL-4 ↑ expression of MHC-II in resting B cells
  - IFN- γ ↓ expression of MHC-II in B cells
- 2) Corticosteroids and Prostaglandins
  - ↓ expression of MHC-II
- 3) Viruses (↓ expression of MHC-I)
  - Human cytomegalovirus (CMV)
  - Hepatitis B virus (HBV)
  - Adenovirus 12 (Ad12)

MHC and immune responsiveness:

- In many cases, the ability of an inbred mouse strain to respond to a given antigen will depend on which alleles the strain carries at its MHC loci…..**low vs high responders!!**

- The reason is that if an antigen cannot bind to an MHC molecule, it **cannot be presented to T cells** and therefore an immune response cannot be made to it.

To respond to an antigen, the **first criterion** that must be met is that the individual must have an MHC molecule that can bind and present the antigen.

The **second criterion** that must be met is that the individual must have T cells capable of responding to the antigen.

The term "restricted" is used in various other ways:

- T cells are MHC-restricted i.e. they must recognize antigen presented on self MHC.

- CD4+ T cells are class II MHC-restricted i.e. they must recognize antigen presented on self class II MHC.

- CD8+ T cells are class I MHC-restricted i.e. they must recognize antigen presented on self class I MHC.

- A particular T cell clone may be $I\text{-}E^k$-restricted i.e. it recognizes its antigen ONLY when presented on self $I\text{-}E^k$.

("restricted" = "recognizes antigen on…")

Associations between MHC and disease

The risk of developing immunological diseases is often influenced by the presence or absence of specific MHC alleles.

TABLE 7-4 SOME SIGNIFICANT ASSOCIATIONS OF HLA ALLELES WITH INCREASED RISK FOR VARIOUS DISEASES

Disease	Associated HLA allele	Relative risk*
Ankylosing spondylitis	*B27*	90
Goodpasture's syndrome	*DR2*	16
Gluten-sensitive enteropathy	*DR3*	12
Hereditary hemochromatosis	*A3*	9.3
	B14	2.3
	A3/B14	90
Insulin-dependent diabetes mellitus	*DR4/DR3*	20
Multiple sclerosis	*DR2*	5
Myasthenia gravis	*DR3*	10
Narcolepsy	*DR2*	130
Reactive arthritis (*Yersinia, Salmonella, Gonococcus*)	*B27*	18
Reiter's syndrome	*B27*	37
Rheumatoid arthritis	*DR4*	10
Sjogren's syndrome	*Dw3*	6
Systemic lupus erythematosus	*DR3*	5

*Relative risk is calculated by dividing the frequency of the HLA allele in the patient population by the frequency in the general population:

$$RR = \frac{(Ag^+/Ag^-)\text{ disease}}{(Ag^+/Ag^-)\text{ control}}$$

SOURCE: SAM CD: A Comprehensive Knowledge Base of Internal Medicine, DC Dale and DD Federman, eds., 1997, Scientific American, New York.

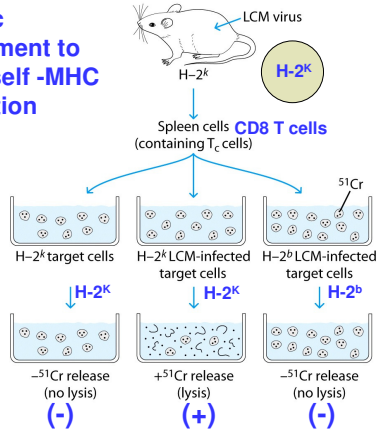
Associations between MHC and disease

Disease	Relative Risk	Allele
• Ankylosing Spondylitis	90	B27
• Hereditary hemochromatosis	90	A3/B14
• Narcolepsy	130	DR2

Self MHC Restriction

- Both MHC-I and MHC-II molecules can only recognize antigens when presented by SELF-MHC molecules.
- No value for individual to have T cells that recognize foreign antigen associated with foreign MHC
- Self MHC restriction occurs in thymus

Classic Experiment to show self -MHC restriction



Role of Antigen-Presenting Cells (APC)

- Helper T cells: recognize antigen after processing and presentation by MHC-II on APC (dendritic cells, macrophages, B cells).

- Cytotoxic T cells: recognize antigen when it is presented on MHC-I.

- Since most nucleated cells in the body express class I MHC, most cells in the body can present antigen to cytotoxic T cells. Although they are presenting antigen, these cells are usually not referred to as "antigen-presenting cells". If they are presenting antigen that will cause them to be killed by cytotoxic T cells, they are referred to as "target cells".

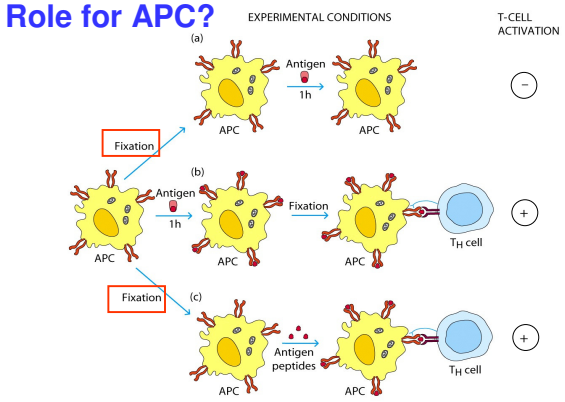
Antigen presenting cells

- **Remember: 1) MHC-II, 2) deliver co-stimulatory signals**
- **Professional APC: DC> MΦ > B cells, why?**
- **DC: Always express high levels of MHC-II molecules and co-stimulatory activity (B7 molecule)**
- **MΦ: requires activation to up-regulate MHC-II molecules and co-stimulatory molecules (B7 molecules)**
- **B cells: always express MHC-II molecules but needs to be activated to express co-stimulatory activity (B7 molecule)**

TABLE 8-1 Antigen-presenting cells

Professional antigen-presenting cells	Nonprofessional antigen-presenting cells	
Dendritic cells (several types)	Fibroblasts (skin)	Thymic epithelial cells
Macrophages	Glial cells (brain)	Thyroid epithelial cells
B cells	Pancreatic beta cells	Vascular endothelial cells

Role for APC?



Ag processing is required

- Classical experiment showing that B and T cells have different requirement for antigen recognition.
- Processing is required for Th activation
- Processing is a metabolic active process

Points Concerning Antigen Processing and Presentation

1. Location of pathogen
- viruses in cytosol, MHC class I pathway, Tc response (**Cytosolic pathway**)
- extracellular bacteria, MHC class II pathway, Th2 response → Ab formation (**Endocytic pathway**)
- intracellular bacteria, MHC class II pathway, Th1 response → cellular response (Endocytic)

Points Concerning Antigen Processing and Presentation

2. Peptides derived from both self and non-self proteins can associate with MHC class I and class II molecules.
3. Chemical nature of MHC groove determines which peptides it will bind.

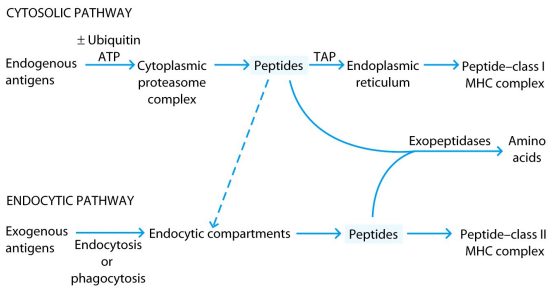
MHC-I and MHC-II associated with peptides processed in different intracellular compartments

A) Class I MHC binds peptides derived from endogenous antigens

B) Class I MHC binds peptides from antigens that have been processed via the cytosolic pathway (derived from the cytoplasm of the cell)

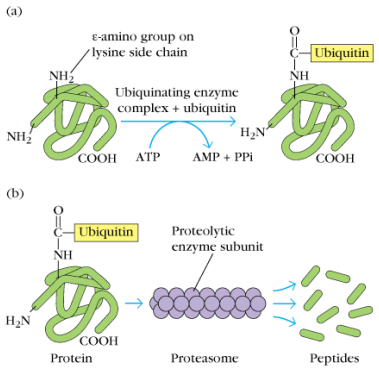
C) Class II MHC molecules bind peptides derived from exogenous antigens. These antigens were internalized by phagocytosis or endocytosis.

D) These peptides are said to have been processed within the endocytic pathway.



Endogenous Pathway

- Peptides are generated by proteasome degradation
- Peptides are transported from cytosol to the RER
- Peptides loading onto MHC-I is aided by chaperones



Kuby Figure 8-5

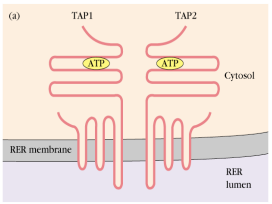
- Size
- Hydrophobicity

- The cytosolic antigen processing pathway - 2. The role of the **TAP (Transporter associated with Antigenic Processing).**

- Peptides from proteasome degradation of cytoplasmic proteins are transported across the membrane of the rough endoplasmic reticulum by a heterodimeric protein designated as TAP.

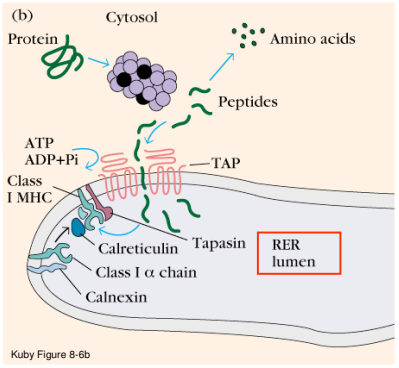
- TAP is composed of two subunits - TAP1 and TAP-2
- TAP-mediated transport is ATP-dependent

The genes for TAP1 and TAP2 are encoded within the MHC.



Kuby Figure 8-6a

The cytosolic antigen processing pathway - 3. Assembly of the class I-peptide complex

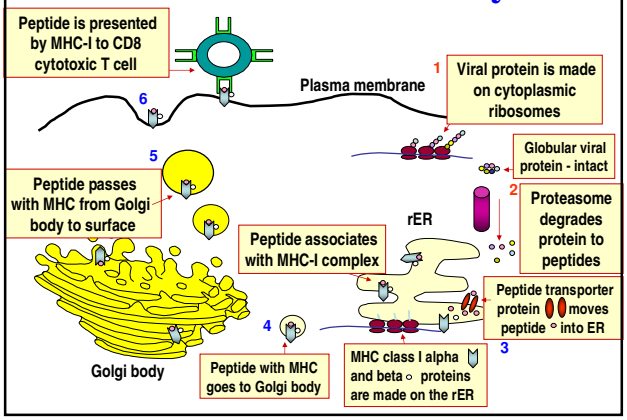


Kuby Figure 8-6b

1. **The class I alpha chain is stabilized by calnexin.**
2. **When the alpha chain binds beta-2-microglobulin:**
   - **calnexin is lost**
   - **calreticulin and tapasin bind**
3. **Tapasin & calreticulin bring the class I molecule into the vicinity of the TAP.**
4. **A cytoplasmic peptide transported through the TAP is loaded onto the class I molecule.**
5. **Class I MHC dissociates from calreticulin and tapasin.**

Class I MHC-peptide complex is transported to Golgi and to the cell surface.

Class I MHC Pathway

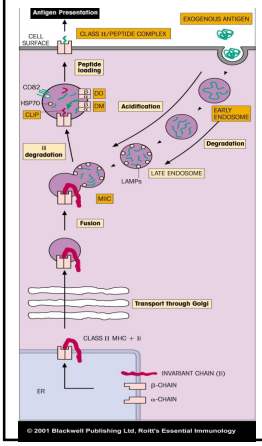


Class II Processing: (Exogenous)

- **The endocytic antigen processing pathway – processing of externally-derived peptides**

- **Antigen can be taken into cells by various means: phagocytosis, endocytosis, pinocytosis, receptor-mediated endocytosis**

- **Antigen taken up in these ways passes through a series of intracellular compartments of increasing acidity - early endosome (pH 6.5-6.0), late endosome (pH 6.0-5.0), phagolysosome (pH <5.0)**



The endocytic antigen processing pathway - processing of externally-derived peptides

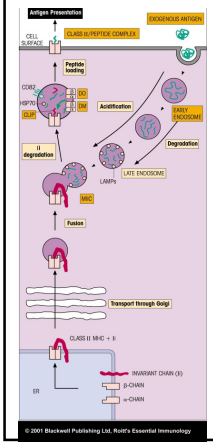
Three major events occur in the endosomal pathway:

- Degradation of material that was taken in – endosome goes through acidification and fusion with lysosome which contain a wide array of degradative enzymes

- Loading of peptides from this material on to class II MHC molecules

- Transport of class II MHC - peptide complexes back to the cell surface

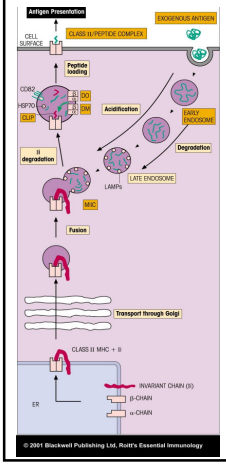
Figure 5-18



The endocytic antigen processing pathway - processing of externally-derived peptides

The endosomal compartment is completely separate from the endoplasmic reticulum ---> So, externally derived peptides are usually not loaded on to MHC-I.

Figure 5-18



The endocytic antigen processing pathway - processing of externally-derived peptides

Class II MHC is synthesized in the ER, but is not loaded with peptides there because it's peptide binding site is blocked by the invariant chain (Ii).

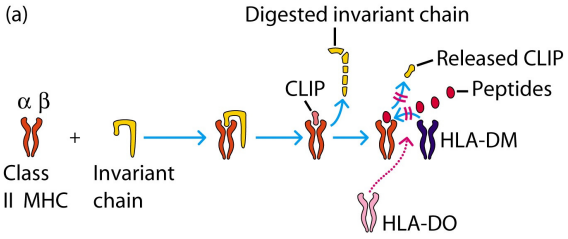
Once class II MHC enters the endosomal compartments, the invariant chain is degraded, leaving a small fragment - CLIP - in the peptide binding site.

CLIP is removed by HLA-DM, which loads a peptide on to class II MHC

HLA-DO inhibits HLA-DM until the cell is activated

Figure 5-18

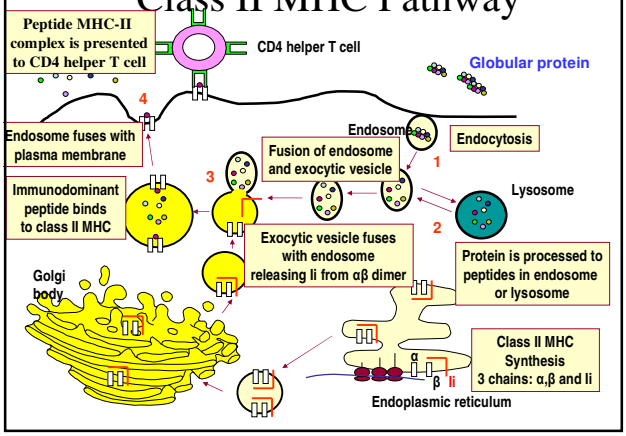
Invariant Chain- guides transport to endocytic Vesicles



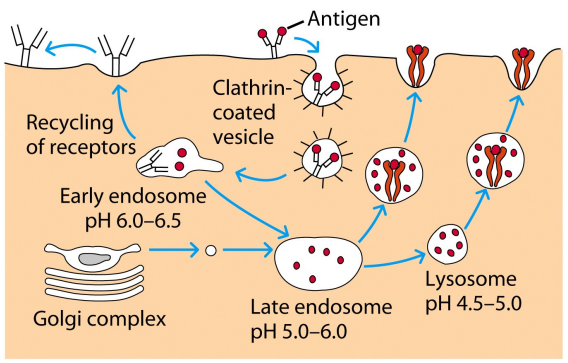
CLIP= Class II-associated invariant chain peptide

HLA-DM – mediates exchange
HLA-DO – inhibits HLA-DM

Class II MHC Pathway



Receptor-Mediated Endocytosis



Presentation of Non-Peptide Antigens

- CD1 molecules (CD1a-d)
- Structurally related to MHC-I
- Encoded outside the MHC region
- Present in APC (DC>MØ>B cells)
- Presents peptides of 12-22 aa in size
- Presents to CD4, CD8 and NK cells
- Present LIPIDS and glycolipids

The End!!