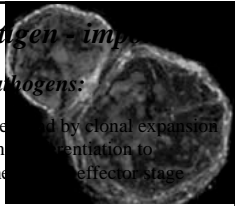


# 1. How T cells recognize antigen - importance

## Adaptive immune response to pathogens:

T cells have clonally specific receptors capable of recognizing AA sequence of nearly any peptide ~9-16 AA long

Resulted by clonal expansion and differentiation to memory and effector stage



**Mechanisms involved based on recognition of peptides bound to Major Histocompatibility Complex (MHC) molecules**

*p-MHC*

The T cell faces several formidable challenges to gain its objective

The solutions that were evolved

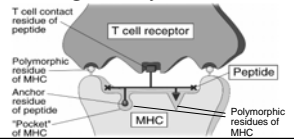
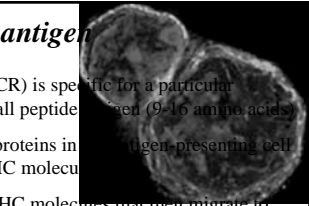
Affect the adequacy of the adaptive immune response

Have profound medical implications...responsible for:

- Autoimmunity and autoimmune disease
- Transplantation rejection
- Complexities of placentalation

## How T cells recognize antigen

- Each clonal T cell receptor (TCR) is specific for a particular sequence of amino acids in a small peptide antigen (9-16 amino acids)
- The peptide is generated from proteins in an antigen-presenting cell and presented to the TCR by MHC molecules
- The soluble peptide binds to MHC molecules that then migrate to the cell surface with the peptide as peptide-MHC complex (p-MHC)
- **Both the amino acids of the bound peptide and the presenting MHC molecule are recognized by the TCR**



*p-MHC*

## The challenge the T cell faces

### Problem 1

Require  $> 10^{13}$  T cell clones each with different TCRs to recognize *the many pathogen peptides*

Peptides of 10 amino acids in length

20 amino acids

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



### Problem 1

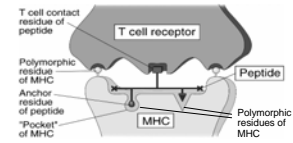
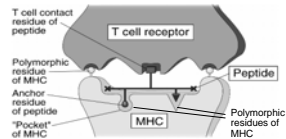
To generate  $\sim 10^{13}$  structurally diverse clonal T cell receptors (TCRs) requires:

- A **somatic recombination mechanism** to randomly form the antigen recognition part of the TCR, **since the genome is not large enough to encode that number of genes**
- A **selection mechanism** to identify the clones that are able to recognize potential pathogenic peptides and **avoid overt self-recognition**

**Problem 2**

A given MHC molecule can bind peptides in only a limited number of ways

*Microbial pathogens can mutate around a stereotyped defense recognition system*



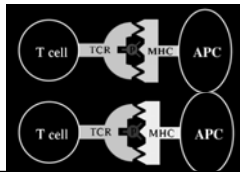
*Microbial pathogens can mutate around a stereotyped defense recognition system*

**Solution...diversify the MHC**

1. Change the peptide-presenting MHC molecules from individual to individual ( generate multiple alleles) so that different persons bind different kinds of peptides

Enables the species to survive by endowing each individual with an essentially unique set of peptide binding mhc molecules

2. Increase number of different MHC structures in an individual (multiple loci)



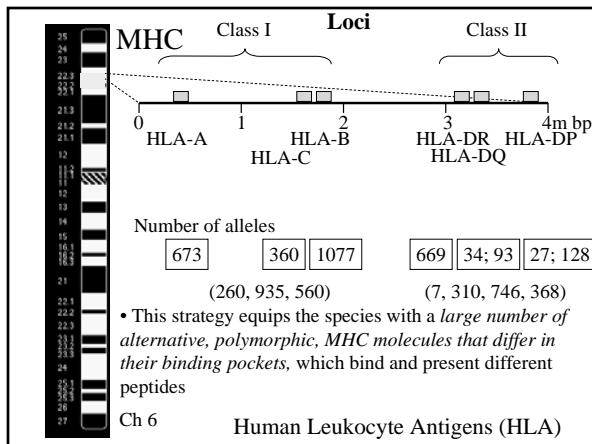
**Problem 3**

*The adaptive immune system must develop T cell clones that:*

*•Can recognize the individual's own MHC molecules and the repertoire of peptides they contain*

*•Specifically bind and recognize pathogen peptides prior to encountering the pathogen*

**Solution:** Use self-peptides as a surrogate for pathogen peptides and select on self-MHC molecules



**Complication:** the TCR of randomly generated T cell clones could either strongly recognize self-peptides presented in self-MHC and mount a self attack, or alternatively be incapable of recognizing one's own MHC

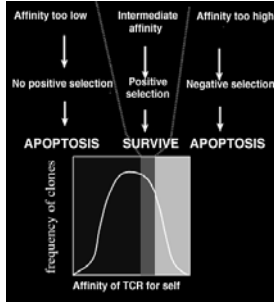
This requires a *clonal selection process* centered in the thymus and driven by self p-MHC to select the repertoire of clones with TCR appropriate for the self-MHC and self-peptides of each individual

“Non”-reactive against self (“Tolerance”)

Reactive against non-self (“Immunity”)

**The T cell selection process (Thymic “education”) has two stages that occur during T cell development**

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



- **Second stage negative selection** deletes *overtly* self reactive clones with high affinity for self-peptide- MHC ...”central tolerance”

T cells at work

**2. Surveillance: How MHC class I and class II molecules function differently in directing the T cell immune response to pathogens**

**Immunologic self** is the nearly unique set of self-peptides and self-MHC molecules that generates, and in turn is recognized, by the individual’s *unique adaptive immune system T cell repertoire*

- Major selective advantages to the species in dealing with infection since there is essentially no set of stereotyped recognition structures shared by different individuals in the species

**Types of surveillance for pathogen peptides**

*There are two fundamental classes of pathogens that the immune system must respond differently to:*

- **Viruses (intracellular bacteria)**
  - A viral peptide on a cell’s MHC molecules signifies to a T cell that it is infected and should be **killed**
- **Extracellular bacteria**
  - A bacterial peptide on a phagocytic cell that ingested an extracellular bacterium signifies to a T cell the phagocyte has ingested a foreign substance and must be **helped** by the activated T cell to eliminate the pathogen

**Immunologic “self” is the basis of graft rejection**

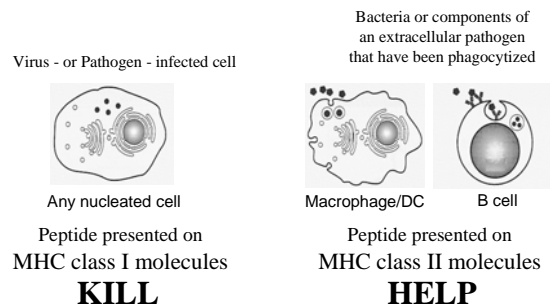
- Other individuals of the same species inherit different MHC alleles and *their cells and tissues are recognized as non-self and attacked by the person’s T cells as if they were pathogens...*

**Histocompatibility**

**Immunologic “self” is the basis for autoimmunity and autoimmune disease**

- Since the entire T cell system is selected on self-peptides and self-MHC, it is inherently autoreactive and if triggered these T cells will be responsible for **Autoimmunity**

Two different classes of MHC molecules direct different T cell immune responses to the two different pathogen types in this surveillance



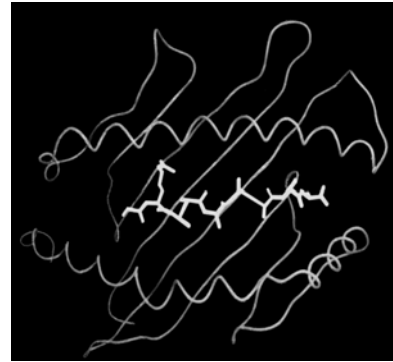
There are two separate lineages of T cells:

**CD8**

**CD4**

**KILL**

**HELP**



Structure of peptide-binding class I MHC domain

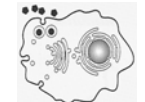
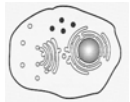
**The immune system makes this distinction by loading and recognizing peptides in either class I or class II MHC**

Challenge:

Intracellular Virus or Pathogen

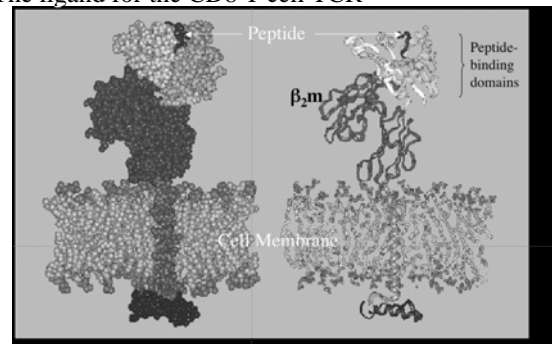
Ingested Bacteria or Endocytic Pathogen

Extracellular Pathogen or Toxin



Presenting cell:	Any cell	Macrophage/DC	B cell
Protein degraded in:	Cytosol	Endocytic vesicles	Endocytic vesicles
Peptides bind to:	MHC class I	MHC class II	MHC class II
Presented to:	CD8 T cells	CD4 T cells	CD4 T cells
Effect on presenting cell of T cell recognition:	Death of cell presenting the viral antigen	Activation of cell to help enhance pathogen killing	Provision of help to B cell for production of antibodies

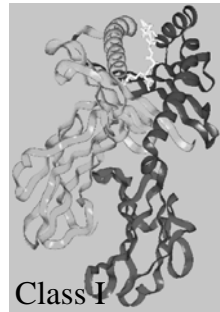
**The ligand for the CD8 T cell TCR**



**MHC Class I Domains**

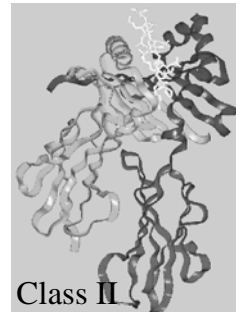
**3. Structure of Class I and II MHC molecules**

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



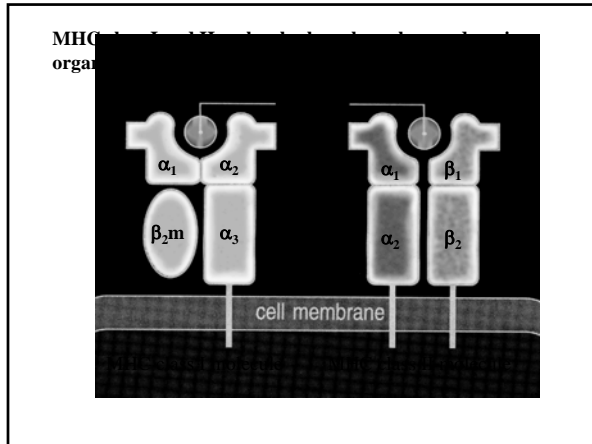
**Class I**

- Expressed on virtually all nucleated cells
- Specifically interacts with CD8



**Class II**

- Expressed on professional APCs: DC, macrophages and B cells
- Specifically interacts with CD4



4. How peptides bind to MHC molecules

The Structure of MHC Molecules: MHC Class I

- The  $\alpha$  chain is ~ 350 AA long
- Three globular domains,  $\alpha 1$ ,  $\alpha 2$  and  $\alpha 3$ , each ~90AA
- $\alpha 1$  and  $\alpha 2$  form the antigen-binding cleft
- $\beta_2$  microglobulin ~100AA, associates with the  $\alpha 3$  domain, not MHC encoded
- ~ 70 AA transmembrane and cytoplasmic portion

peptides bind to Class I MHC

Cluster of tyrosine residues recognized by TCR

positively 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  $\alpha$ -chain and  $\beta$ -chain both encoded within the MHC
- Each chain is made of two globular domains, each ~90AA
- $\alpha 1$  and  $\beta 1$  domains form the antigen-binding cleft

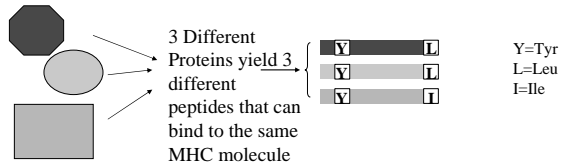
TCR How peptides bind to MHC class I

Rules for binding to MHC class I molecules

- Usually peptides are 9 amino acids in length
- Always oriented with NH<sub>2</sub> 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.

## Rules for peptide binding to MHC class I molecules

Role of side chains



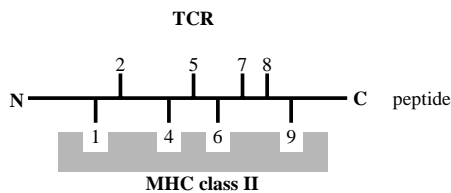
A MHC Class I molecule selects homologous peptides derived from different proteins that have P2 and P9 side chains composed of homologous amino acids, e.g. tyrosine and leucine or isoleucine

## 5. Genetic polymorphisms of MHC genes

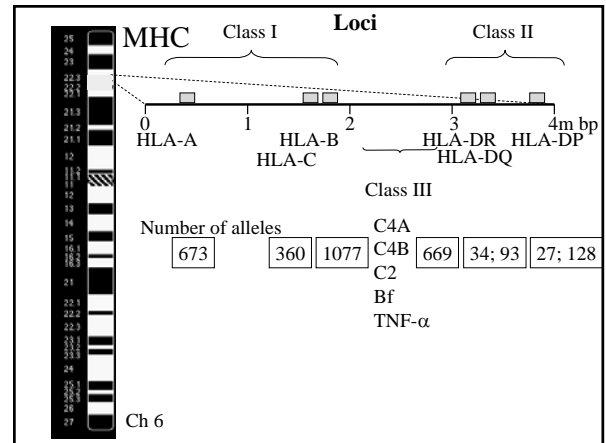
### HLA Genetics

## How peptides bind

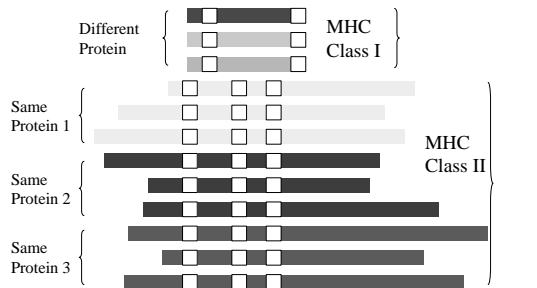
### Rules for binding to MHC class II molecules



- 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



## 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 NH<sub>2</sub> termini to the left

## Diversity of MHC class I and II genes

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***



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

- No practical biologic limit on the number of alleles for the species

- Frequency-dependant selection**- The individual with the rarest allele has the best chance to survive an infection in an epidemic

- Heterozygote advantage**- the individual with more MHC structures can present more, different pathogen peptides

*Duplication of a locus incurs a risk*

- Each duplication results in a new set of antigen-presenting structures
- Each MHC type selects its own allele-specific TCR clonal repertoire capable of recognizing additional pathogen peptides
- However, each duplication increases the size of immune self and 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)