Adaptive immunity

How T cells recognize antigen

Starting point:

2. Diversity in antigen recognition is accomplished, in part, by rearrangements in the TCR loci. This occurs in the thymus
3. The T cell repertoire determines the spectrum of antigens that can be recognized in an individual's lifetime. The nature of this repertoire is determined by the Major Histocompatibility Complex (MHC), which binds peptide antigen

TCR The generation of the repertoire of T cell clones in the adaptive immune system of each individual must solve two profound challenges:

• The MHC molecules that present peptides to the T cells differ among individuals; thousands of alleles

• The huge diversity of (pathogen) peptides

Peptide of 10 amino acids in length

20 amino acids

# of different peptides = 20^{10} =~ $\mathbf{10^{13}}$

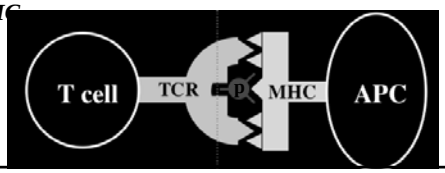
Require > $\mathbf{10^{13}}$ T cell clones each with different TCRs to recognize this array of peptides presented by different MHC molecules

How T cells recognize antigen -concepts

• Each clonal T cell receptor (TCR) is specific for a particular sequence of amino acids in a peptide antigen

• The peptide is generated from proteins in the antigen-presenting cell and presented to the TCR by MHC molecules

• ***Both the amino acids of the bound peptide and the presenting MHC molecule are recognized by the TCR: p-MHC***



The generation of > $\mathbf{10^{13}}$ T cell clones, each with different TCRs

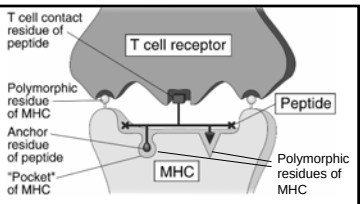
• Necessitates a ***somatic recombination mechanism*** to generate the large number of structurally diverse clonal TCRs, because not enough DNA in genome to encode this number of different TCR genes

However, the TCR of randomly generated T cell clones could either be incapable of recognizing one's own MHC, or alternatively strongly recognize self-peptides

• This, in turn requires a ***clonal selection process*** centered in the thymus and driven by p-MHC to select the repertoire of clones with TCR appropriate for the self-MHC and self-peptides of each individual

P-MHC

(HLA)



• A key feature of the adaptive immune system is that the *MHC molecules differ markedly among individuals*

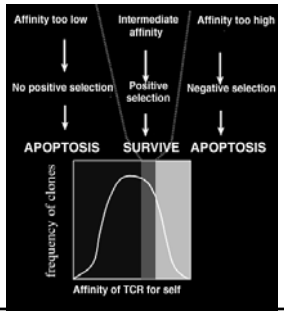
• The specificity of peptide binding is determined by *pockets* in the MHC molecules that only bind certain amino acid side chains

• This evolutionary strategy equips the species with a *large number of alternative MHC molecules that differ in their binding pockets*, and thus bind and present different peptides

• The MHC genes are by far the most polymorphic in the genome

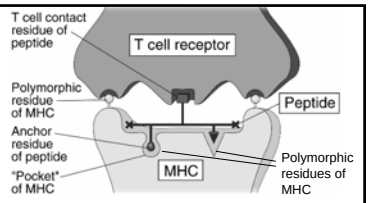
The selection process (Thymic "education") has two stages

• **First stage** selects T cell clones that recognize self-peptide in an individual's own MHC molecules - ***positive selection***



• **Second stage *negative selection*** eliminates *overtly* self reactive clones with high affinity for self-peptide- MHC **…"central tolerance"**

(Self-peptides are used as a surrogate for foreign peptides)



Immunologic self is the set of self-peptides and self-MHC molecules that generates the individual's adaptive immune system T cell repertoire

(One of the major functions of the natural killer cell population is to detect decreases in the expression of the MHC portion of this "self")

A viral peptide on a cell signifies it is infected and should be killed, while a pathogen's peptide on a phagocytic cell signifies the cell has ingested a foreign substance and must be helped to eliminate the pathogen

These differing tasks require the adaptive immune system to be organized into two sets of T cells each specialized for either killing or helping the cell presenting the antigen and two sets of directing MHC molecules, respectively loaded with (viral) peptides or peptides from the ingested pathogen

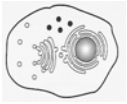
The set of self-MHC molecules varies from individual to individual because of MHC polymorphism

As a result the TCR repertoire selected on self peptide-self MHC is unique for each individual

- Major selective advantages to the species since there is essentially no set of stereotyped recognition structures shared by different individuals in the species
- However because the adaptive immune system is patterned on self, it sets the stage for the development of autoimmune disease

Two different classes of MHC molecules direct the different immune responses to the two different pathogen types

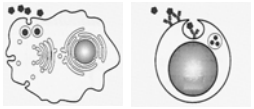
Virus - or Pathogen - infected cell



Any nucleated cell

Peptide presented on MHC class I molecules

Bacteria or components of an extracellular pathogen that have been internalized



Macrophage/DC B cell

Peptide presented on MHC class II molecules

Primary immune response

The T cell clones *generated by selection on self-peptides* that recognize, but are relatively unresponsive to self (tolerance), are then used in each adaptive immune response to identify non-self peptides typically encoded by pathogens

The non-self peptides are analogously presented by self-MHC molecules and are recognized by TCR of T cell clones as "not quite-self" (altered self) when triggered by innate immune signals

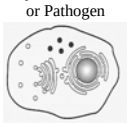
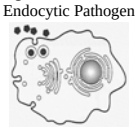
MHC class I molecules primarily present peptides that originate from the cell's cytoplasm

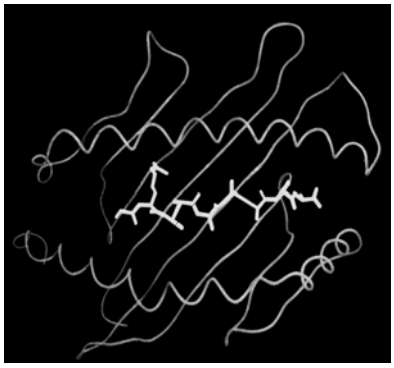
- Typically serve as a surveillance mechanisms for viral infection
- Expressed on the surface of virtually all nucleated cells

MHC class II molecules primarily present peptides that originate outside of the cell and are ingested by the cell during endocytosis

- Typically serve as a surveillance mechanisms for bacterial infection
- Expressed on the surface of cells specialized for phagocytosis - "professional antigen presenting cells"

The immune system makes this distinction by loading and recognizing peptides in either class I or class II MHC

Challenge:	Cytosolic Virus or Pathogen	Ingested Bacteria or Endocytic Pathogen	Extracellular Pathogen or Toxin
			
Presenting cell:	Any cell	Macrophage/DC	B cell
Peptide degraded in:	Cytosol	Endocytic vesicles	Endocytic vesicles
Peptides bind to:	**MHC class I**	**MHC class II** (or I)	**MHC class II**
Presented to:	CD8 T cells	CD4 T cells (or CD8)	CD4 T cells
Effect on presenting cell of T cell recognition:	Death of cell presenting the viral antigen	Activation of cell to enhance pathogen killing	Provision of help to B cell for production of antibodies

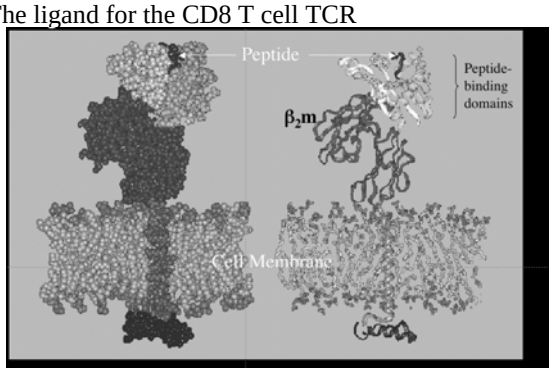


Structure of peptide-binding class I MHC domain

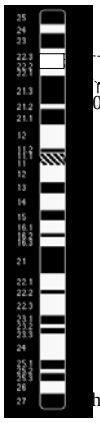
Structure of MHC molecules

What are the structural features of the MHC that determine peptide binding?

The ligand for the CD8 T cell TCR



MHC Class I Domains



Organization of the MHC

Two classes of peptide presenting MHC molecules are encoded by the HLA ABC and D genes

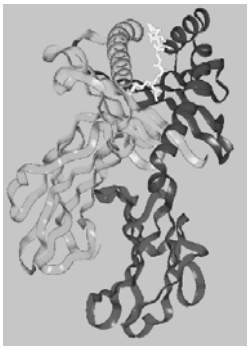
0 1 2 3 4m bp

Class II	Class III	Class I
HLA-DR	C4A	HLA-A
HLA-DQ	C4B	HLA-B
HLA-DP	C2	HLA-C
	Bf	
	TNF-α	

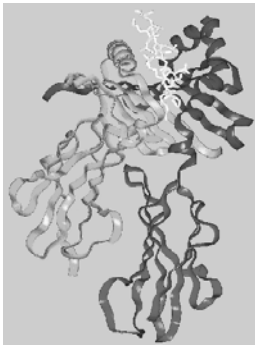
Human Leukocyte Antigens (HLA)

h 6

The overall structure of class I and class II MHC is rather similar

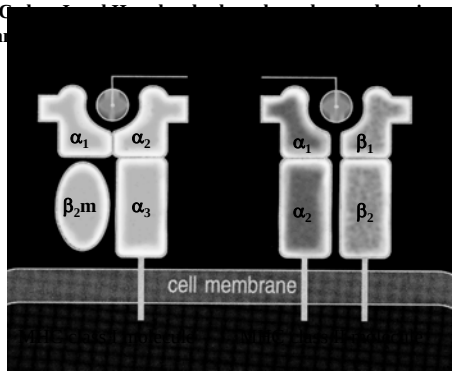


Class I



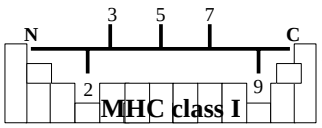
Class II

MHC
organ



TCR

How peptides bind

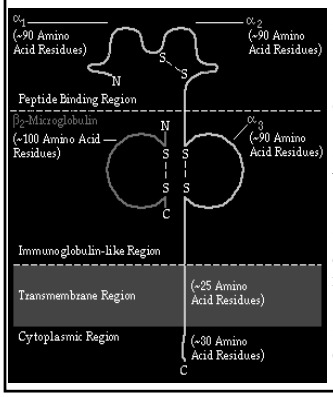


MHC class I

Rules for binding to MHC class I molecules

- Usually peptides are 9 amino acids in length
- Always oriented with NH2 terminus to the left
- Most often are anchored by interactions of the side chains of their 2nd (P2) and 9th (P9) amino acids to MHC pockets that confers specificity for amino acids with similar physical properties, e.g. size, charge, hydrophobicity, etc.

The Structure of MHC Molecules: MHC Class I



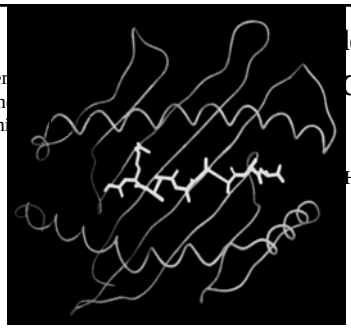
- The α chain is~ 350 AA long
- Three globular domains, α1, α2 and α3, each ~90AA
- α1 and α2 form the antigen-binding cleft
- β_2 microglobulin ~100AA, associates with the α3 domain, not MHC encoded
- ~ 70 AA transmembrane and cytoplasmic portion

le orientation

Cluster
tyrosin
recogni

Class I MHC

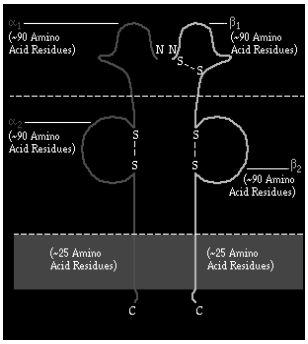
H



+ charged amino acids interact with negative COOH

The bound peptide must be oriented in the same direction in the MHC to allow the TCR clone specific for the peptide to identify it

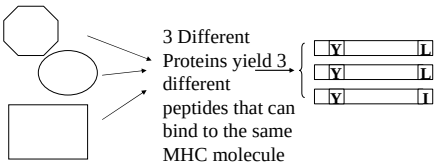
The Structure of MHC Molecules: MHC Class II



- Composed of two similar membrane spanning proteins, the α-chain and β–chain both encoded within the MHC
- Each chain is made of two globular domains, each ~90AA
- α1 and β1 domains form the antigen-binding cleft

Rules for peptide binding to MHC class I molecules

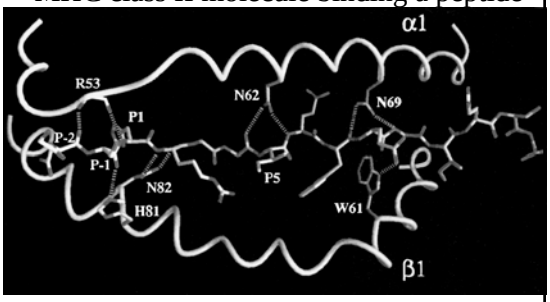
Role of side chains



Y=Tyr
L=Leu
I=Ile

A MHC Class I molecule selects homologous peptides derived from different proteins that have P2 and P9 side chains composed of homologous amino acids, e.g. tyrosine and leucine or isoleucine

MHC class II molecule binding a peptide

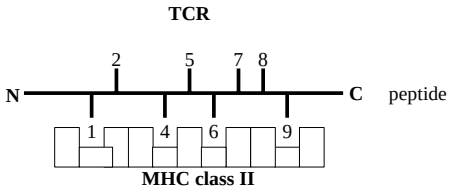


Genetic polymorphisms of MHC genes

HLA Genetics

How peptides bind

Rules for binding to MHC class II molecules



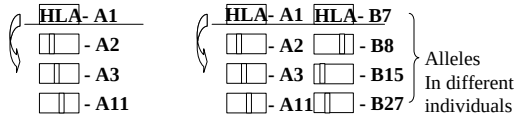
- Side chains in the middle of the peptide tether it to pockets via multiple hydrogen bonds, van der Waals and electrostatic forces
- The peptide ends are free and the peptide length is variable
- Interactions with the peptide backbone orient the peptide as in class I molecules

Keep the diversity of MHC class I and II genes straight!

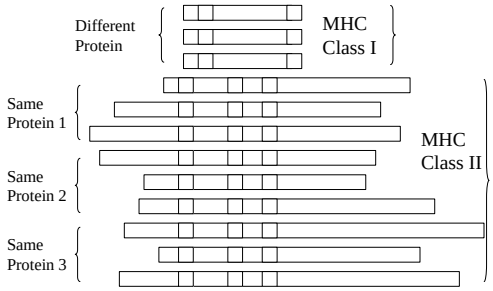
Arises from two mechanisms:

Duplication of a gene locus in an individual resulting in multiple loci, *polygeny*

Development of multiple alleles at a locus among individuals in the species, *polyallelism*



Different rules for peptide binding to class II MHC molecules



Peptides binding class II molecules vary in length, are anchored in the middle, but are also always oriented with NH2 termini to the left

MHC polymorphism is all about survival, it is an evolutionary response to the structural diversity and mutation potential of microorganisms

There are over 700 MHC class II alleles and over 1000 MHC class I alleles

HLA-DR (494)	HLA-A (396)
HLA-DQ (94)	HLA-B (699)
HLA-DP (123)	HLA-C (198)

No practical biologic limit on the number of alleles (July 2005)

- However each duplication of a locus increases "self-MHC"
- Each MHC type selects its own allele-specific TCR clonal repertoire
- Each duplication mandates more negative clonal selection across all repertoires during repertoire formation, reducing the size of the repertoire for each allele

Practical maximum is ~ three loci each for class I and class II

HLA-DR	HLA-A
HLA-DQ	HLA-B
HLA-DP	HLA-C

(Remember both maternal and paternal alleles are expressed)

Nomenclature: The genetic "unit" of the HLA system is the allele, with each defined by its own DNA nucleotide sequence

Allele	E.g. HLA-B*0801	"Specificity"
	*0802	HLA-B8
	…	
	*0821	
	*2701	
	*2702	
	*2703	HLA-B27
	…	
	*2725	

But to make things "simpler", alleles can be grouped in families, e.g. HLA-B*27
"specificity", is an old nomenclature used when human alloantibodies were used to first detect HLA serologic "specificities" or "antigens"

Consequences of polyallelism for transplantation

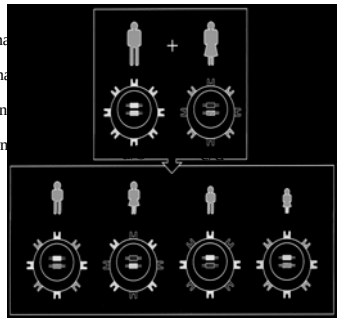
The differences MHC between individuals means that the cells of a donor who differs from the recipient by any of the MHC alleles are recognized as non-self by the T cells of the recipient and are attacked as if they were a foreign substance

This difference is the origin of the name "major histocompatibility complex" that reflects the first recognized role for these molecules as the primary genetically determined targets for graft rejection or compatibility

The chance of finding an unrelated individual with the same HLA alleles ranges from 1 in 300,000 to less than 1 in a 1,000,000

Codominant expression of MHC alleles

- a=paterna
- b=paterna
- c=matern
- d=matern



Nomenclature

Genotype: the collection of genes in an individual, usually referring to a small segment of a chromosome

Alleles: the alternative forms of a gene found in different individuals

Allotypes or allomorphs: the different protein forms encoded by alleles

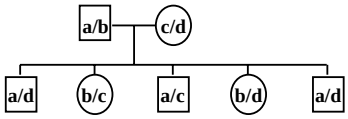
Haplotype: the genes (alleles) contributed by one parent, usually referring to alleles of both class I and class II loci

Gene loci exhibit linkage, a measure of their genetic distance

Linkage disequilibrium: certain alleles in a haplotype are found together significantly more (or less) frequently than expected by chance

HLA genetics in transplantation

A graft is compatible only if there is a complete match at all MHC alleles, i.e. a two haplotype match for all MHC loci



Note that in a family the parents always differ by one haplotype from the children, while children may share 0, 1 or 2 haplotypes
In situations where a transplant is required, the family is first typed to find 2 haplotype matches, then unrelated individuals are studied

Different MHC alleles confer different functional properties on the adaptive immune system by specifying molecules that have different peptide binding abilities

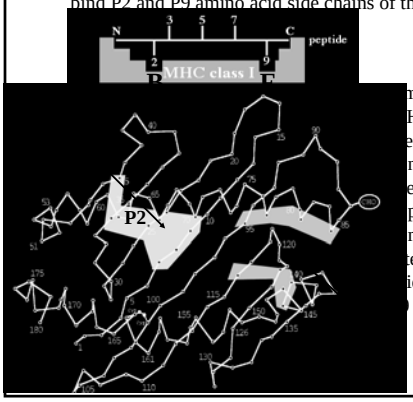
MHC alleles regulate immune responsiveness by influencing the number of peptides in a protein that can be recognized (Example HIV envelope protein)

Allele:	**HLA-B*27052**	**HLA-B*3501**	**HLA-B*0702**
Motif	XRXXXXXX[KRYL]	XPXXXXXXY	XPXXXXXXL

Peptides able to bind each allelic HLA molecule

IRGKVQKEY	KRRVVQREK	DPNPQEVVL
IRPVVSTQL	ARILAVERY	KPCVKLTPL
TRPNNNTRK	ERDRDRSIR	RPVVSTQLL
IRIQRGPGR	LRSLCLFSY	SPLSFQTHL
SRAKWNNTL	TRIVELLGR	IPRRIRQGL
LREQFGNNK	CRAIRHIPR	
FRPGGGDMR	IRQGLERIL	
WRSELYKYK		
# of peptides	**15**	**0**	**6**

Location of MHC class I pockets termed "B" and "F" that bind P2 and P9 amino acid side chains of the peptide



mino acids of the HC molecule that form e B and F pockets nfer the main ecificity for binding ptides through mplementary teractions with amino ids at position P2 and in the peptide

What peptides are found in MHC molecules?

•Elution of peptides from MHC molecules reveals that class I molecules typically bind 2000-10,000 different peptides per cell

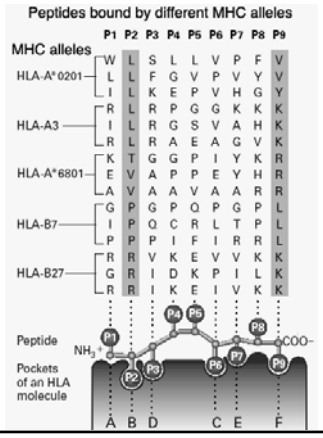
•Each of these peptides has a dominant motif reflecting the relatively conserved anchor residues, e.g. for HLA-B27

Motif XRXXXXXX[KRYL]

•Most peptides are fragments of conventional cell proteins, e.g.

HRAQVIYTR 40S ribosomal protein
RRIKEIVKK Heat shock protein 89
ARLFGIRAK Breast basic conserved protein
RRFFPYYVY Proteasome subunit C5
GRWPGSSLY Lamin B receptor

•Even the most abundant peptide species accounts for only 1% of the total peptides bound, so the T cell has its work cut out



Key Concept

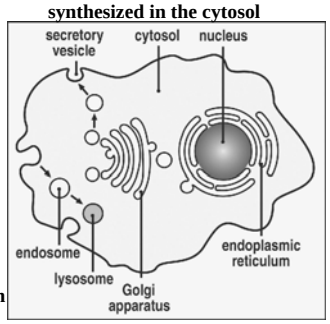
The difference in amino acid sequence around the peptide binding pockets of various allelic forms of MHC class I molecules confer the property of binding completely different self or non-self peptides

How do peptides get loaded onto the correct MHC molecule?

How do cytosolic peptides from virally infected cells get loaded on class I and not class II molecules to trigger killing by CD8T cells?

How do peptides from endocytosed proteins get loaded on class II and not class I molecules to elicit macrophage activation and B cell help?

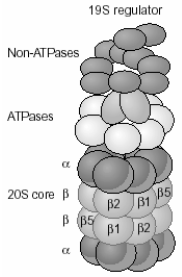
Class I peptide loading from peptides synthesized in the cytosol



Class II peptide loading from peptides produced by lysosomal degradation of endocytosed organism

The endocytic and synthetic pathways are usually quite separate

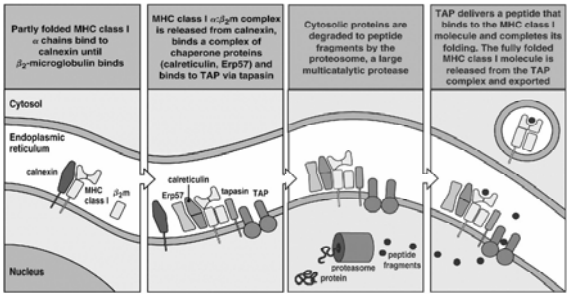
The proteasome is composed of the proteasome catalytic core and regulatory complexes that bind and unfold ubiquitylated substrates



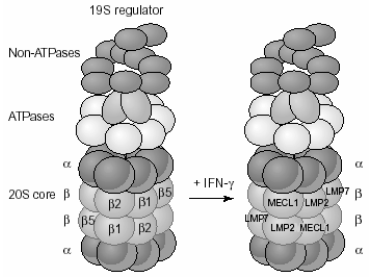
3 of the 7 subunits in each ring of the 20s core confer the proteolytic activity

The immunomodulatory cytokine IFN-γ made by NK, CD8 and some CD4 T cells upregulates the synthesis of three new proteasome subunits with different proteolytic activities that replace the three constitutively active proteolytic subunits

MHC class I molecules are synthesized and bind cytosol -derived peptides during assembly within the ER

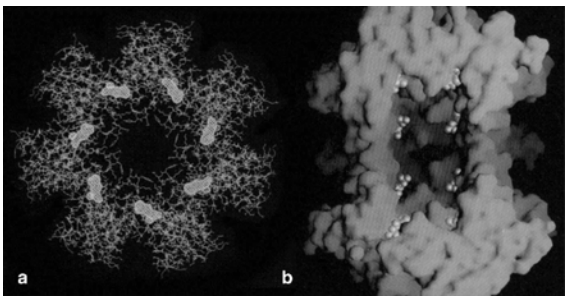


Generation of the immunoproteosome



3 immunosubunits LMP2 (iβ1), LMP7 (iβ5) and MECL-1 (iβ2) replace the constitutive β1, β5 and β2 subunits, changes specificity of proteasome towards production of hydrophobic peptides that have greater binding affinity for MHC pockets

Proteosome- digests ubiquitin-tagged cytosolic proteins



Production of 9 AA peptides for class I MHC

Proteasome makes precise cuts only at the C termini of the peptides

Other peptidases nibble back the N termini until the peptide fits exactly, e.g. the IFN-γ-inducible leucine aminopeptidase (LAP)

The peptide production system is not coordinated with the peptide binding specificity of the individual's MHC class I molecules

Peptide and β_2 microglobulin subunit are required to stabilize the MHC class I molecule

Empty MHC class I molecules are unstable to prevent "friendly fire" killing of bystander cells by the uptake of random peptides

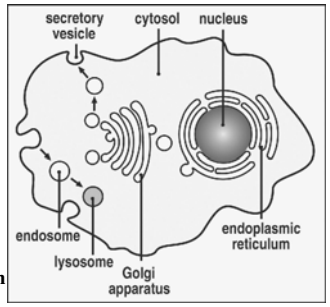
Invariant chain (Ii)

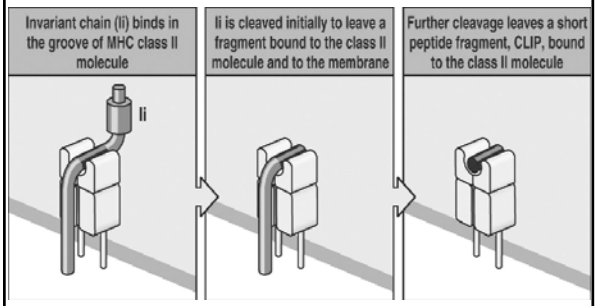
A chaperone that complexes with MHC class II molecules during their synthesis in the endoplasmic reticulum that solves two problems, since MHC II molecules are also synthesized in the er

• Ii Blocks the class II peptide binding groove during synthesis and prevents loading by peptides destined for class I molecules

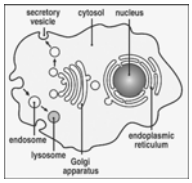
• A recognition sequence on the Ii transmembrane portion directs the nascent MHC II molecule to travel to the acidic endosomal compartment where it will be loaded with the degraded ingested exogenous peptides

Class II peptide loading from peptides produced by lysosomal degradation of endocytosed organism



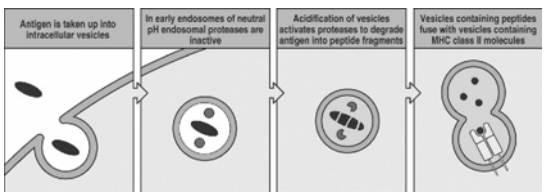


Within the acidic endosome, Ii is degraded to CLIP (Class II-associated invariant chain peptide) by specific endosomal acidic cysteine proteases (cathepsins)



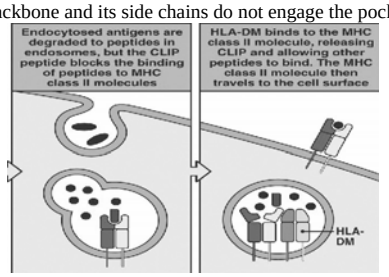
Class II loading is centered in the vesicular system

Acidic endosomal proteases digest ingested proteins into peptides that will load MHC class II molecules



This process does not require the precise proteolysis needed in the class I system, since the peptide terminii are not constrained by MHC class II

CLIP is only bound to the MHC groove by its peptide backbone and its side chains do not engage the pockets



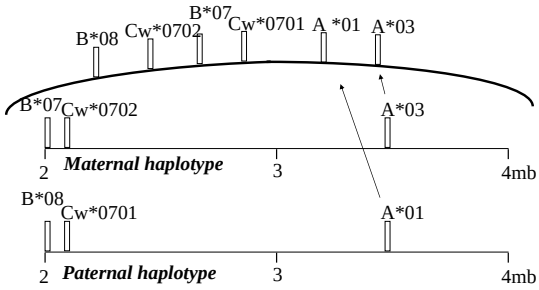
• HLA-DM, an ancient but non-classical class II molecule catalyzes the release of CLIP and the binding of high affinity peptides via interaction of peptide amino acid side-chains with MHC pockets

• Without Ii the MHC class II molecule now traffics to the cell membrane

Expression MHC molecule on the cell surface

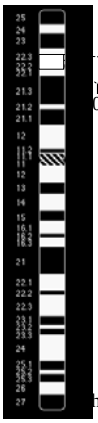
(Putting all of the information together)

Codominant expression of MHC class I genes results in 6 different types of class I molecules on the surface of each cell

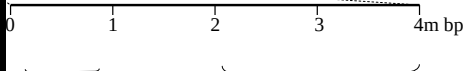


MHC class I region

10-20,000 molecules of each type are present on most cells



Organization of the MHC



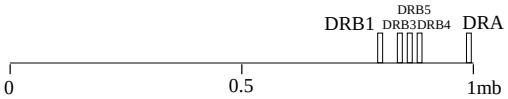
Class II
HLA-DR
HLA-DQ
HLA-DP

Class I
HLA-A
HLA-B
HLA-C

n 6

Polygenic human MHC class II (HLA-DR) genes

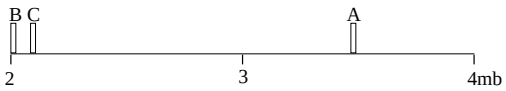
MHC class II loci	Specificity (Antigen)	Allele designation	# of alleles
HLA-DRA α-chain		DRA*0101	2
HLA-DRB1 β-chain	DR1, 2,…	DRB1*0101,…	**494**



MHC class II region

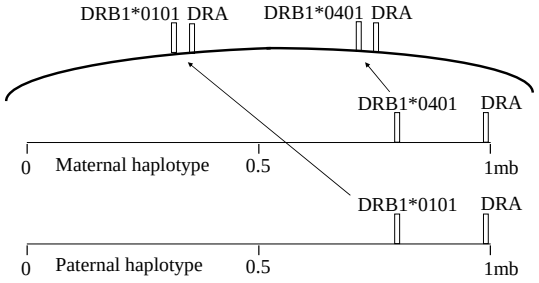
MHC class I (HLA-A,B,C) genes

MHC class I loci	Specificity (Antigen)	Allele designation	# of alleles
HLA-**A** α-chain	A1, A2,…	A*0101,…	396
HLA-**B** α-chain	B7, B8,…	B*0702,…	699
HLA-**C** α-chain	Cw1, Cw2...	Cw*0101,...	198



(β_2 microglobulin encoded on chromosome 15)

Codominant expression of MHC II genes results in the presence of 2 different HLA- DR molecules on the cell surface



MHC class II region

(DRA are monomorphic)

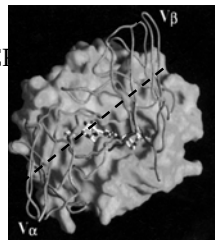
Maximum number of different types of HLA molecules expressed on the cell surface

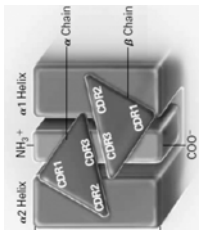
	Nucleated cells	Antigen presenting cells
Class I (HLA-A)	2	2
Class I (HLA-B)	2	2
Class I (HLA-C)	2	2
Class II (HLA-DR)	0	2*
Class II (HLA-DQ)	0	4
Class II (HLA-DP)	0	4
Total	6	16

Each of these MHC molecules selects its *own T cell repertoire* that only recognizes peptides presented by that particular type of MHC molecule

T cell receptors interact both with the MHC molecule and with the bound peptide

TCR



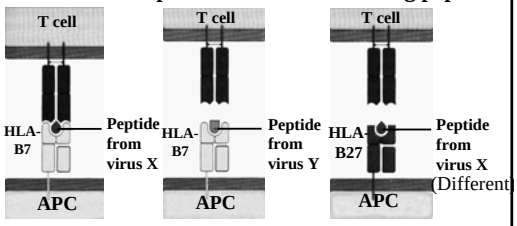


MHC I-CD8 TCR ***CDR3 hypervariable regions*** of TCR α and β-chain primarily contact peptide

Recognition of p-MHC by the TCR

(Putting all of the information together)

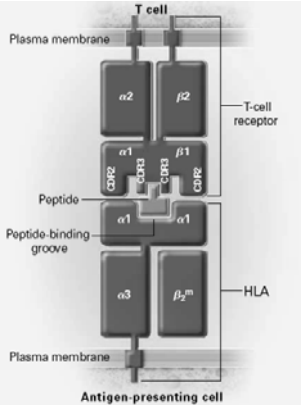
Because the TCR recognizes both peptide and MHC molecule, T cell recognition of MHC-peptide is both MHC restricted and specific for the immunizing peptide



(Different)

Target killed: ***Yes*** **No** **No**

In each experiment the T cell is from a HLA-B7 person who recovered from infection by virus "X".
The APC target cell is either infected with virus X or Y and is from an individual who is either HLA-B7 or HLA-B27



T cell receptors interact both with the MHC molecule and with the bound peptide

Summary points

During development the T cell repertoires of an individual are separately selected on self-peptides presented by 3 types of class I and 3 types of class II MHC molecules. Later during an immune response these same T cells recognize "not quite self"/non self peptides presented on these MHC molecules and clonally expand

MHC molecules are codominantly expressed, with class I molecules found on the surface of all nucleated cells and class II molecules on professional antigen presenting cells

The alleles of the MHC genes specify different amino acids in MHC pockets that bind peptide side chains, and this confers specificity on MHC molecules to bind different peptides

As a consequence individuals vary markedly in what particular peptides the T cell recognizes

Class I and class II MHC molecules differ markedly in the details of how they bind peptides and the biochemical pathways the peptides take to be loaded on the MHC. These differences assure that the correct CD4 or CD8 adaptive immune response is made to a peptide

The presence of a “not quite self”/non self peptide on a MHC class I molecule renders the cell a target of a cytotoxic CD8 T cell, while a peptide in a class II molecule evokes macrophage activation and B cell help