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

Respond by clonal expansion and differentiation to memory & effector stage

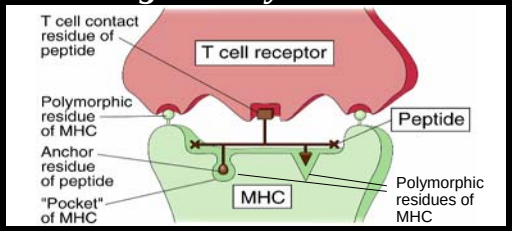
Mechanisms involved based on recognition of peptides bound to Major Histocompatibility Complex (MHC) molecules

p-MHC

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 the 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 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 placentation

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} =**~ 10^{13}**

Problem 1

To generate **~ 10^{13}** structurally diverse clonal T cell receptors (TCRs) requires:

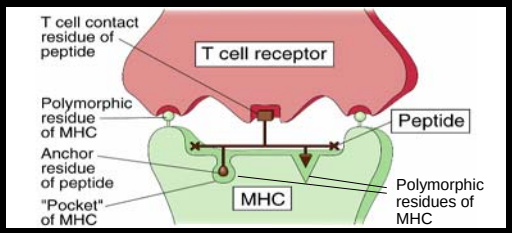
- A ***somatic recombination mechanism*** to randomly form the antigen recognition part of the TCR, **since the genome is not large enough to encode that number of genes**

- 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



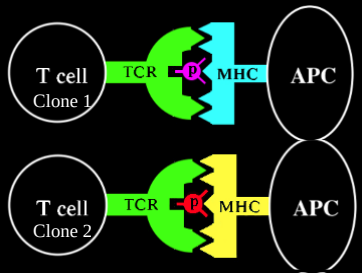
Microbial pathogens can mutate around a stereotyped defense recognition system

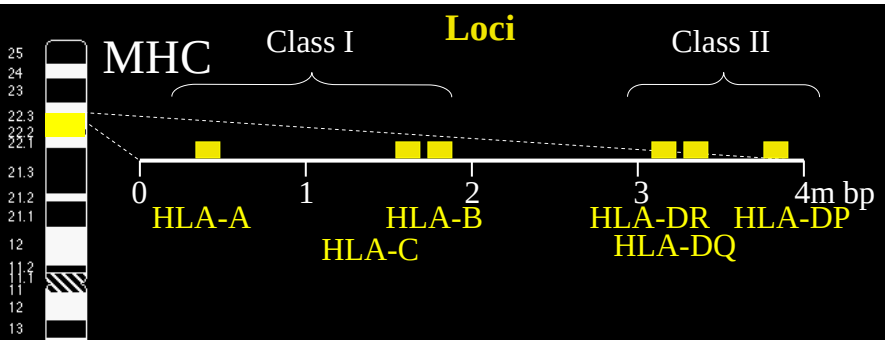
Solution…diversify the MHC

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)





Number of alleles

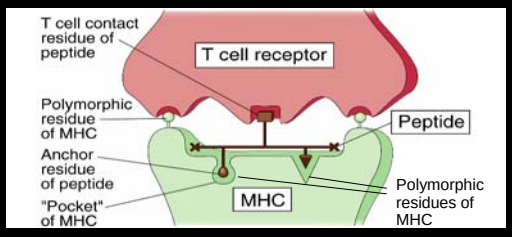
673	360	1077	669	34; 93	27; 128

(260, 935, 560) (7, 310, 746, 368)

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

Ch 6

Human Leukocyte Antigens (HLA)



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***

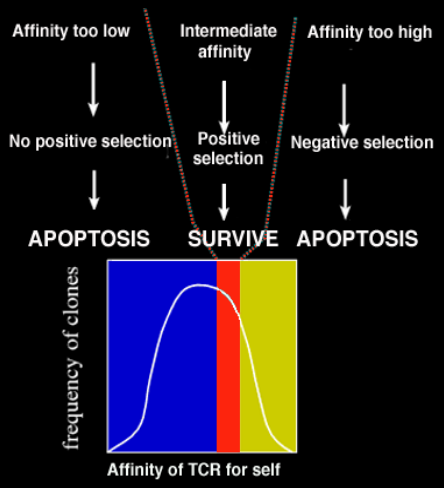
This requires a ***clonal selection process*** centered in the thymus and driven by self p-MHC to ***select the repertoire of clones with TCR appropriate for the self-MHC and self-peptides of each individual***

"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"**

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

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**

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

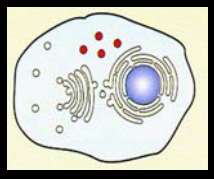
Types of surveillance for pathogen peptides

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

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

Virus - or Pathogen - infected cell

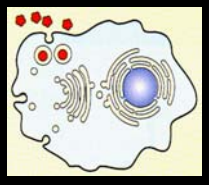


Any nucleated cell

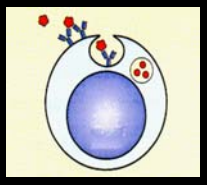
Peptide presented on MHC class I molecules

KILL

Bacteria or components of an extracellular pathogen that have been phagocytized



Macrophage/DC



B cell

Peptide presented on MHC class II molecules

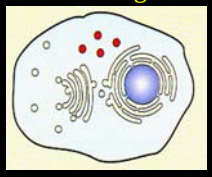
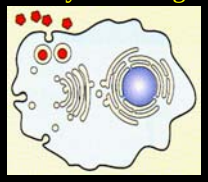
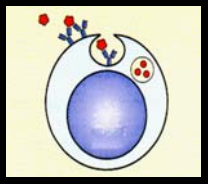
HELP

There are two separate lineages of T cells:

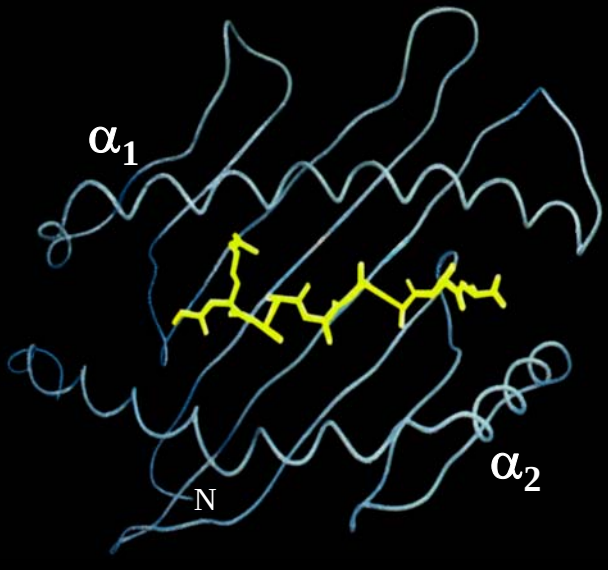
CD8 **CD4**

KILL **HELP**

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

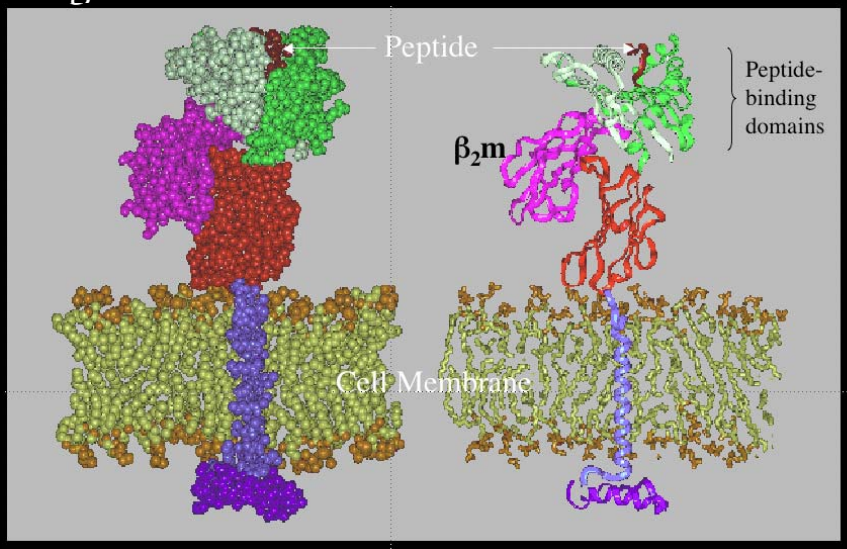
Challenge:	Intracellular Virus or Pathogen	Ingested Bacteria or Endocytic Pathogen	Extracellular Pathogen or Toxin
			
Presenting cell:	Any cell	Macrophage/DC	B cell
Protein degraded in:	**Cytosol**	**Endocytic vesicles**	**Endocytic vesicles**
Peptides bind to:	**MHC class I**	**MHC class II**	**MHC 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

3. Structure of Class I and II MHC molecules



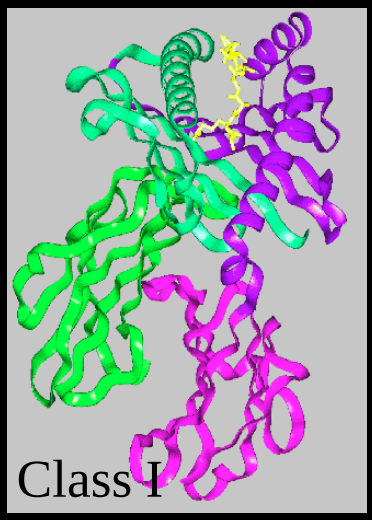
Structure of peptide-binding class I MHC domain

The ligand for the CD8 T cell TCR



MHC Class I Domains

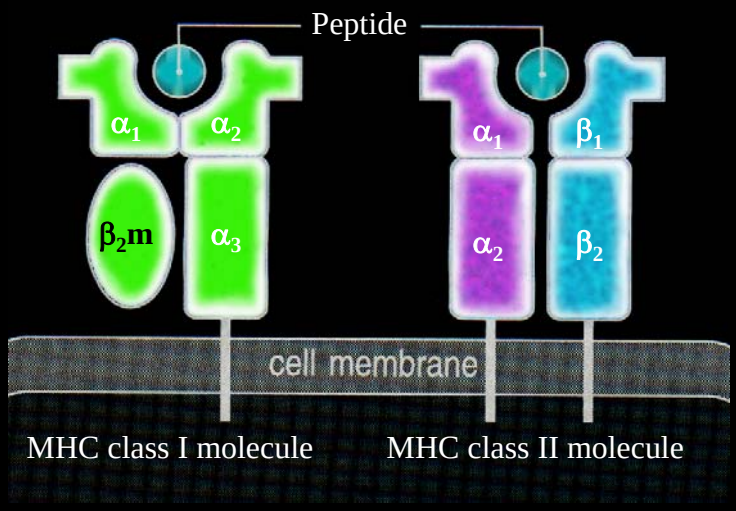
The overall structure of class I and class II MHC is rather similar



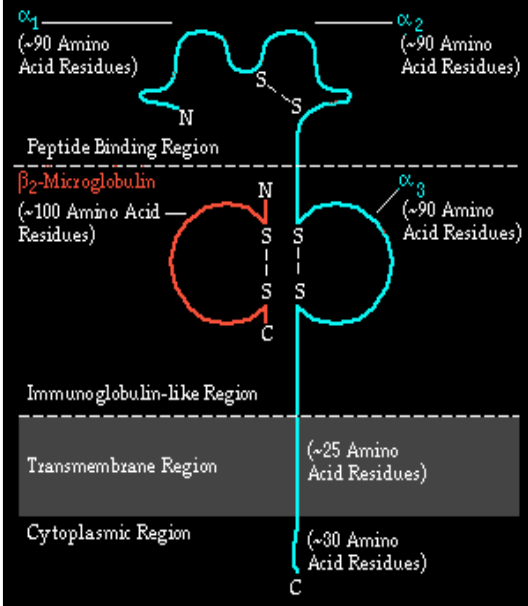
- Expressed on virtually all nucleated cells
- Specifically interacts with CD8

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

MHC class I and II molecules have homologous domain organization, but different chain structure

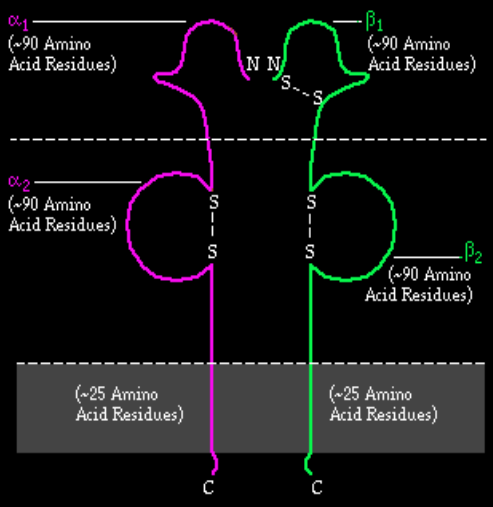


The Structure of MHC Molecules: MHC Class I



- 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

The Structure of MHC Molecules: MHC Class II

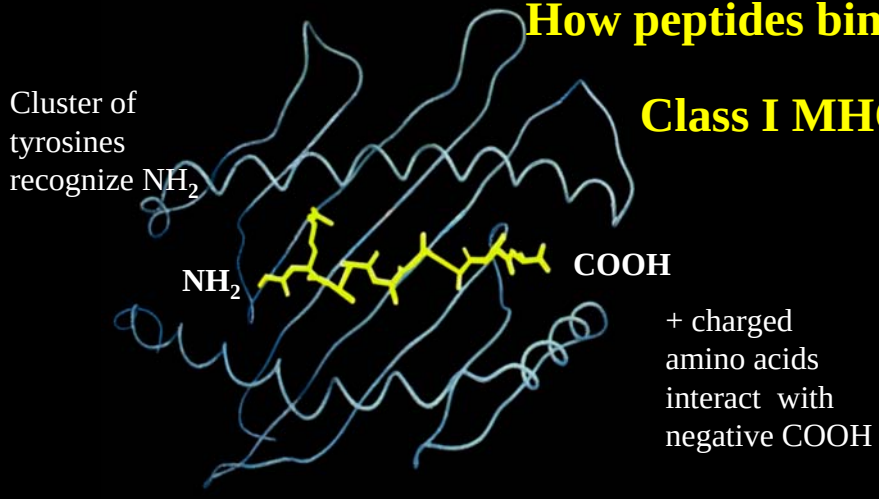


- Composed of two similar membrane spanning proteins, the α-chain and β–chain both encoded within the MHC
- Each chain is made of two globular domains, each ~90AA
- α1 and β1 domains form the antigen-binding cleft

4. How peptides bind to MHC molecules

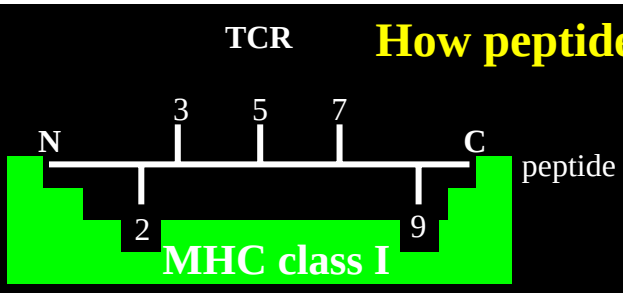
How peptides bind

Class I MHC



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

How peptides bind

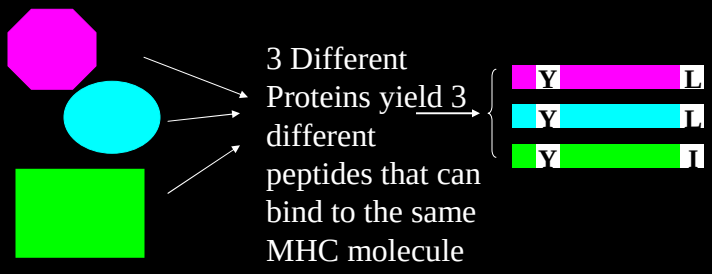


Rules for binding to MHC class I molecules

- 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.

Rules for peptide binding to MHC class I molecules

Role of side chains



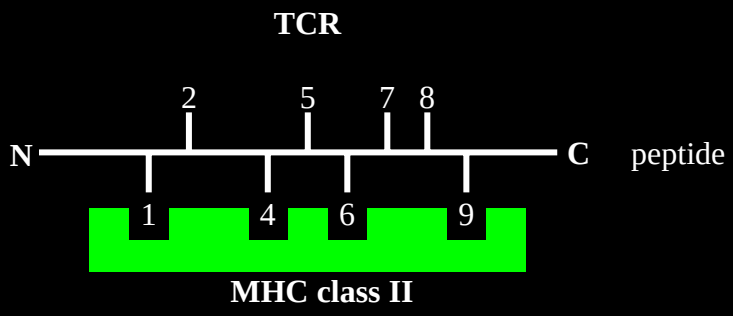
3 Different Proteins yield 3 different peptides that can bind to the same MHC molecule

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

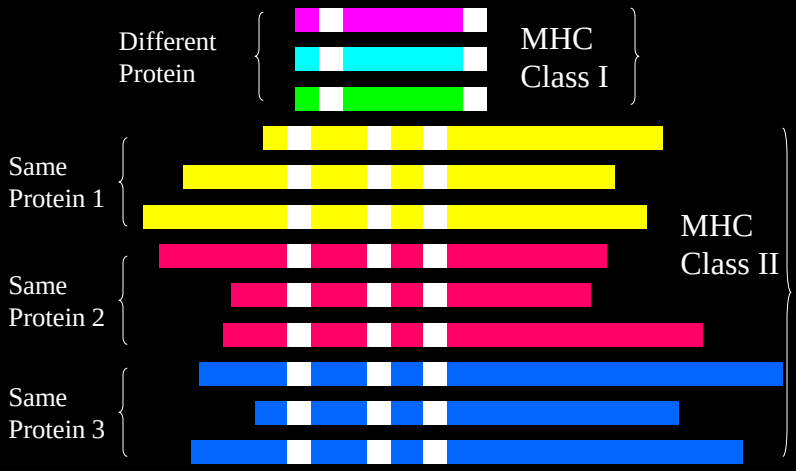
How peptides bind

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

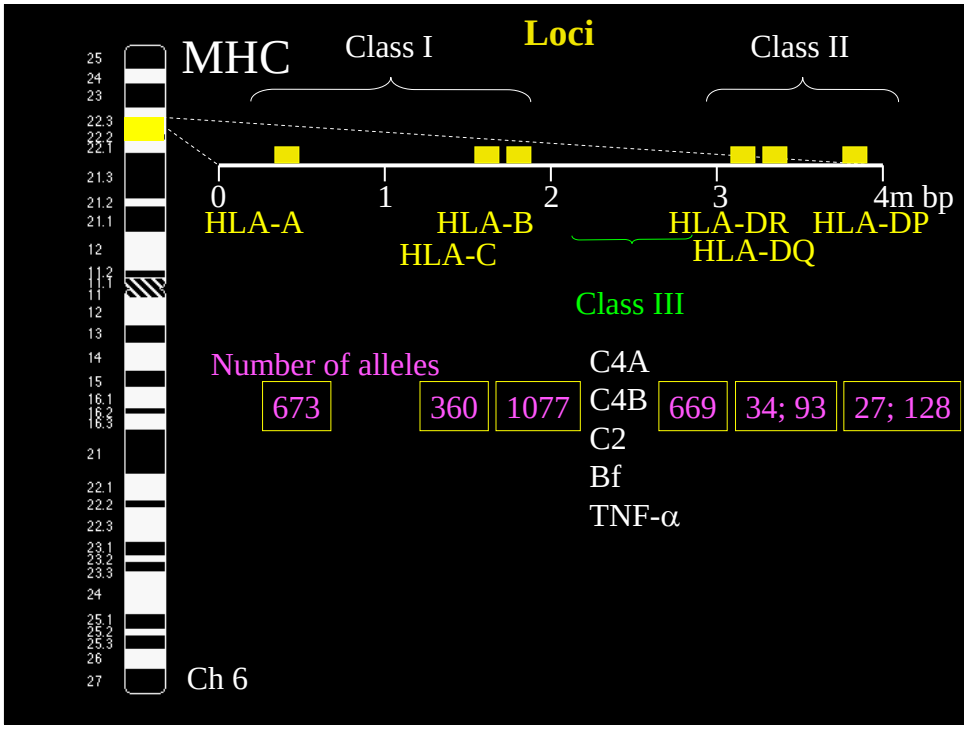
Different rules for peptide binding to class II MHC molecules



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

5. Genetic polymorphisms of MHC genes

HLA Genetics

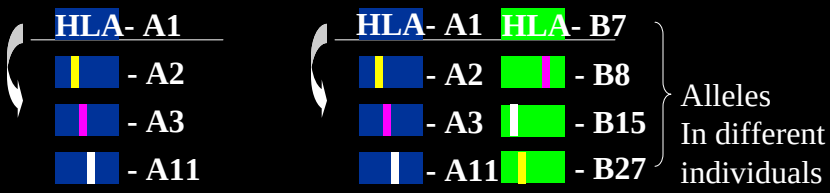


Diversity of MHC class I and II genes

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)