The Major Histocompatibility Complex of Genes

Topic 4

The Major Histocompatibility Complex

Outline of Lectures

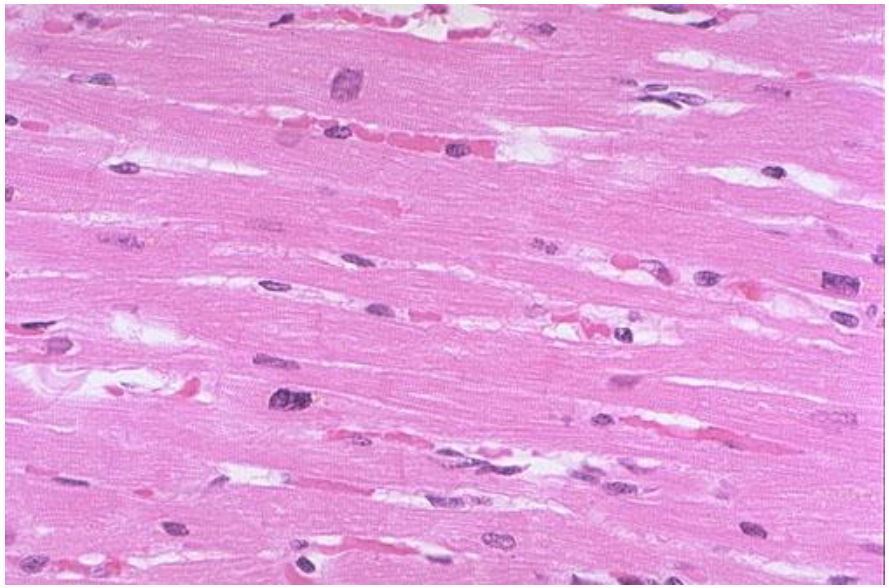
- The immunological reasons for transplant rejection
- How the MHC was discovered using inbred strains of mice
- That T cells recognise MHC molecules
- What is meant by the term Antigen Presentation
- The structure function relationships of MHC molecules
- The principles of the interactions between peptide antigens and MHC molecules
- The structure and organisation of human and mouse MHC loci
- The meaning of polymorphism and polygenism in the MHC

Transplant rejection

Early attempts to transplant tissues failed
Rejection of transplanted tissue was associated with inflammation

and lymphocyte infiltration

IMMUNE GRAFT REJECTION



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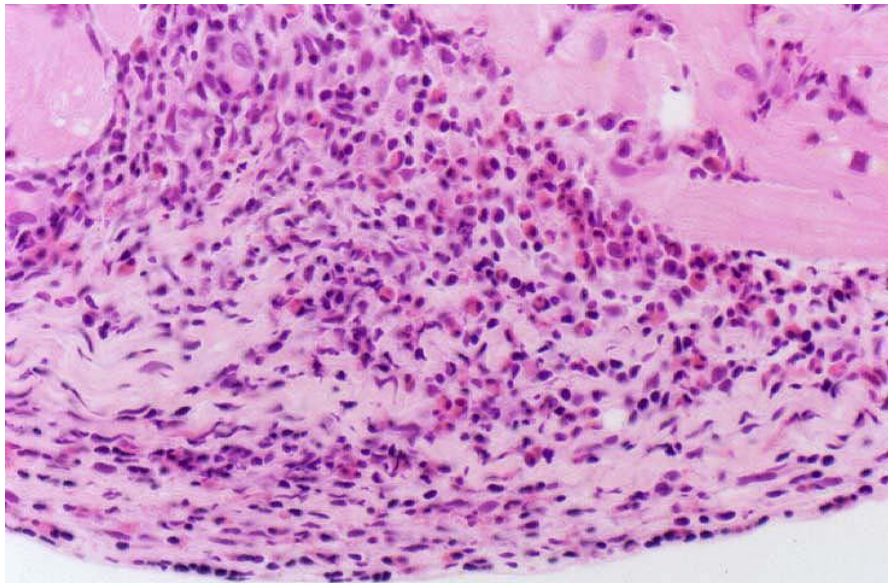

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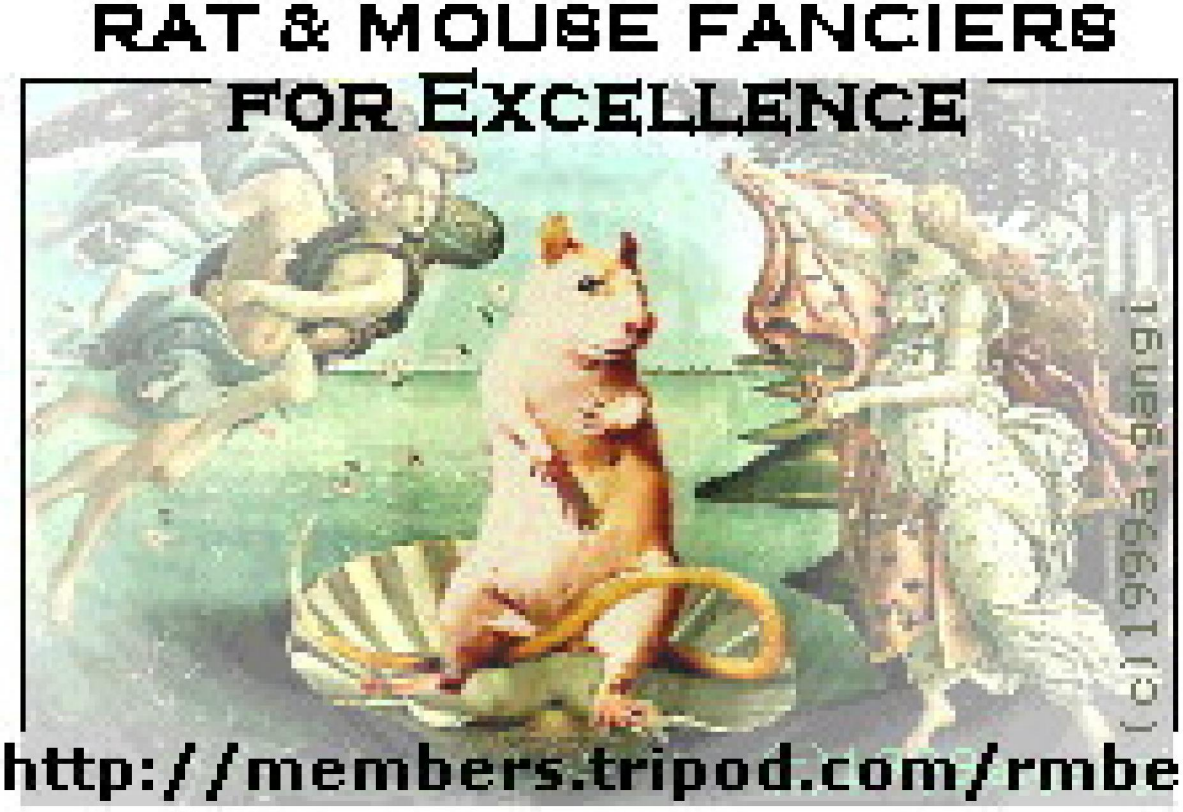
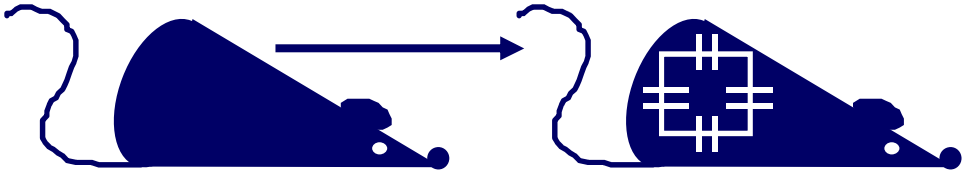
The origin of Immunogenetics



Genetic basis of transplant rejection

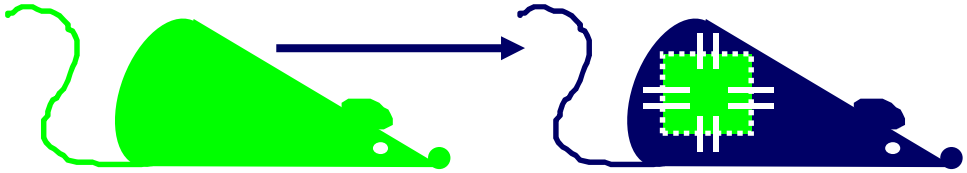
Inbred mouse strains - all genes are identical

Transplantation of skin between strains showed that rejection or acceptance was dependent upon the genetics of each strain



ACCEPTED

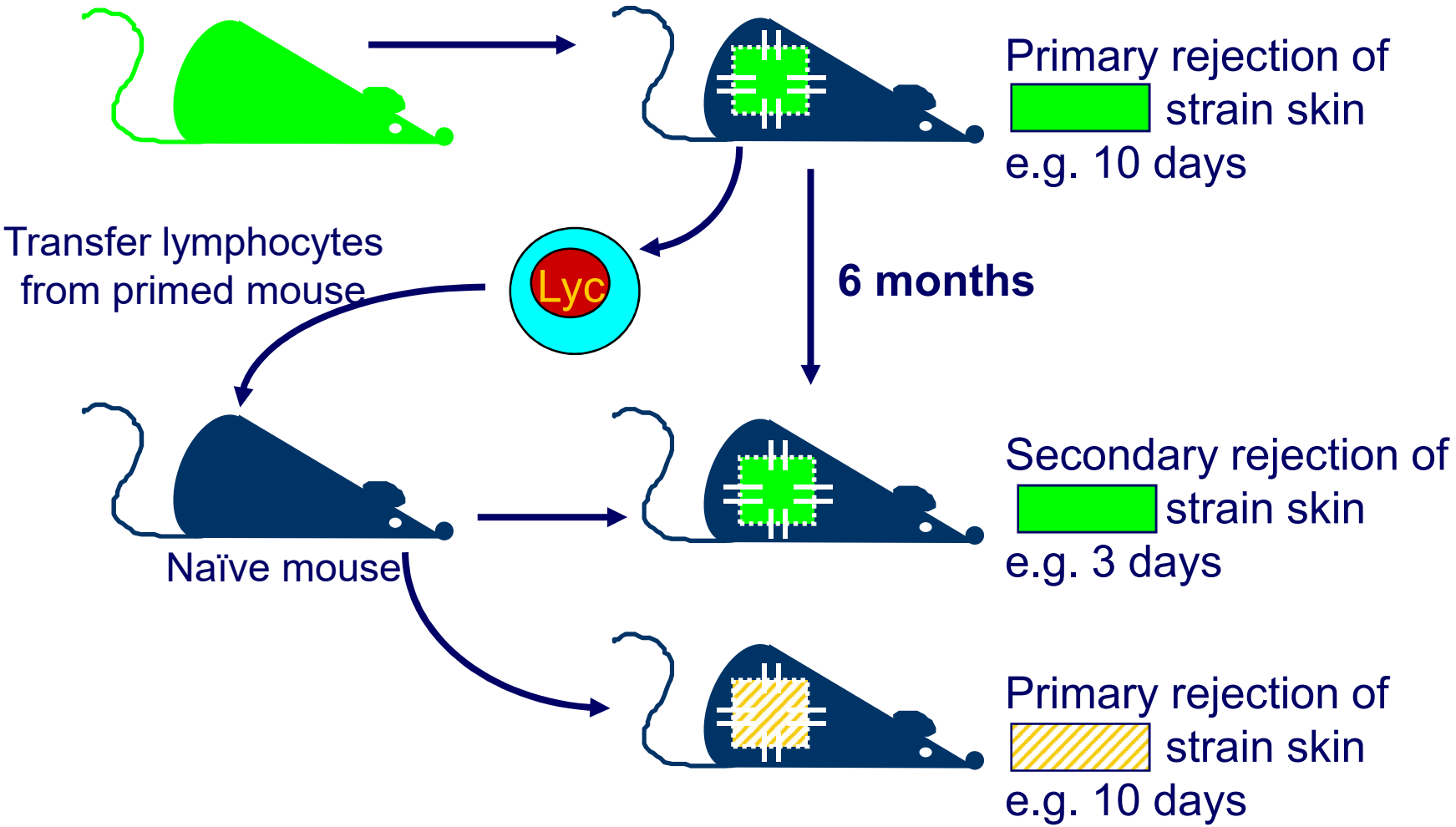
Skin from an inbred mouse grafted onto the same strain of mouse



REJECTED

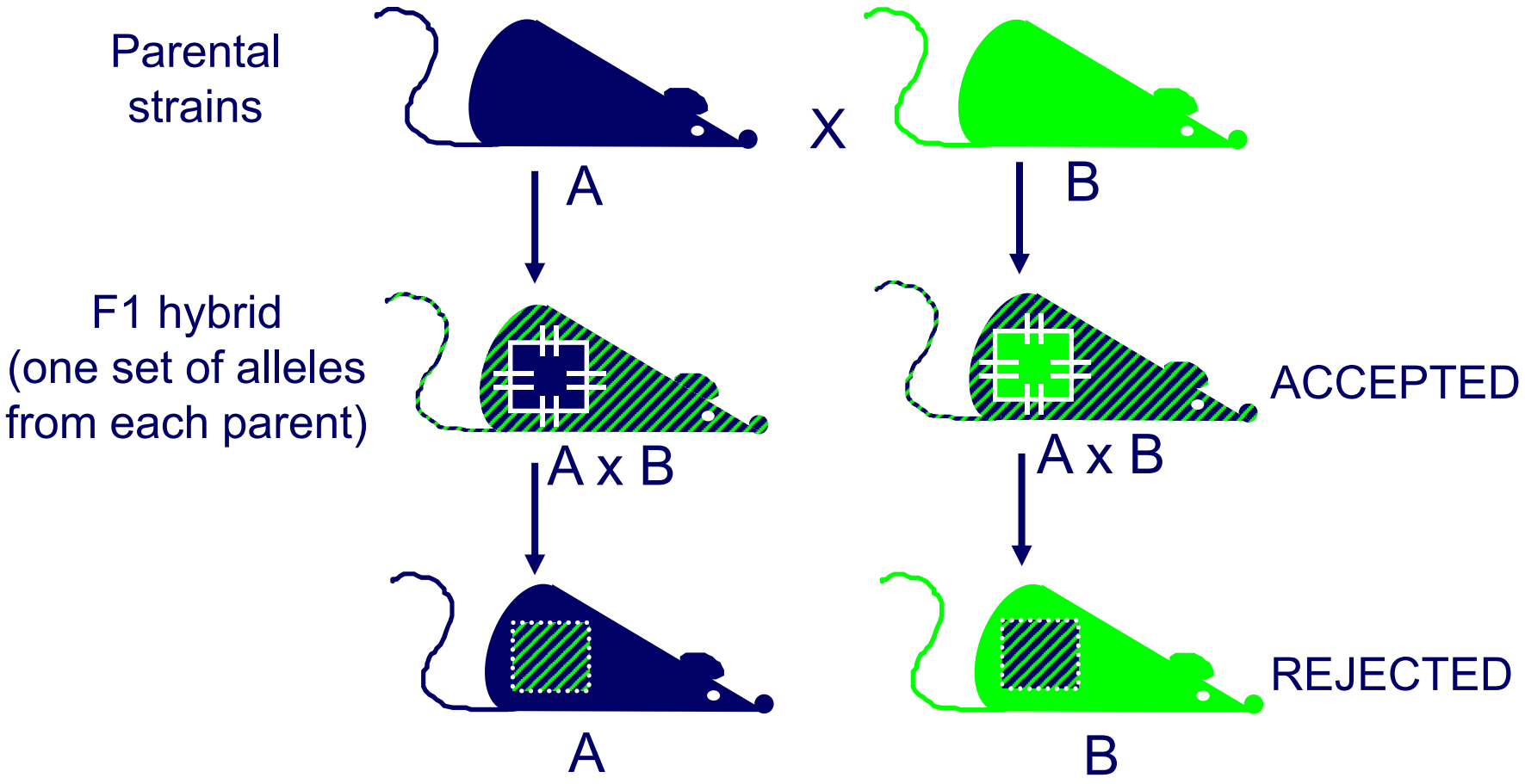
Skin from an inbred mouse grafted onto a different strain of mouse

Immunological basis of graft rejection



Transplant rejection is due to an antigen-specific immune response with immunological memory.

Immunogenetics of graft rejection



Mice of strain (A x B) are immunologically tolerant to A or B skin

Skin from (A x B) mice carry antigens that are recognised as foreign by parental strains

Major Histocompatibility Complex – MHC

In mice the MHC is called H-2

Rapid graft rejection between strains segregated with Antigen-2, encoded as part of the MHC 'haplotype'

(A set of linked genes inherited as a unit)

Inbred mice identical at H-2 did not reject skin grafts from each other

MHC genetics in mice is simplified by inbred strains

In humans the MHC is called the Human Leukocyte Antigen system – HLA

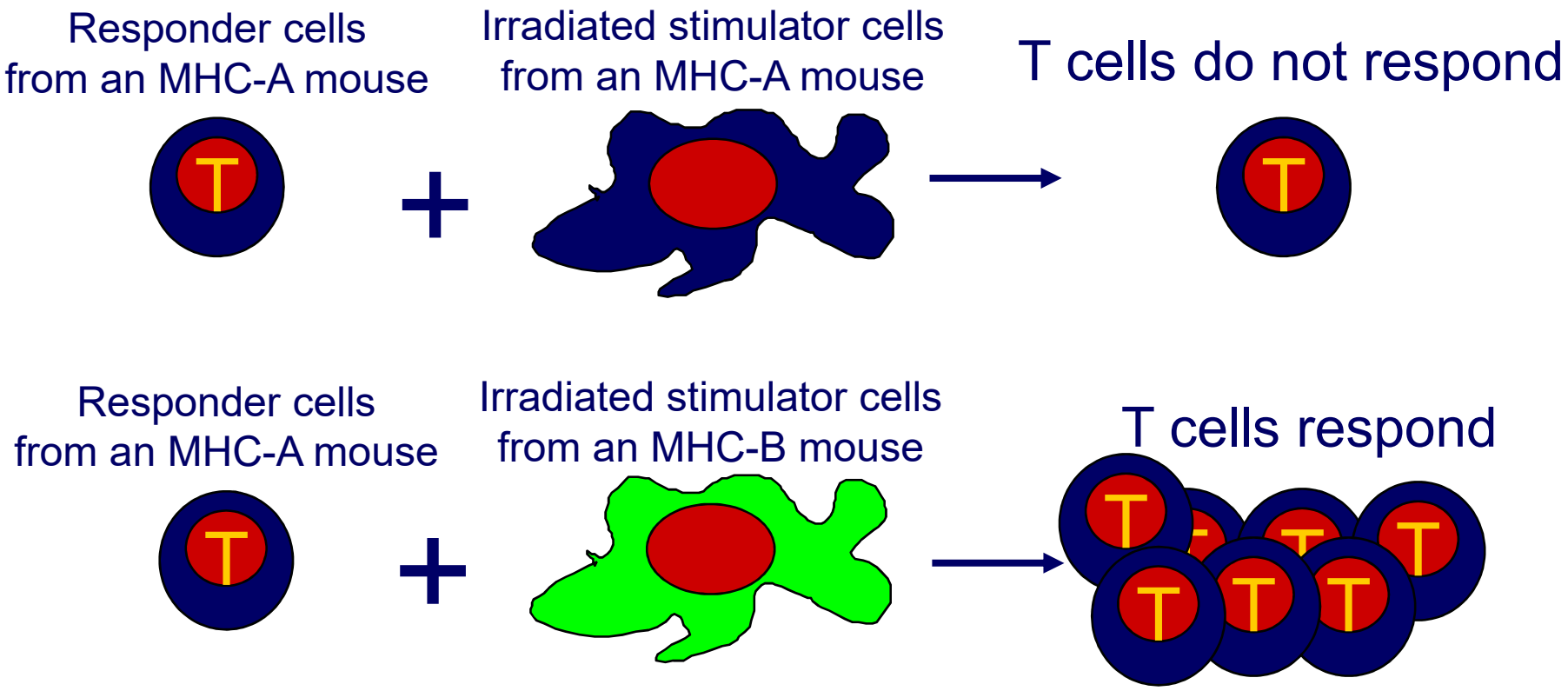
Only monozygous twins are identical at the HLA locus

The human population is extensively out bred

MHC genetics in humans is extremely complex

T cells respond to MHC antigens

Graft rejection in vivo is mediated by infiltrating T lymphocytes
The in-vitro correlate of graft rejection is the
MIXED LYMPHOCYTE REACTION



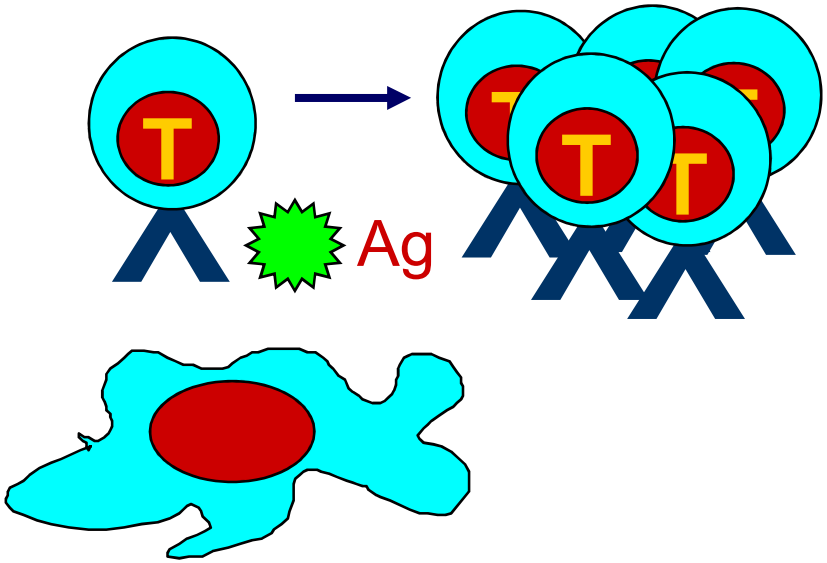
MHC antigens are involved in the activation of T cells

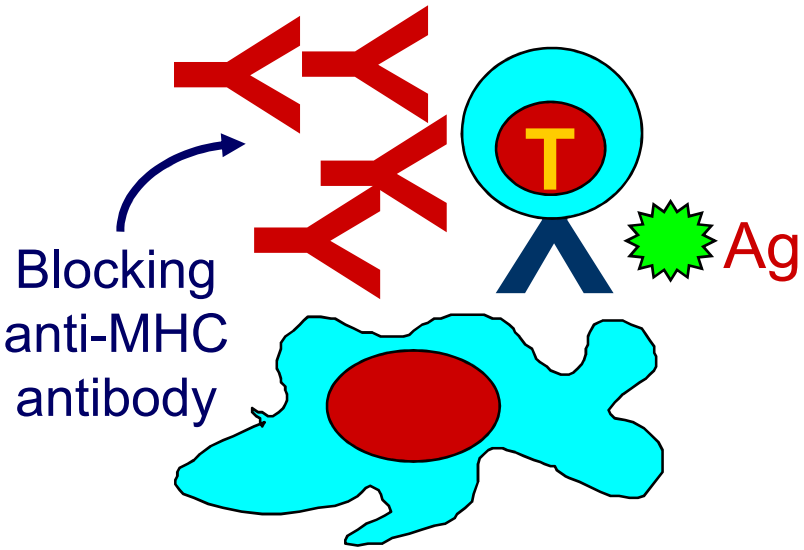
MHC directs the response of T cells to foreign antigens

Graft rejection is an unnatural immune response

MHC antigens PRESENT foreign antigens to T cells

Cells that present antigen are ANTIGEN PRESENTING CELLS

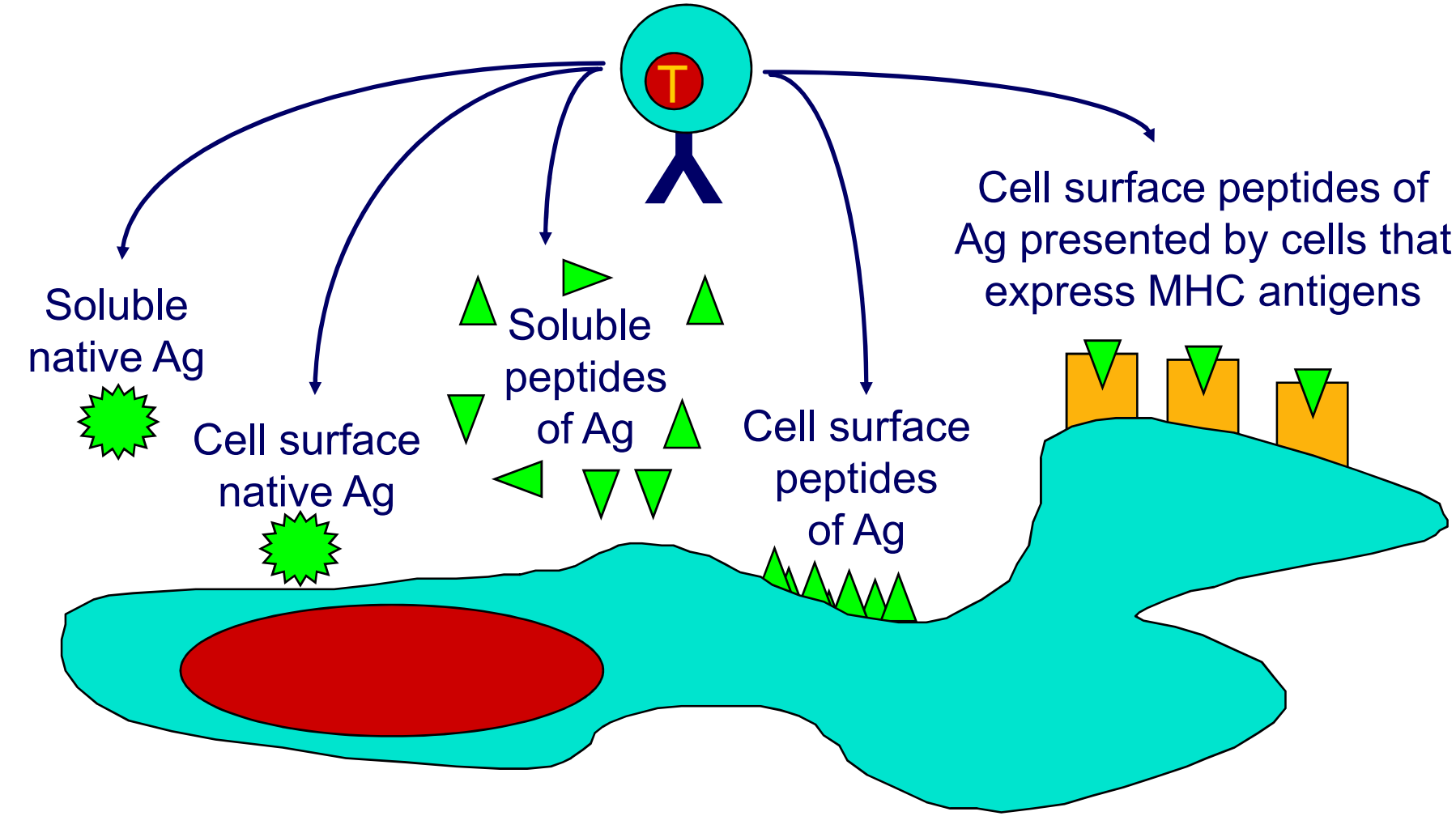




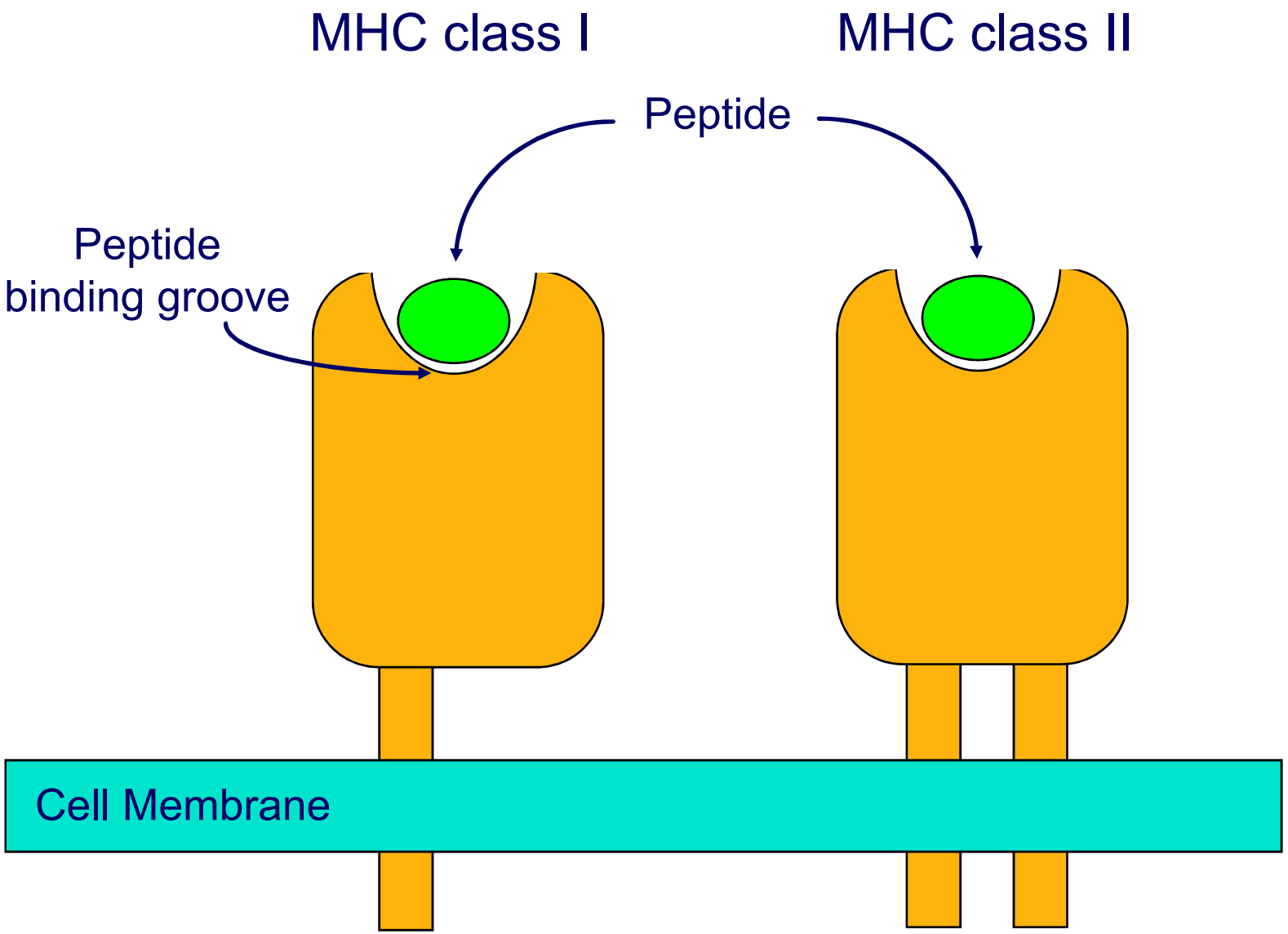
Anti response

No anti response

Antigen recognition by T cells requires peptide antigens and presenting cells that express MHC molecules



MHC molecules



Differential distribution of MHC molecules

Tissue	MHC class I	MHC class II
T cells	+++	+/-
B cells	+++	+++
Macrophages	+++	++
Other APC	+++	+++
Thymus epithelium	+	+++
Neutrophils	+++	-
Hepatocytes	+	-
Kidney	+	-
Brain	+	-
Erythrocytes	-	-

Cell activation affects the level of MHC expression.

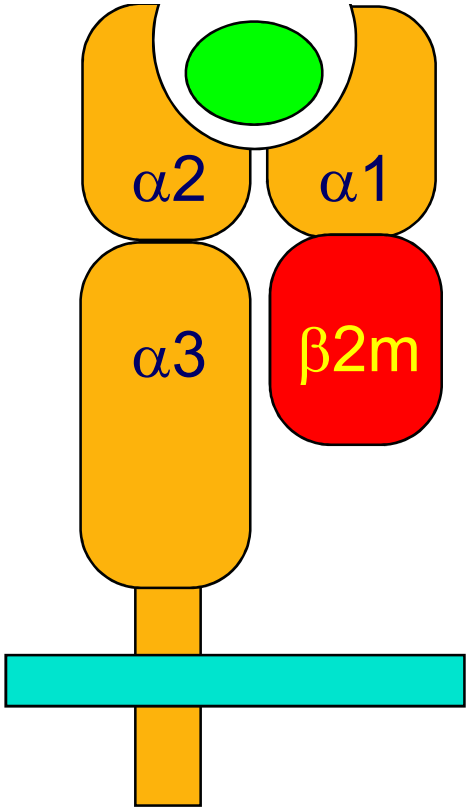
The pattern of expression reflects the function of MHC molecules:

- Class I is involved in the regulation of anti-viral immune responses
- Class II involved in regulation of the cells of the immune system

Anucleate erythrocytes can not support virus replication - hence no MHC class I.

Some pathogens exploit this - e.g. *Plasmodium* species.

Overall structure of MHC class I molecules



MHC-encoded α-chain of 43kDa

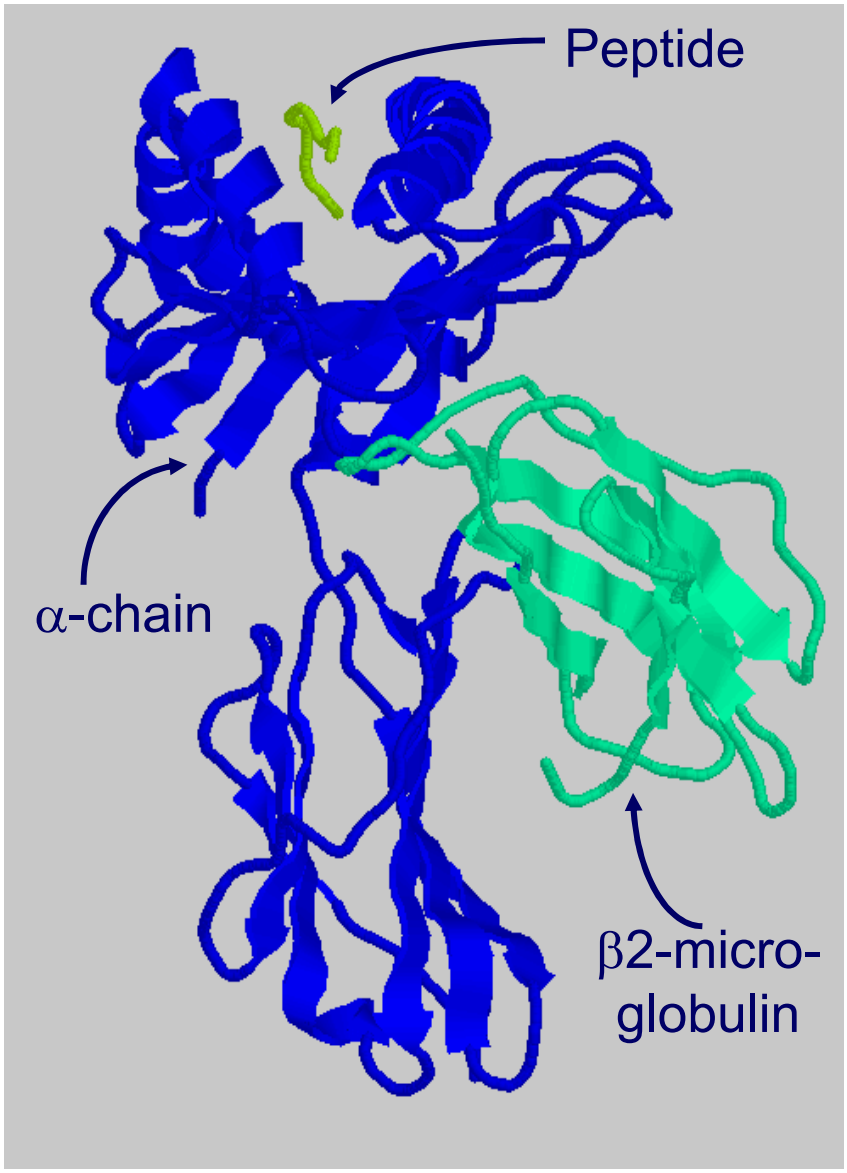
α-chain anchored to the cell membrane

Peptide antigen in a groove formed from a pair of α-helicies on a floor of anti-parallel β strands

β2-microglobulin, 12kDa, non-MHC encoded, non-transmembrane, non covalently bound to α-chain

α3 domain & β2m have structural & amino acid sequence homology with Ig C domains Ig GENE SUPERFAMILY

MHC class I molecule structure

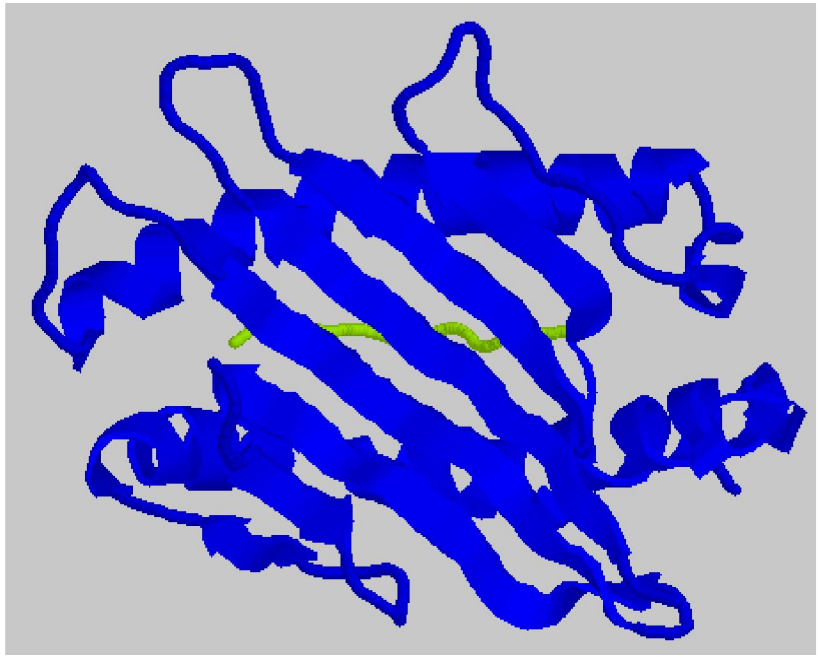


Chains

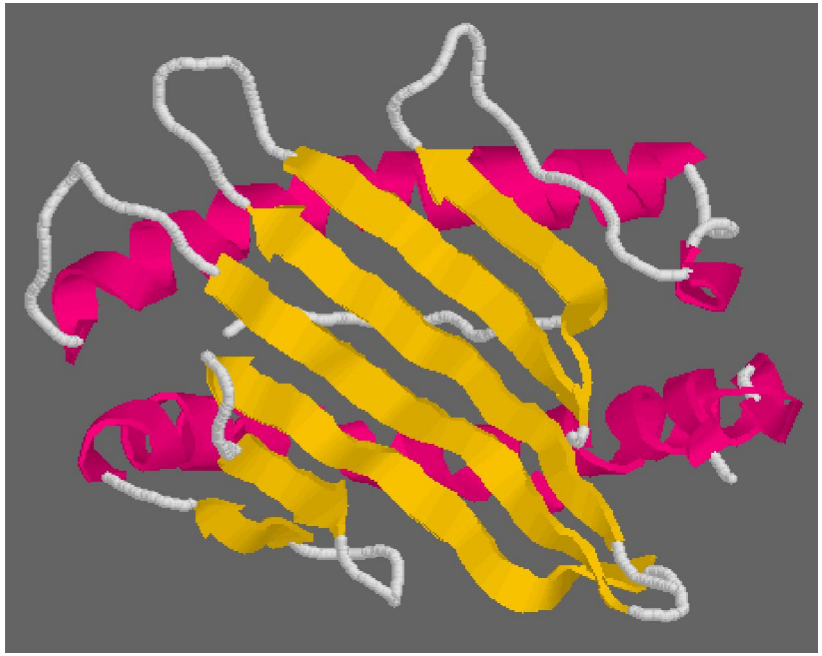
Structures

Structure of MHC class I molecules

α1 and α2 domains form two segmented α-helices on eight anti-parallel β-strands to form an antigen-binding cleft.



Chains

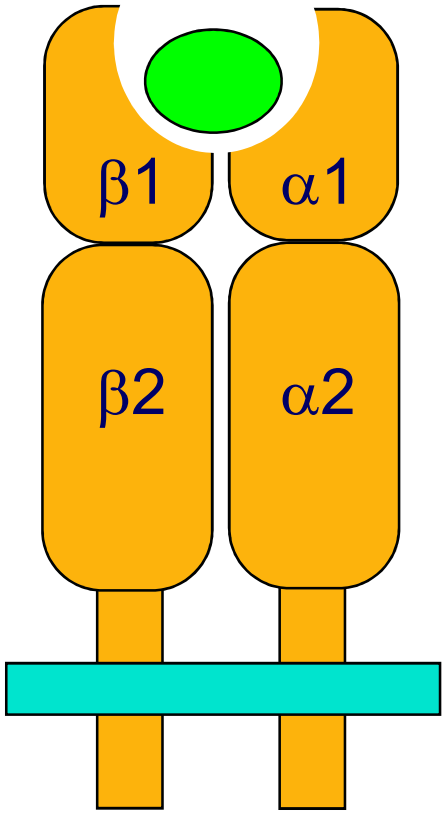


Structures

Properties of the inner faces of the helices and floor of the cleft determine which peptides bind to the MHC molecule

View structures

Overall structure of MHC class II molecules



MHC-encoded, α-chain of 34kDa
and a β-chain of 29kDa

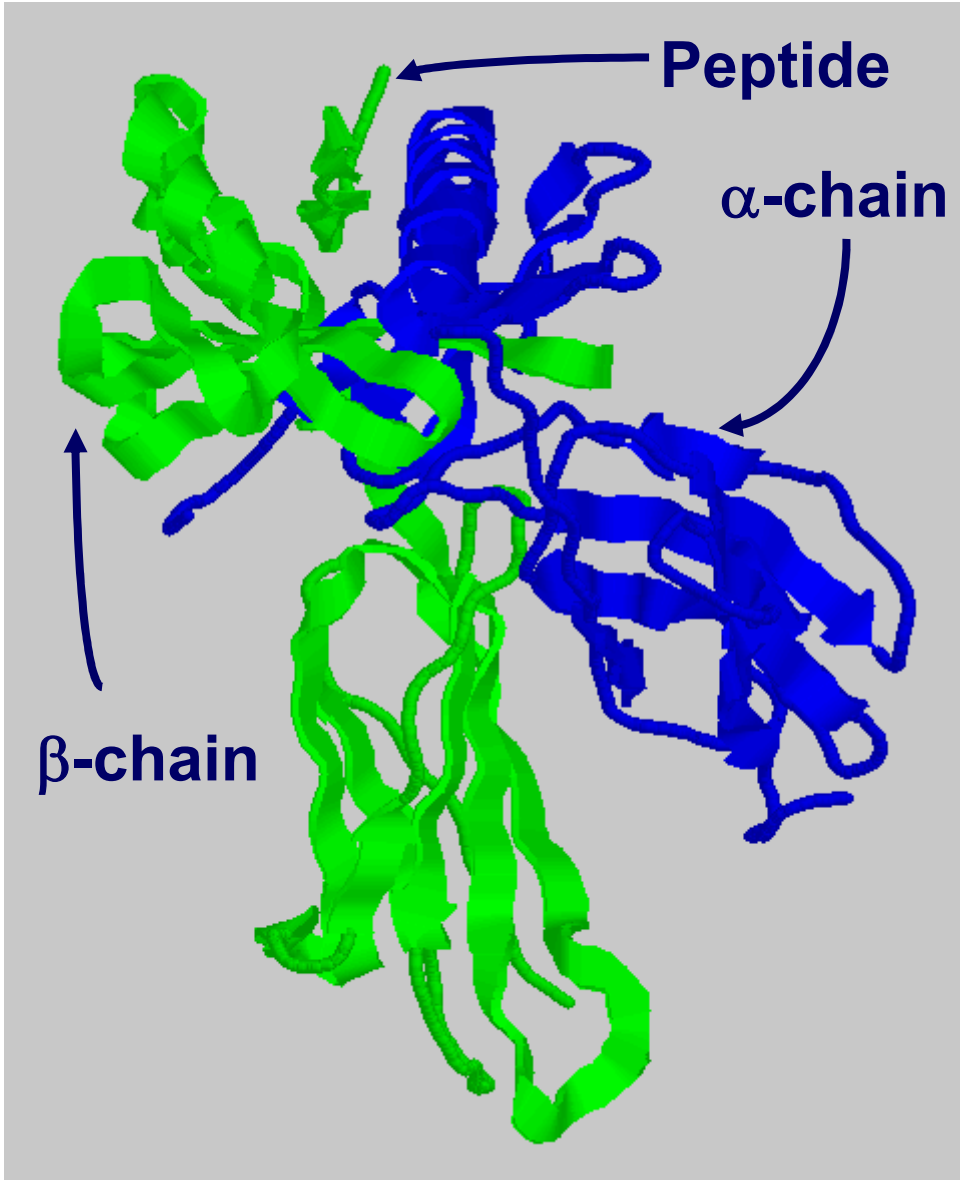
α and β chains anchored to the cell membrane

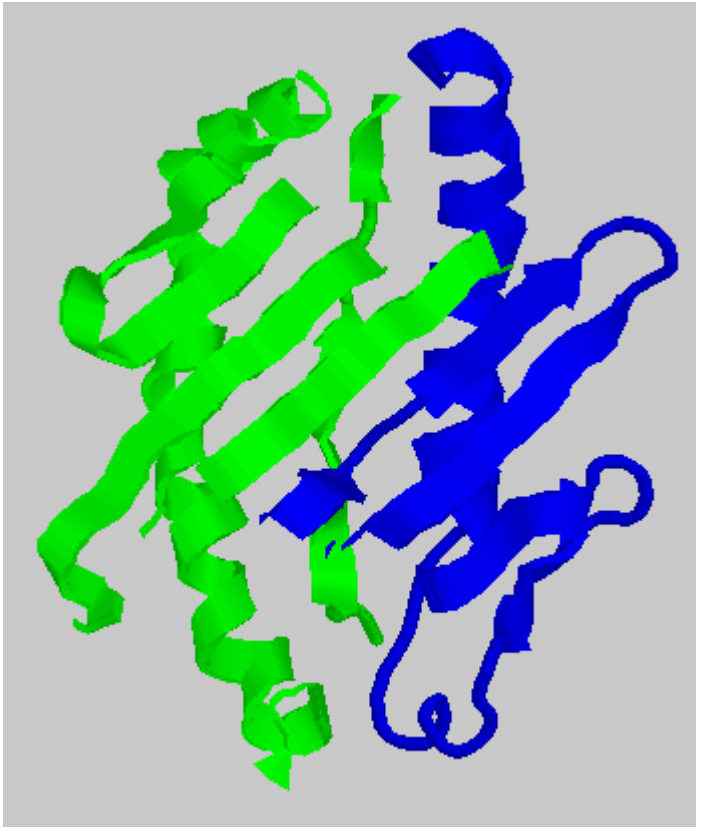
No β-2 microglobulin

Peptide antigen in a groove formed from a pair of α-helicies on a floor of anti-parallel β strands

α2 & β2 domains have structural & amino acid sequence homology with Ig C domains Ig GENE SUPERFAMILY

MHC class II molecule structure

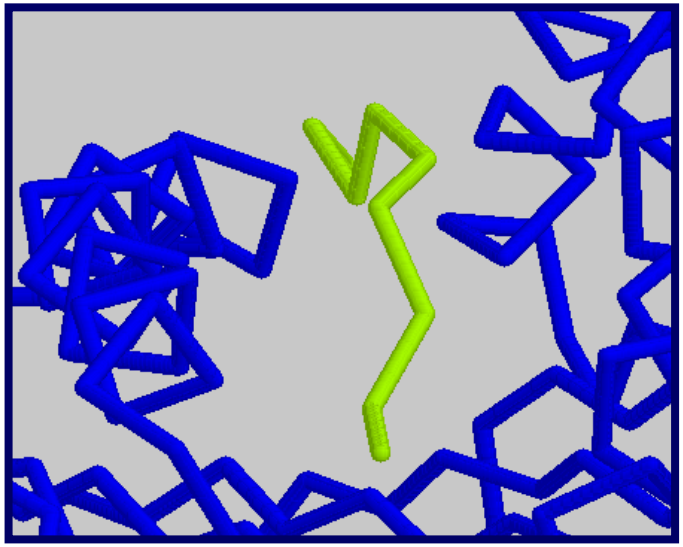


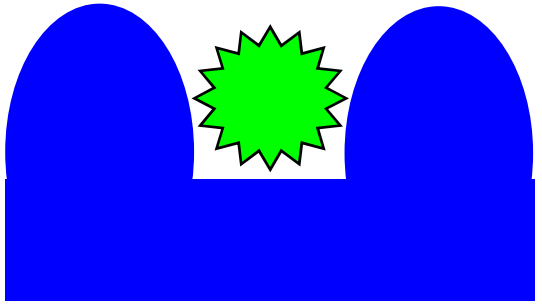


Cleft is made of both α and β chains

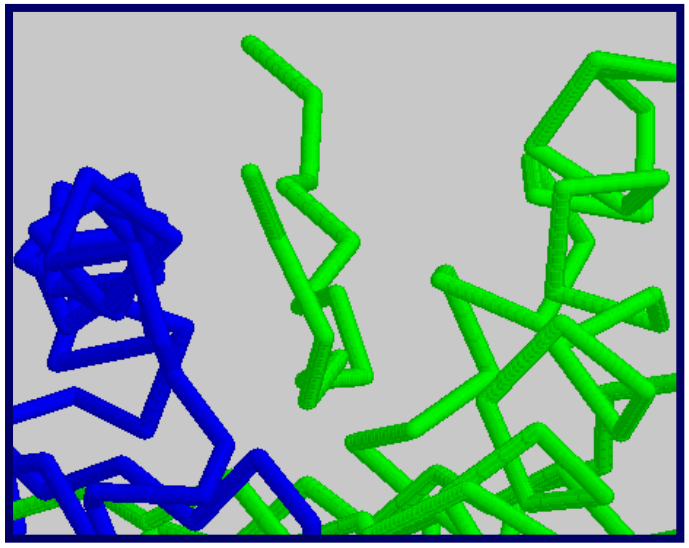
View structures

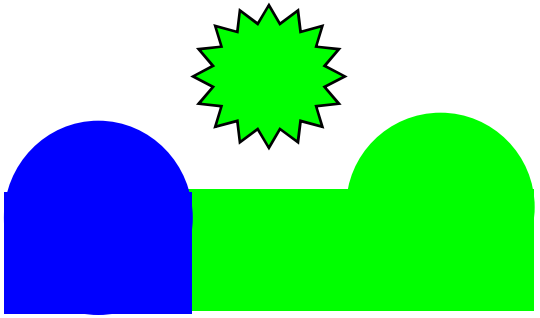
Cleft geometry





MHC class I

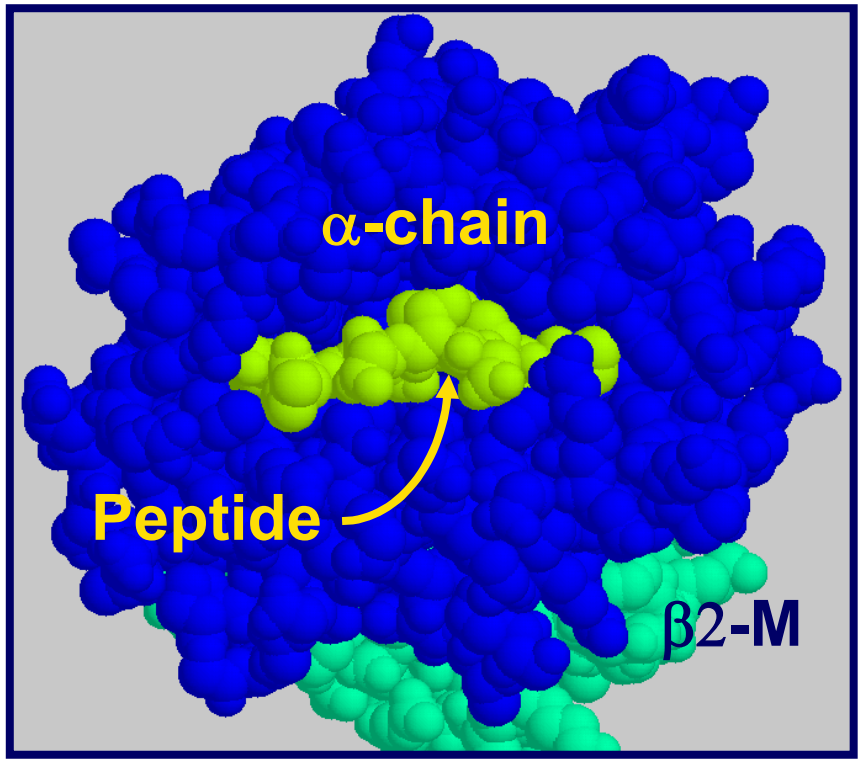


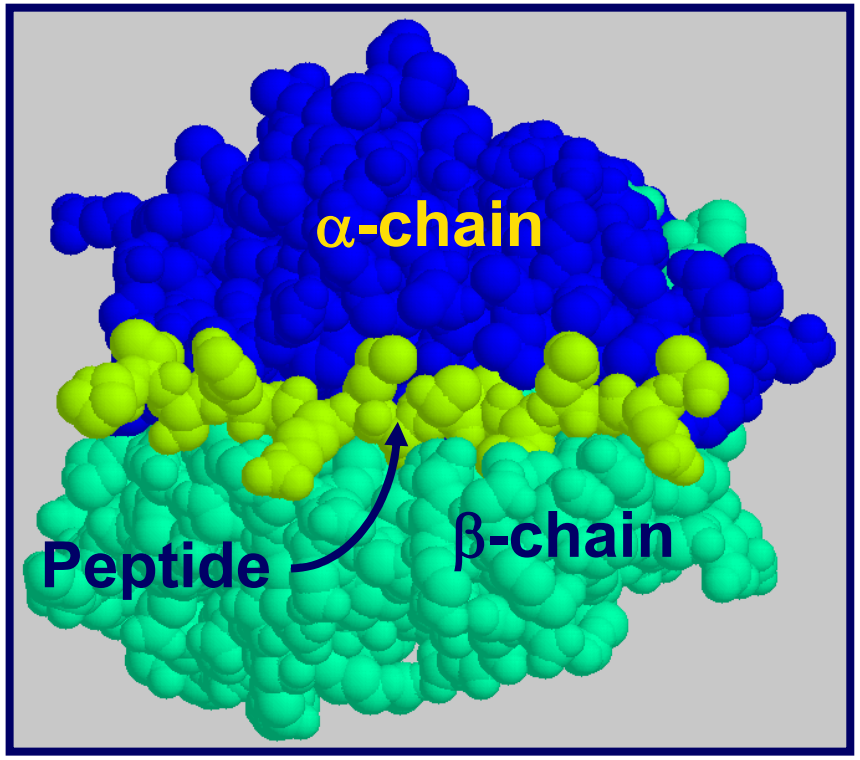


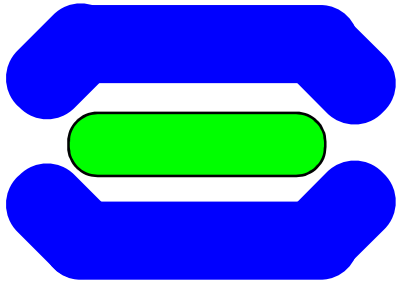
MHC class II

Peptide is held in the cleft by non-covalent forces

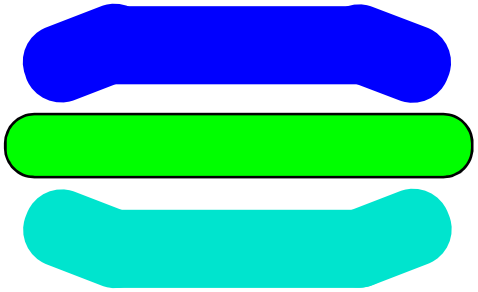
Cleft geometry







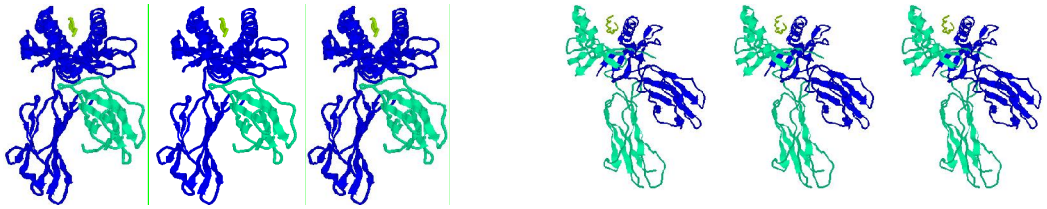
MHC class I accommodate peptides of 8-10 amino acids



MHC class II accommodate peptides of >13 amino acids

MHC-binding peptides

Each human usually expresses:
3 types of MHC class I (A, B, C) and
3 types of MHC class II (DR, DP,DQ)



The number of different T cell antigen receptors is estimated to be

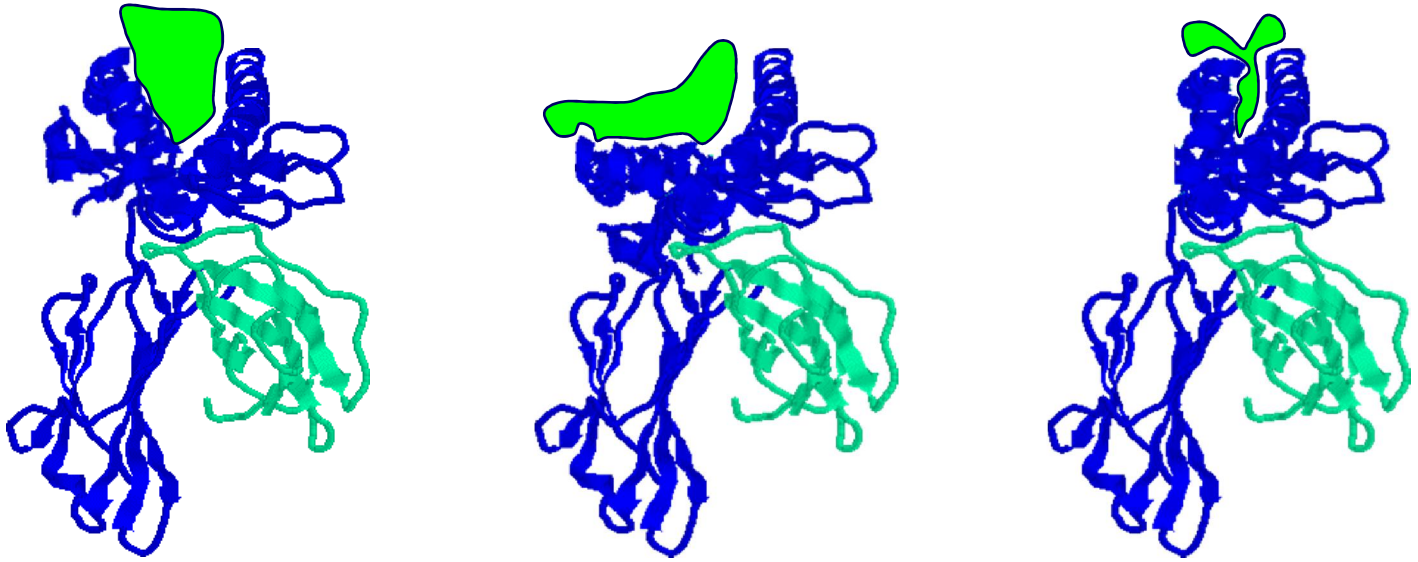
1,000,000,000,000,000

Each of which may potentially recognise a different peptide antigen

How can 6 invariant molecules have the capacity to bind to 1,000,000,000,000,000 different peptides?

A flexible binding site?

A binding site that is flexible enough to bind any peptide?



NO because: at the cell surface, such a binding site would be unable to

- allow a high enough binding affinity to form a trimolecular complex with the T cell antigen receptor
- prevent exchange of the peptide with others in the extracellular milieu

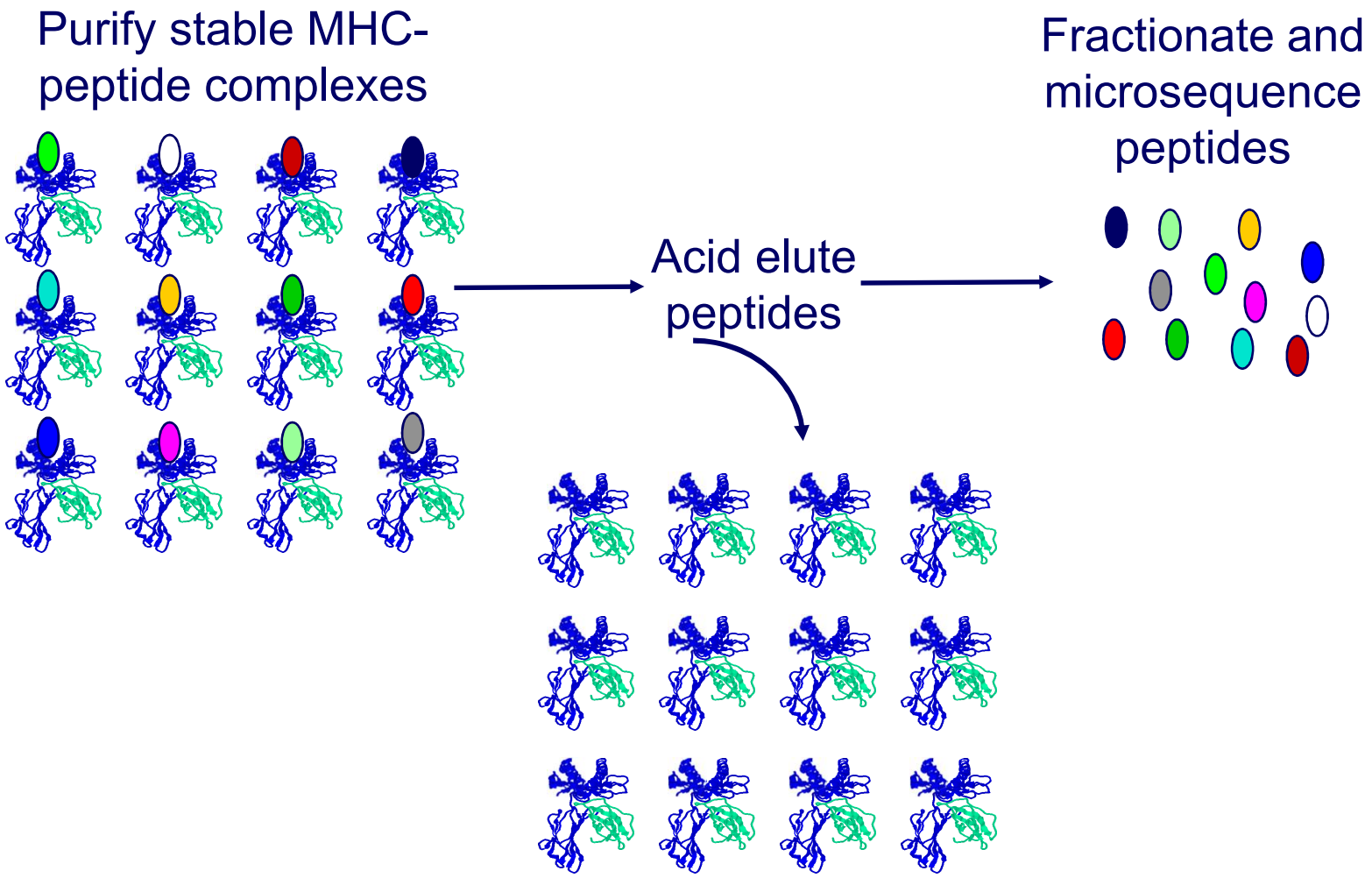
A flexible binding site?

A binding site that is flexible at an early, intracellular stage of maturation
Formed by folding the MHC molecules around the peptide.

Allows a single type of MHC molecule to

- bind many different peptides
- bind peptides with high affinity
- form stable complexes at the cell surface
- Export only molecules that have captured a peptide to the cell surface

Peptides can be eluted from MHC molecules



Eluted peptides from MHC molecules have different sequences but contain motifs

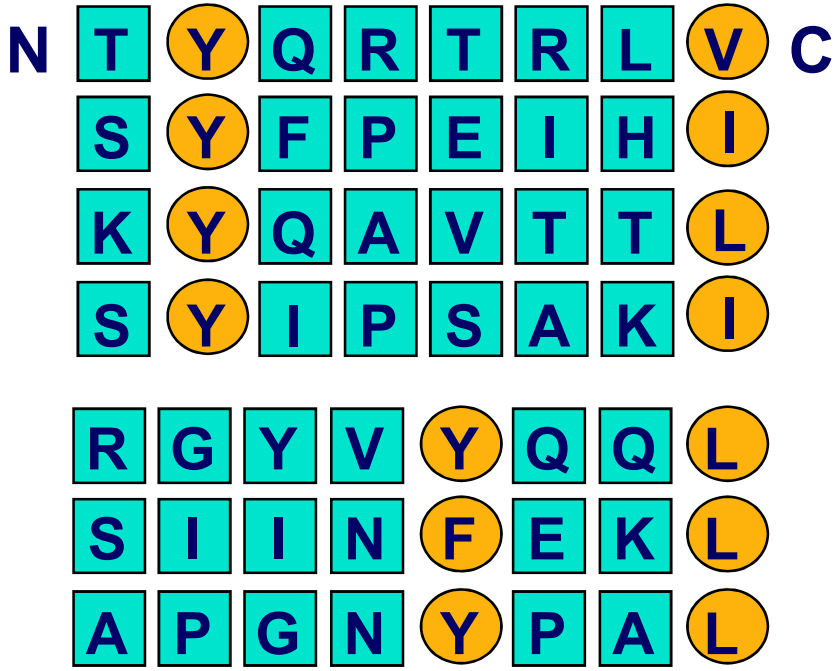
Peptides bound to a particular type of MHC class I molecule have conserved patterns of amino acids

A common sequence in a peptide antigen that binds to an MHC molecule is called a MOTIF

Amino acids common to many peptides tether the peptide to structural features of the MHC molecule
ANCHOR RESIDUES

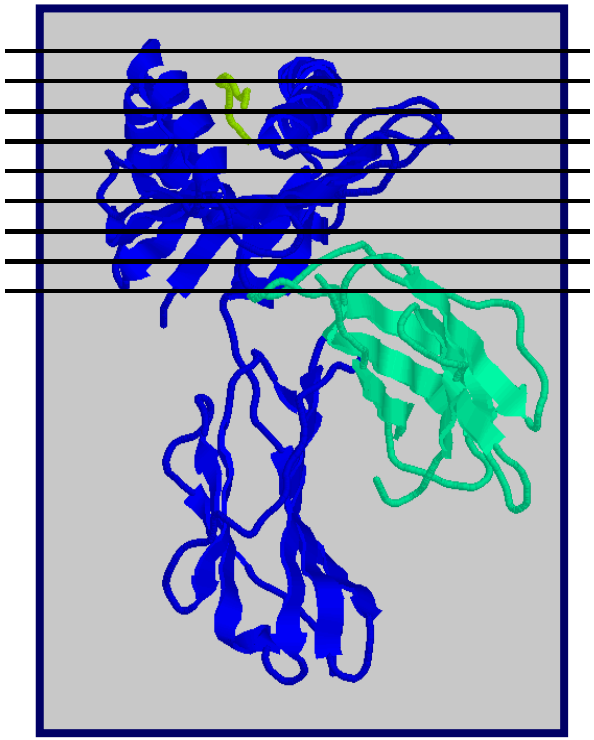
Tethering amino acids need not be identical but must be related
Y & F are aromatic
V, L & I are hydrophobic

Side chains of anchor residues bind into POCKETS in the MHC molecule

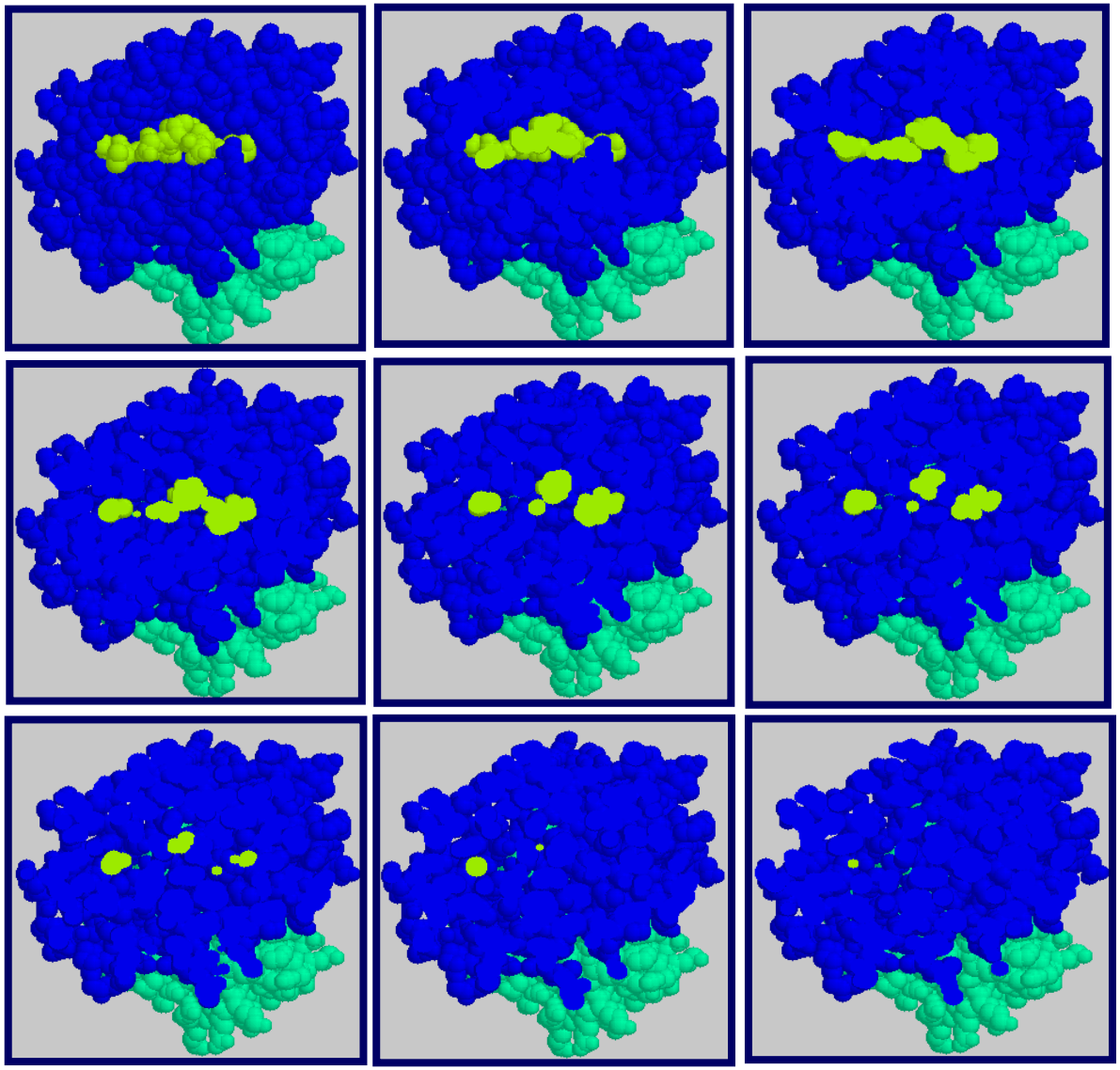


Different types of MHC molecule bind peptides with different patterns of conserved amino acids

Peptide binding pockets in MHC class I molecules

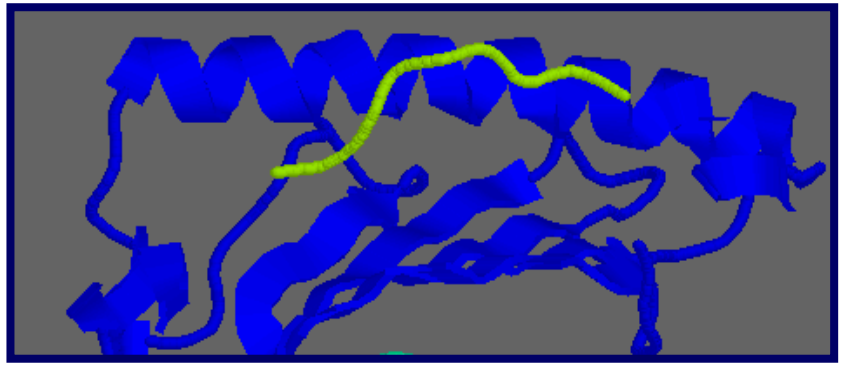


Slices through MHC class I molecules, when viewed from above reveal deep, well conserved pockets

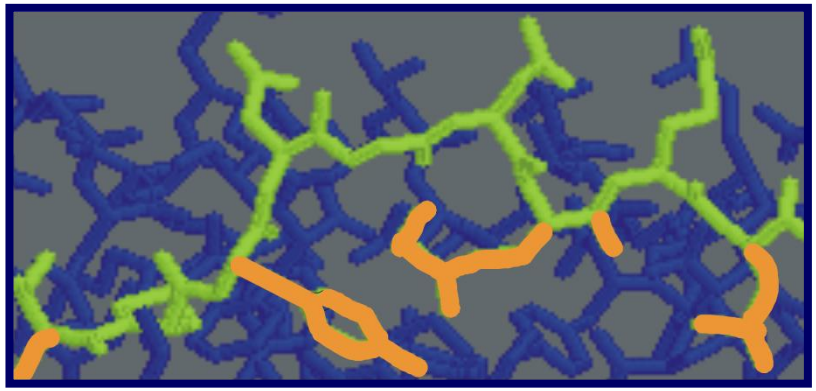


Anchor residues and T cell antigen receptor contact residues

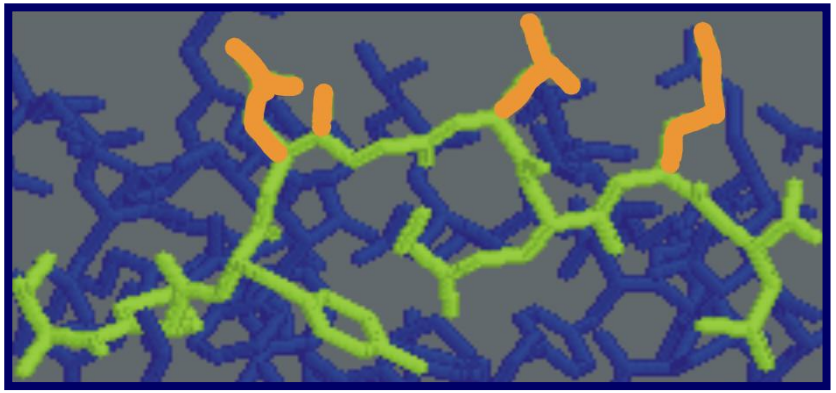
Cell surface



MHC class I
Sliced between
α-helicies to reveal
peptide

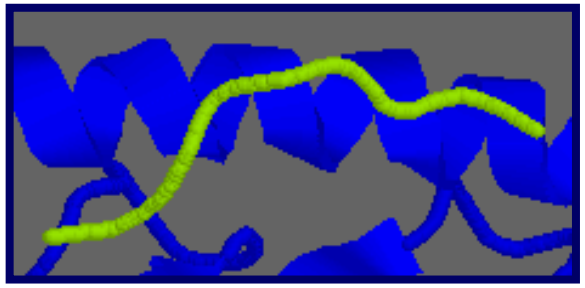


MHC anchor residue
side-chains point down

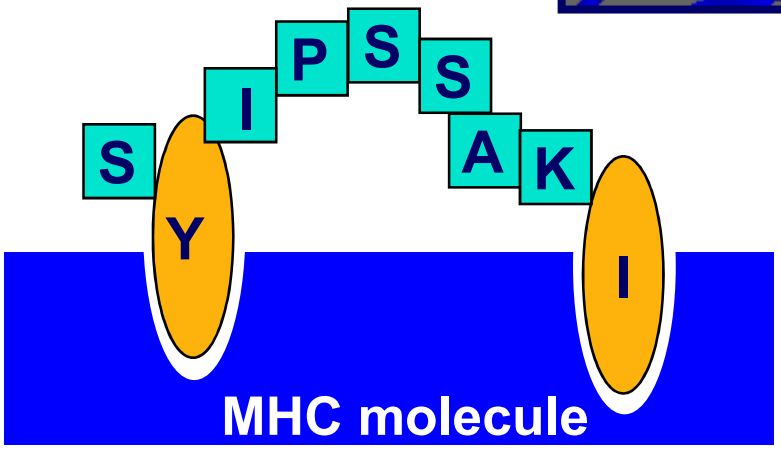


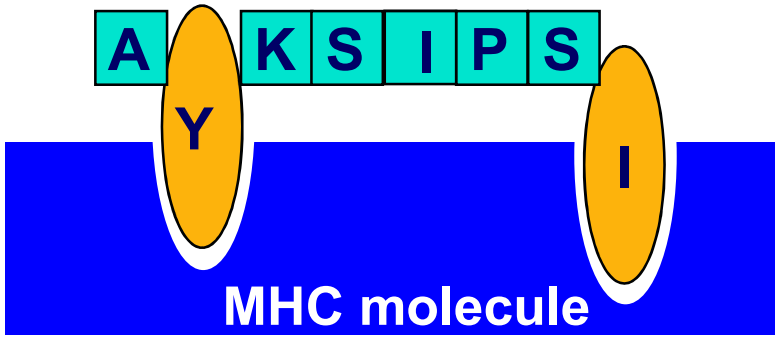
T cell antigen receptor contact
residue side-chains point up

MHC molecules can bind peptides of different length



Arched peptide

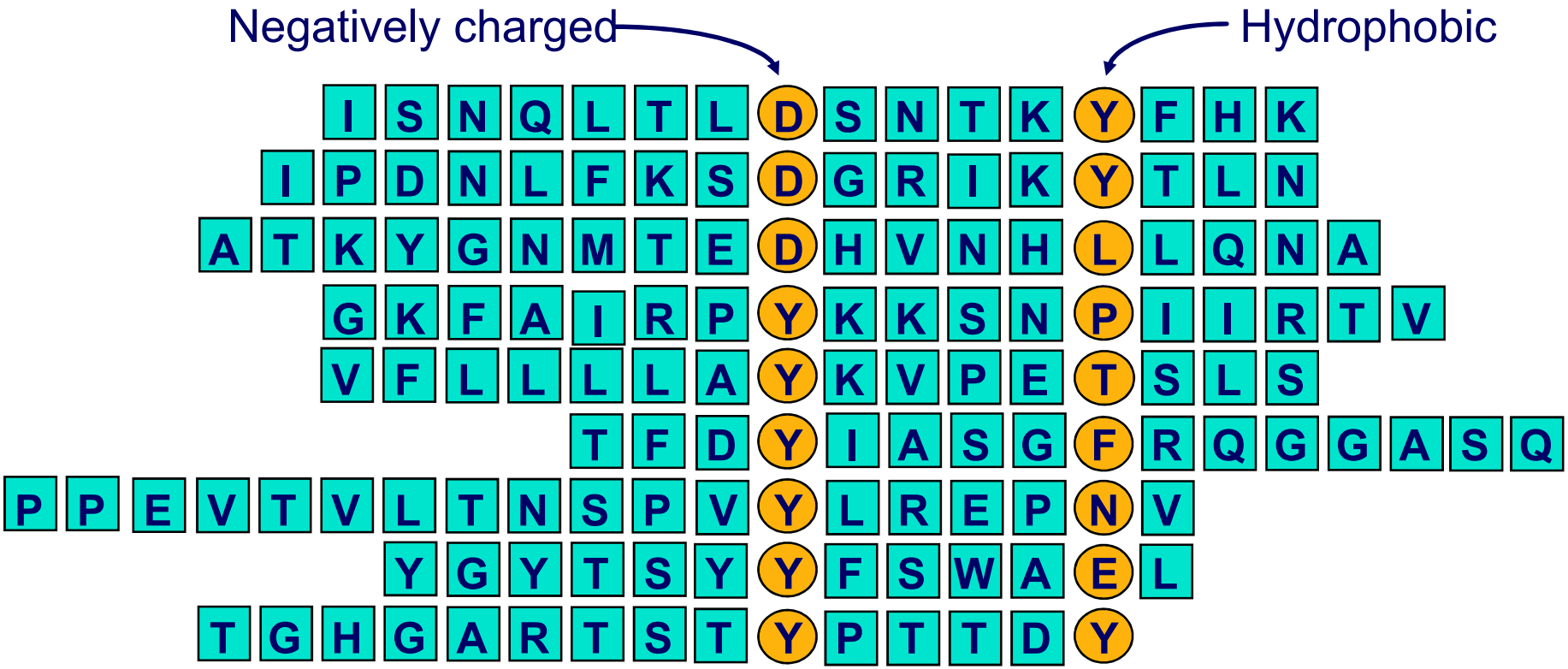




Complementary anchor residues & pockets provide the broad specificity of a particular type of MHC molecule for peptides

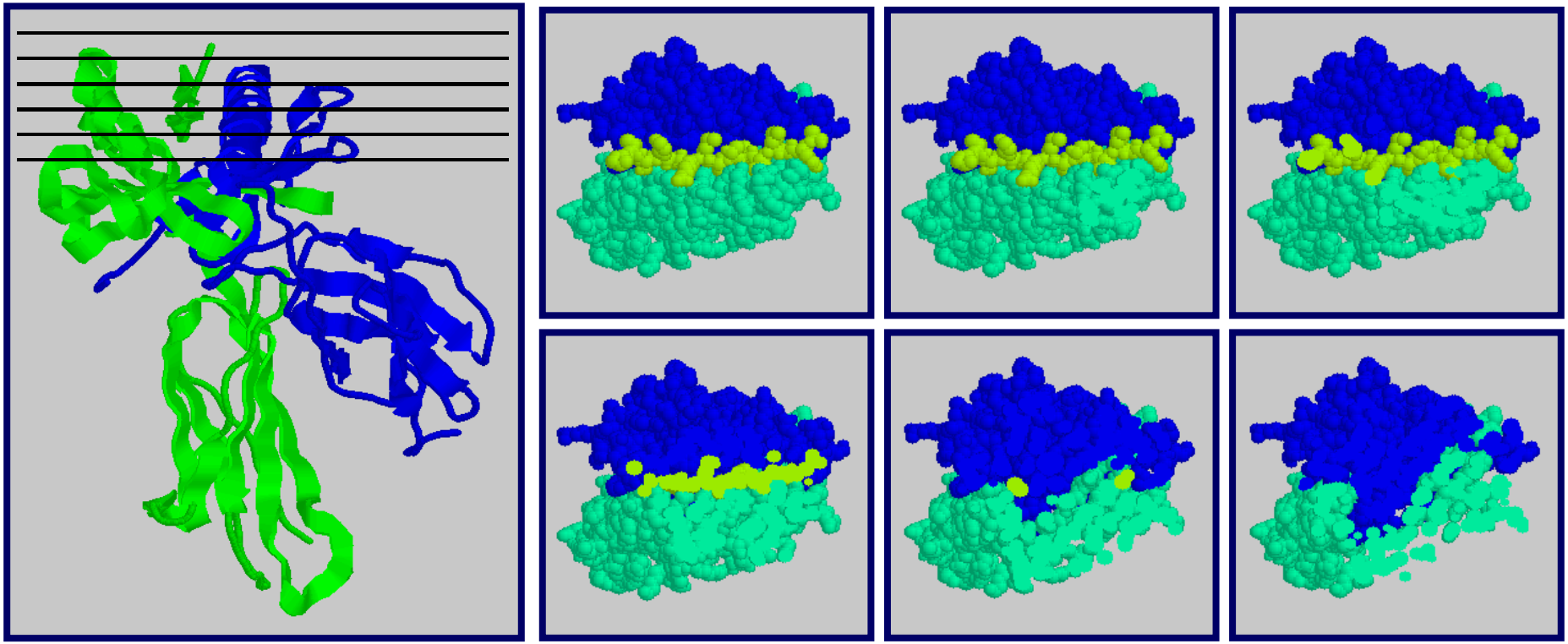
Peptide sequence between anchors can vary
Number of amino acids between anchors can vary

Peptide antigen binding to MHC class II molecules



- Anchor residues *are not* localised at the N and C termini
- Ends of the peptide are in extended conformation and may be trimmed
- Motifs are less clear than in class I-binding peptides
- Pockets are more permissive

Peptide binding pockets in MHC class II molecules



Slices through MHC class II molecules, when viewed from above reveal shallow, poorly conserved pockets compared with those in MHC class I molecules

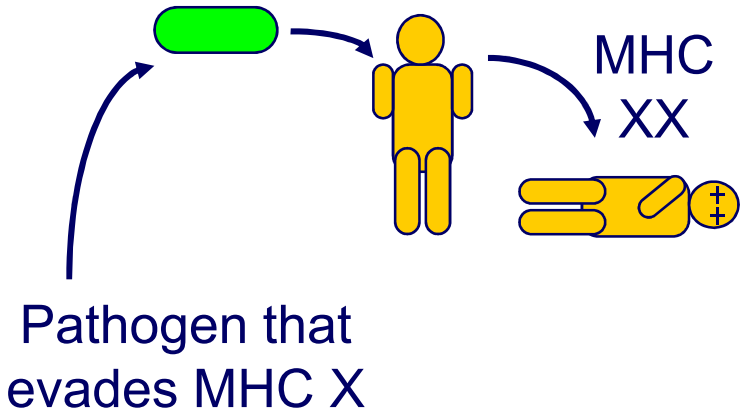
How can 6 invariant molecules have the capacity to bind to 1,000,000,000,000,000 different peptides with high affinity?

- Adopt a flexible “floppy” conformation until a peptide binds
- Fold around the peptide to increase stability of the complex
- Tether the peptide using a small number of anchor residues
- Allow different sequences between anchors and different lengths of peptide

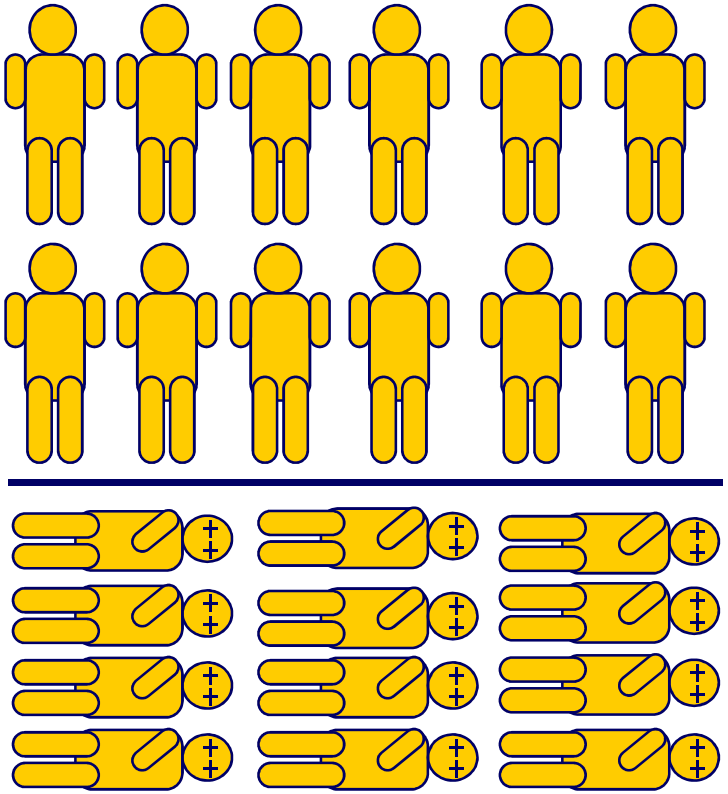
MHC molecules are targets for immune evasion by pathogens

- Without T cells there is no effective immune response
- Ag–specific T cells are activated by peptide/MHC complexes
- There is therefore strong selective pressure for pathogens to mutate genes encoding antigens so that they can evade the formation of peptide/MHC complexes
- The MHC has two strategies to prevent evasion by pathogens
  - More than one type of MHC molecule in each individual
  - Extensive differences in MHC molecules between individuals

Example: If MHC X was the only type of MHC molecule

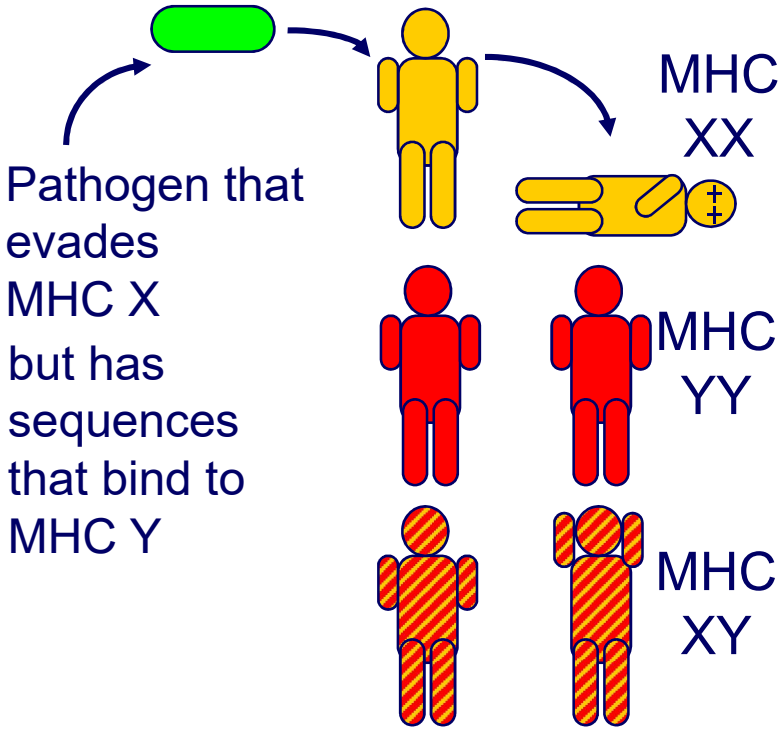


Survival of individual threatened

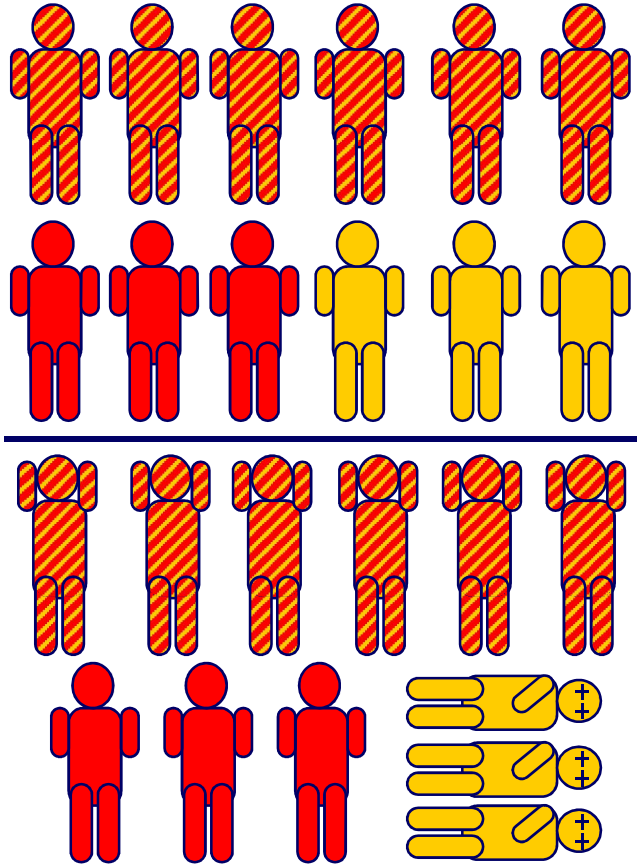


Population threatened with extinction

Example: If each individual could make two MHC molecules, MHC X and Y

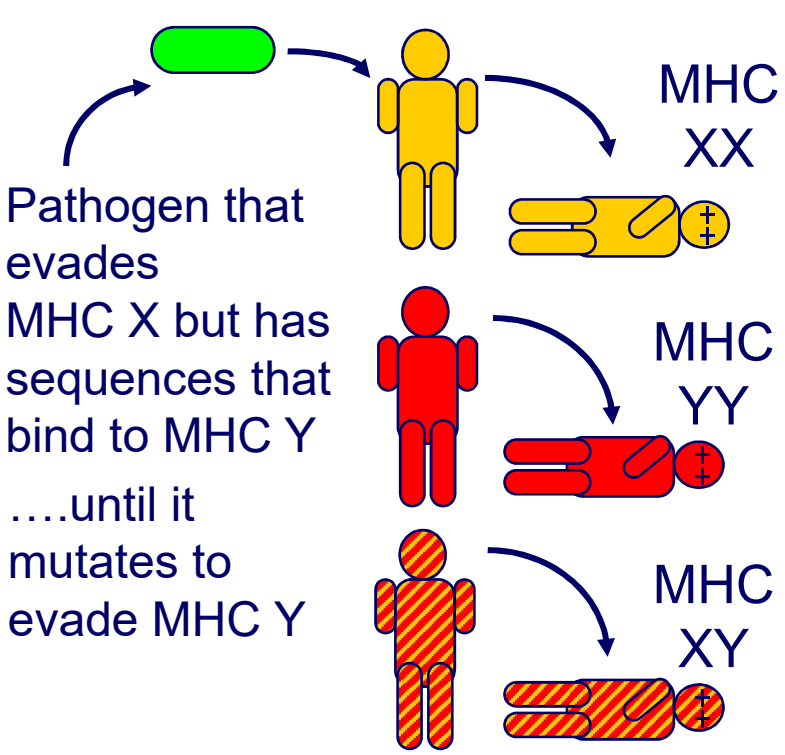


Impact on the individual depends upon genotype

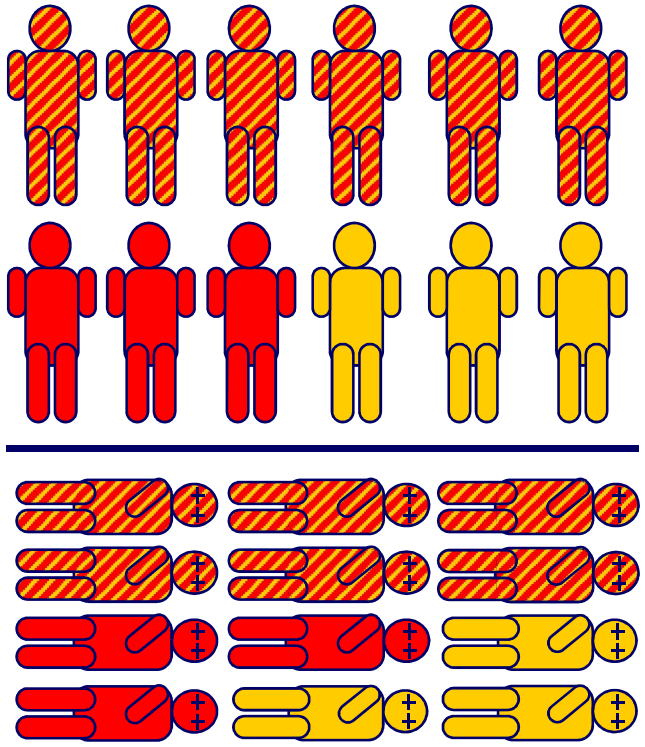


Population survives

Example: If each individual could make two MHC molecules, MHC X and Y……and the pathogen mutates



Survival of individual threatened



Population threatened with extinction

The number of types of MHC molecule can not be increased *ad infinitum*

Populations need to express variants of each type of MHC molecule

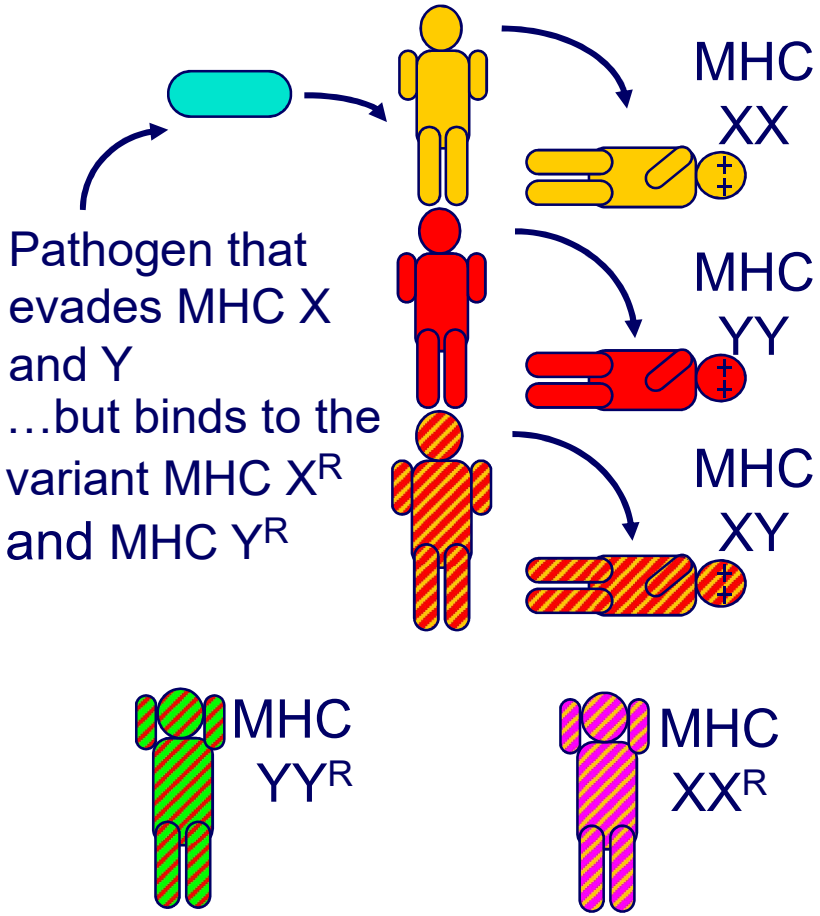
- Populations of microorganisms reproduce faster than humans
- Mutations that change MHC-binding antigens or MHC molecules can only be introduced to populations after reproduction
- The ability of microorganisms to mutate in order to evade MHC molecules will always outpace counter evasion measures that involve mutations in the MHC
- The number of types of MHC molecules are limited

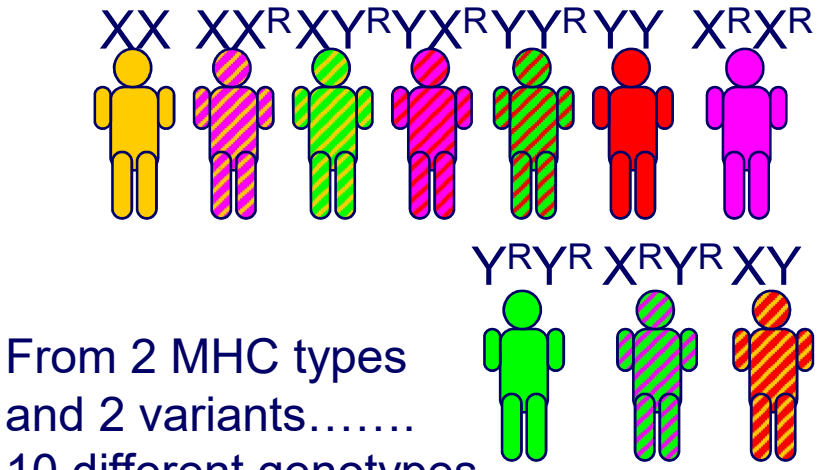
To counteract the superior flexibility of pathogens:

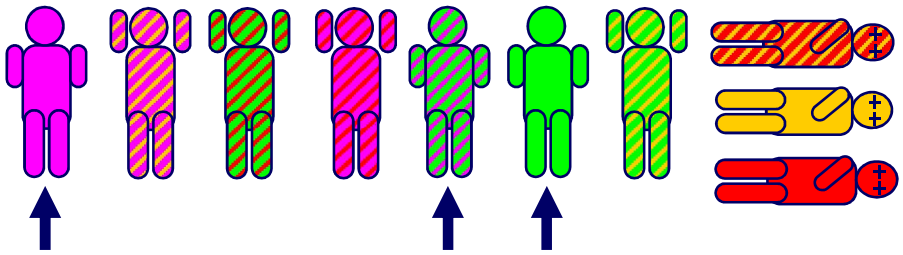
Human populations possess many variants of each type of MHC molecule

Variant MHC may not protect every individual from every pathogen. However, the existence of a large number of variants means that the population is prevented from extinction

Variant MHC molecules protect the population







Variants – alleles - of each type of MHC gene encode proteins that increase the resistance of the population from rapidly mutating or newly encountered pathogens without increasing the number of types of MHC molecule

Molecular basis of MHC types and variants

POLYGENISM

Several MHC class I and class II genes
encoding different types of MHC molecule
with a range of peptide-binding specificities.

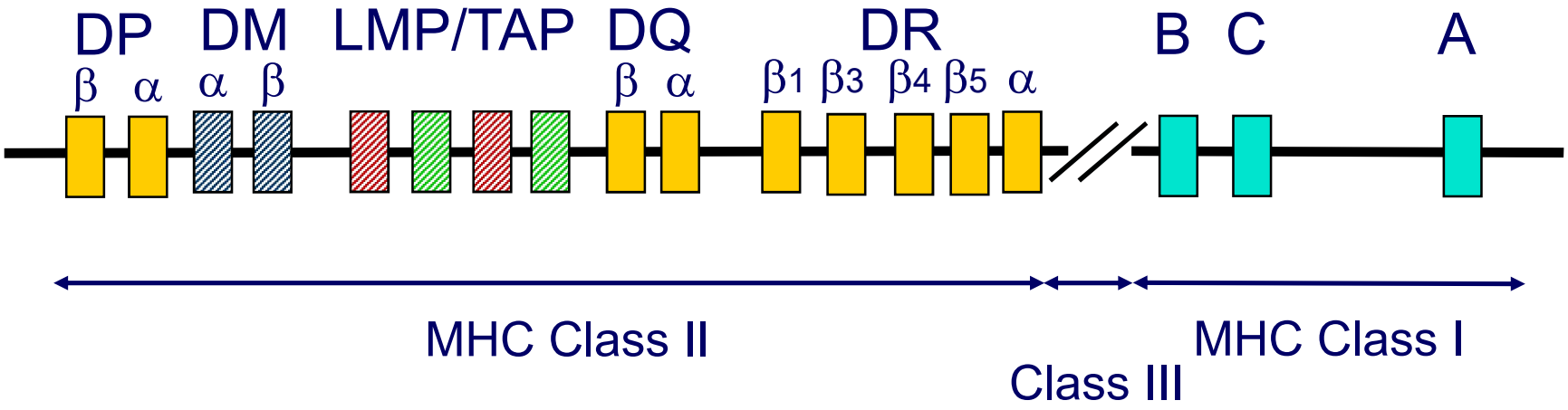
POLYMORPHISM

Variation >1% at a single genetic locus in a
population of individuals
MHC genes are the most polymorphic known

The type and variant MHC molecules do not vary in the lifetime of the individual

Diversity in MHC molecules exists at the population level
This sharply contrasts diversity in T and B cell antigen receptors which are in a constant state of flux within the individual.

Simplified map of the HLA region



Polygeny

CLASS I: 3 types HLA-A, HLA-B, HLA-C (sometimes called class Ia genes)

CLASS II: 3 types HLA-DP HLA-DQ HLA-DR.

3 extra DRβ genes in some individuals can allow 3 extra HLA-DR molecules

Maximum of 9 types of antigen presenting molecule allow interaction with a wide range of peptides.

Detailed map of the HLA region

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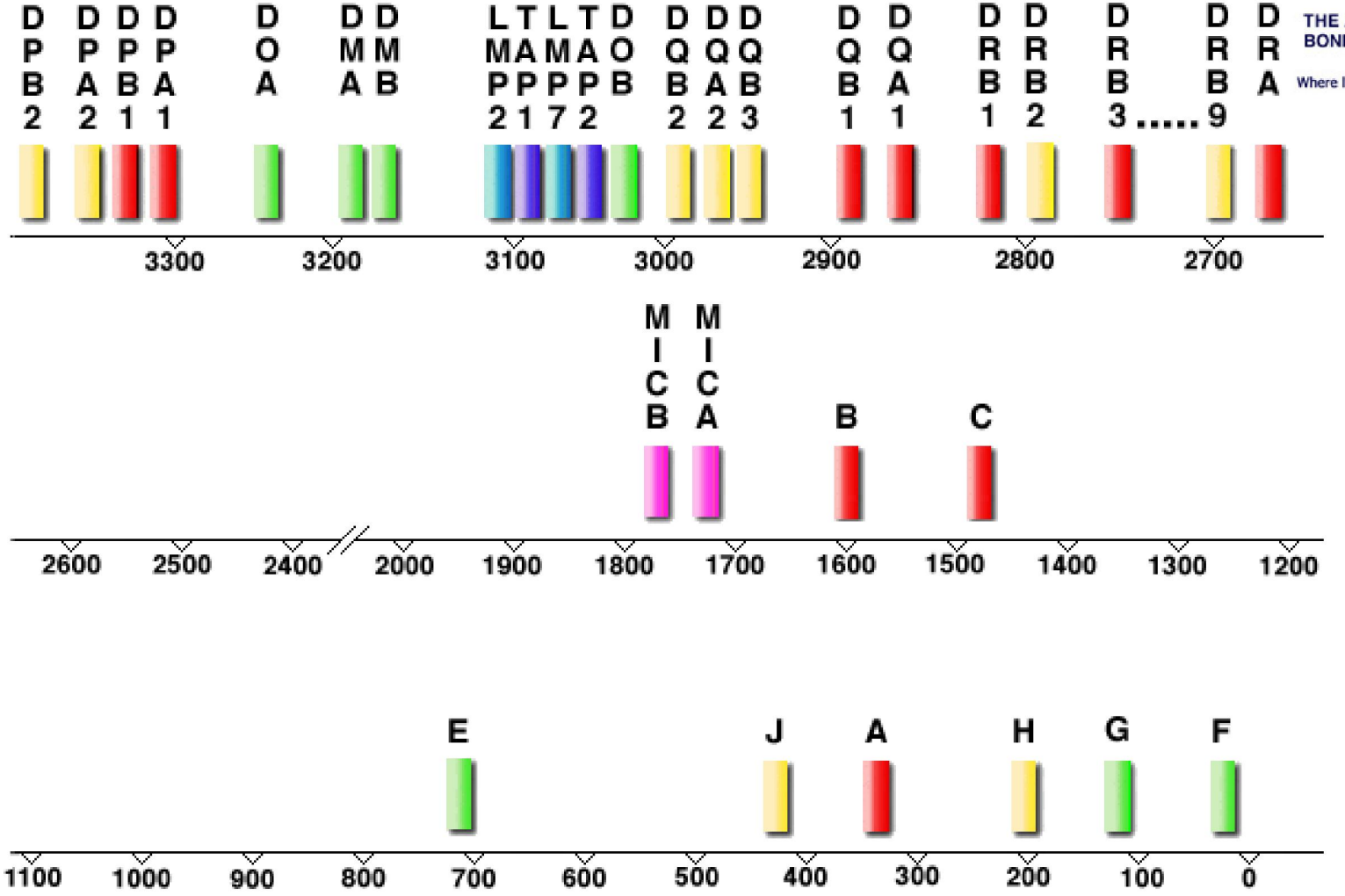
Where leukaemia meets its match



http://www.anthonynolan.org.uk/HIG/data.html September 2005 update

Map of the Human MHC from the Human Genome Project

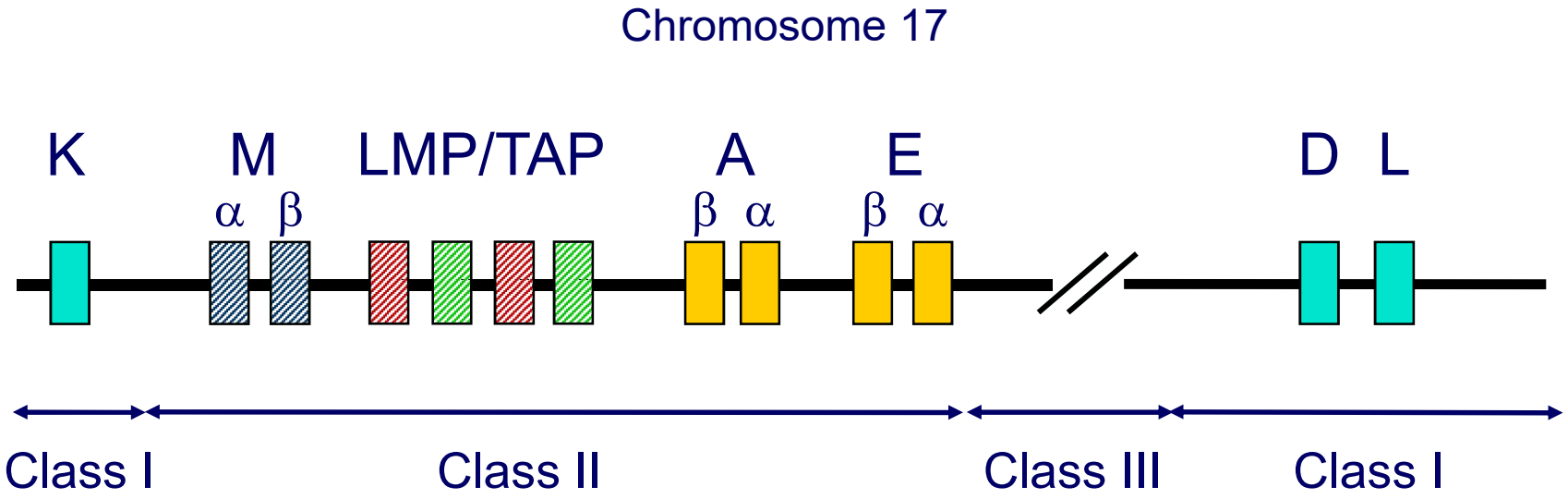
3,838,986 bp
224 genes
on chromosome 6

The Sanger Centre

The MHC sequencing consortium
Nature **401,** 1999

http://webace.sanger.ac.uk/cgi-bin/ace/pic/6ace?name=MHC&class=Map&click=400-1

Simplified map of the mouse MHC



Similar organisation to the human MHC except:

- one class I gene is translocated relative to human MHC
- 2 pairs of genes encoding class II molecules
- no alternative class II β chains

Other genes in the MHC

MHC Class 1b genes

Encoding MHC class I-like proteins that associate with β-2 microglobulin:

HLA-G binds to CD94, an NK-cell receptor. Inhibits NK attack of foetus/ tumours

HLA-E binds conserved leader peptides from HLA-A, B, C. Interacts with CD94

HLA-F function unknown

MHC Class II genes

Encoding several antigen processing genes:

HLA-DMα and β, proteasome components LMP-2 & 7, peptide transporters

TAP-1 & 2, HLA-DOα and DOβ

Many pseudogenes

MHC Class III genes

Encoding complement proteins C4A and C4B, C2 and FACTOR B

TUMOUR NECROSIS FACTORS α AND β

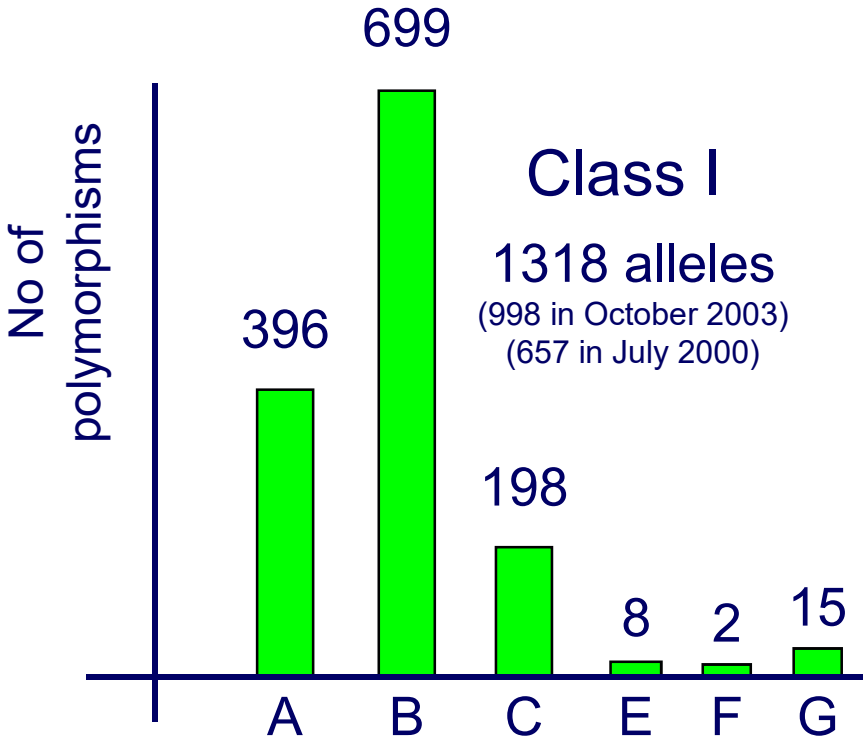
Immunologically irrelevant genes

Genes encoding 21-hydroxylase, RNA Helicase, Caesin kinase

Heat shock protein 70, Sialidase

Polymorphism in MHC Class I genes

Variation >1% at a single genetic locus in a population of individuals
In the human population, over 1300 MHC class I alleles have been identified - some are null alleles, synonyms or differ in regions outside the coding region

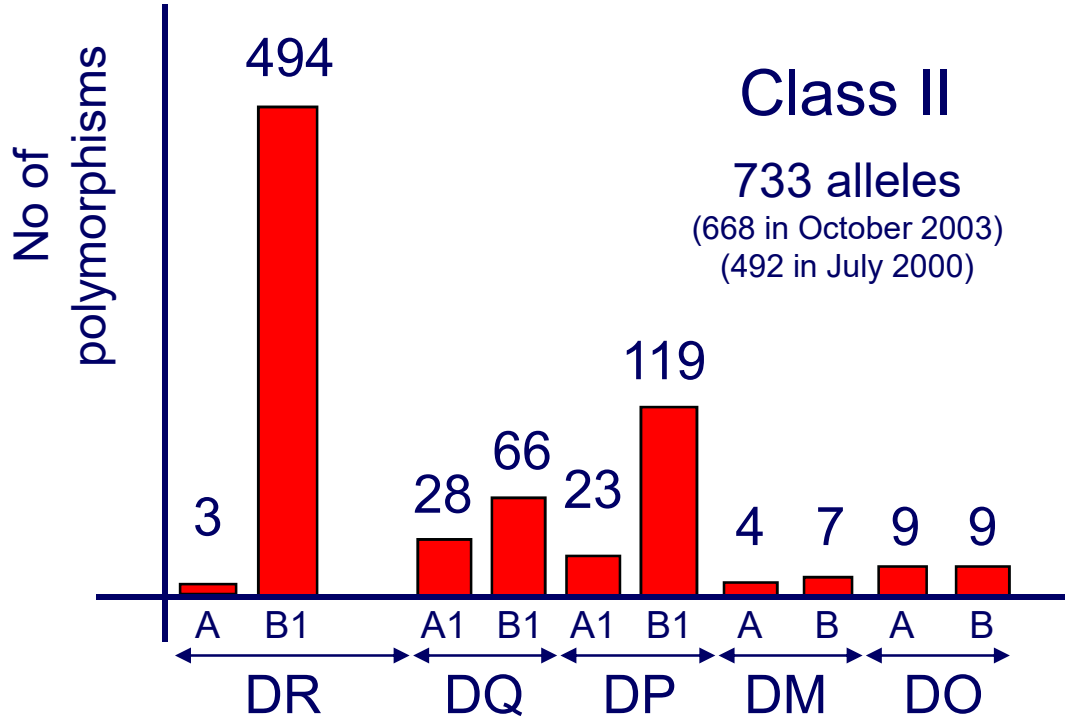


THE ANTHONY NOLAN BONE MARROW TRUST
Where leukaemia meets its match

Data from www.anthonynolan.org.uk/HIG/index.html September 2005

Polymorphism in MHC Class II genes

Over 700 human MHC class II alleles have been identified - some are null alleles, synonyms or differ in regions outside the coding region

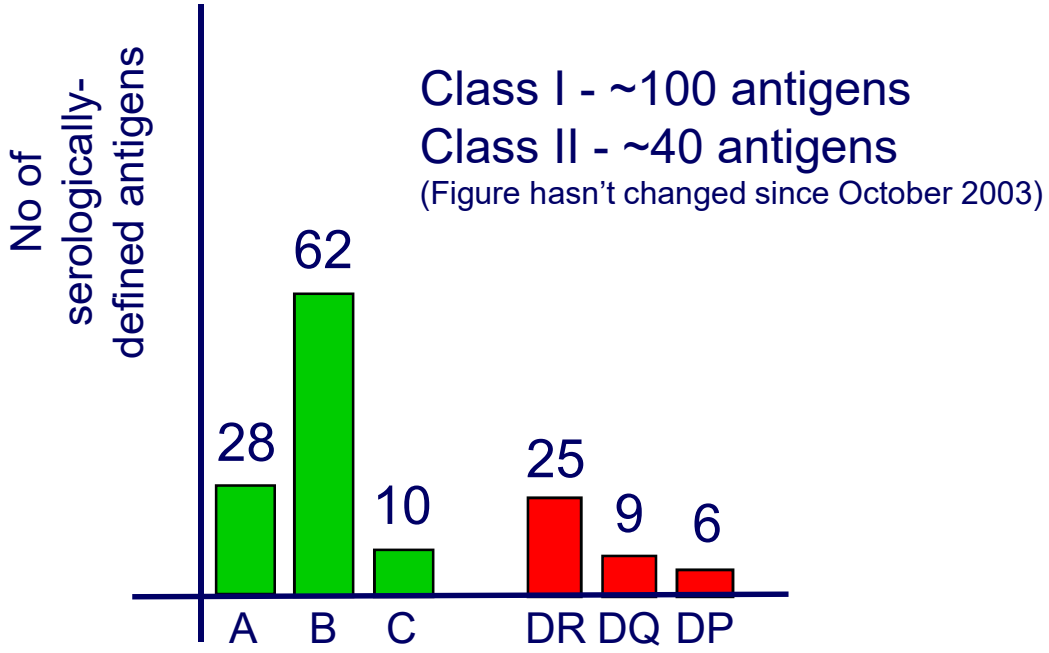


THE ANTHONY NOLAN BONE MARROW TRUST
Where leukaemia meets its match

Data from www.anthonynolan.org.uk/HIG/index.html September 2005

Diversity of MHC Class I and II antigens

Because so many MHC class I & II alleles are null, or contain synonymous mutations, the diversity of MHC molecules that can be identified by antibodies i.e. SEROLOGICALLY, is considerably fewer than that by DNA sequencing



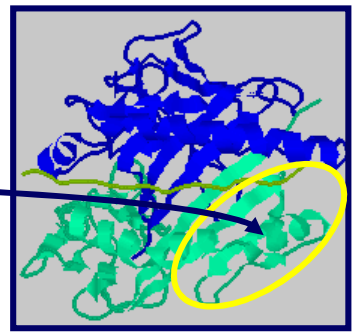
THE ANTHONY NOLAN BONE MARROW TRUST
Where leukaemia meets its match

Data from www.anthonynolan.org.uk/HIG/index.html September 2005

Most polymorphisms are point mutations

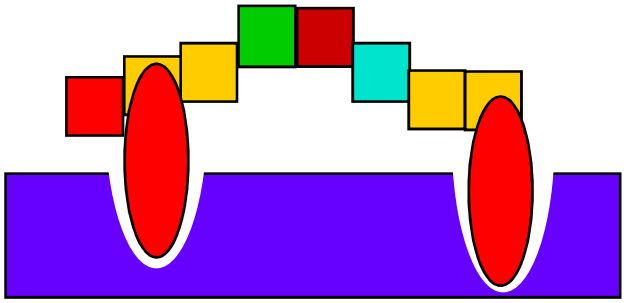
30 HLA-DPβ allele sequences between
Nucleotides 204 and 290
(amino acids 35-68)

Polymorphic nucleotides encode amino acids
associated with the peptide binding site

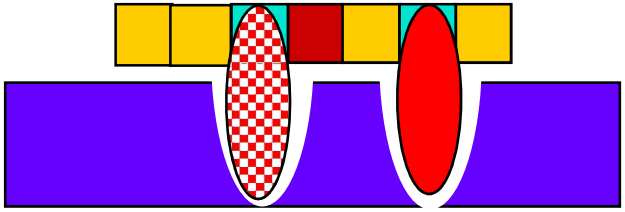


	Y-F	A-V							Silent												E-A	A-D	A-E								I-L			
DPB1*01011	TAC	GCG	CGC	TTC	GAC	AGC	GAC	GTG	GGG	GAG	TTC	CGG	GCG	GTG	ACG	GAG	CTG	GGG	CGG	CCT	GCT	GCG	GAG	TAC	TGG	AAC	AGC	CAG	AAG	GAC	ATC	CTG	GAG	GAG
DPB1*01012	---	---	---	---	---	---	---	---	--A	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
DPB1*02012	-T-	-T-	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	-A-	-A-	---	---	---	---	---	---	---	---	---	---	---	---
DPB1*02013	-T-	-T-	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	-AC	-A-	---	---	---	---	---	---	---	---	---	---	---	---
DPB1*0202	CT-	-T-	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	-AG	---	---	---	---	---	---	---	---	---	---	---	---	---
DPB1*0301	-T-	-T-	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	-A-	-A-	--C	---	---	---	---	---	---	---	C--	---	---	---
DPB1*0401	-T-	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
DPB1*0402	-T-	-T-	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	-A-	-A-	---	---	---	---	---	---	---	---	---	---	---	---
DPB1*0501	CT-	-T-	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	-AG	---	---	---	---	---	---	---	---	---	---	---	---	---
DPB1*0601	-T-	-T-	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	-A-	-A-	--C	---	---	---	---	---	---	---	C--	---	---	---
DPB1*0801	-T-	-T-	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	-A-	-A-	---	---	---	---	---	---	---	---	---	---	---	---
DPB1*0901	-T-	-T-	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	-A-	-A-	--C	---	---	---	---	---	---	---	---	---	---	---
DPB1*1001	-T-	-T-	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	-A-	-A-	---	---	---	---	---	---	---	---	---	---	---	---
DPB1*11011	---	---	---	---	---	---	---	---	--A	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	C--	---	---	---
DPB1*11012	---	---	---	---	---	---	---	---	--A	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	C--	---	---	---
DPB1*1301	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
DPB1*1401	-T-	-T-	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	-A-	-A-	--C	---	---	---	---	---	---	---	C--	---	---	---
DPB1*1501	---	---	---	---	---	---	---	---	--A	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	C--	---	---	---
DPB1*1601	-T-	-T-	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	-A-	-A-	---	---	---	---	---	---	---	---	---	---	---	---
DPB1*1701	-T-	-T-	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	-A-	-A-	--C	---	---	---	---	---	---	---	---	---	---	---
DPB1*1801	-T-	-T-	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	-A-	-A-	---	---	---	---	---	---	---	---	---	---	---	---
DPB1*1901	-T-	-T-	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	-AG	---	---	---	---	---	---	---	---	---	---	---	---	---
DPB1*20011	-T-	-T-	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	-A-	-A-	--C	---	---	---	---	---	---	---	C--	---	---	---
DPB1*20012	-T-	-T-	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	-A-	-A-	--C	---	---	---	---	---	---	---	C--	---	---	---
DPB1*2101	CT-	-T-	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	-AG	---	---	---	---	---	---	---	---	---	---	---	---	---
DPB1*2201	CT-	-T-	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	-AG	---	---	---	---	---	---	---	---	---	---	---	---	---
DPB1*2301	-T-	-T-	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
DPB1*2401	-T-	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	-AG	---	---	---	---	---	---	---	---	---	---	---	---	---
DPB1*2501	-T-	-T-	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	-A-	-A-	---	---	---	---	---	---	---	---	C--	---	---	---
DPB1*26011	---	---	---	---	---	---	---	---	--A	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
DPB1*26012	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---

Polymorphism in the MHC affects peptide antigen binding



MHC allele A



MHC allele B

Changes in the pockets, walls and floor of the peptide binding cleft alter peptide MHC interactions and determine which peptides bind.

S Y I P S A K I MHC allele A

R G Y V Y Q Q L MHC allele B

Products of different MHC alleles bind a different repertoire of peptides

Replacement substitutions occur at a higher frequency than silent substitution

Suggests that selective pressures may operate on MHC polymorphism

Evolution of pathogens to evade MHC-mediated antigen presentation

60% of individuals in south east China & Papua New Guinea express HLA-A11

HLA-A11 binds an important peptide of Epstein Barr Virus
Many EBV isolates from these areas have mutated this peptide so that it can not bind to HLA-A11 MHC molecules

Evolution of the MHC to eliminate pathogens

In west Africa where malaria is endemic HLA-B53 is commonly associated with recovery from a potentially lethal form of malaria

How diverse are MHC molecules in the population?

IF

- each individual had 6 types of MHC
- the alleles of each MHC type were randomly distributed in the population
- any of the 1,200 alleles could be present with any other allele

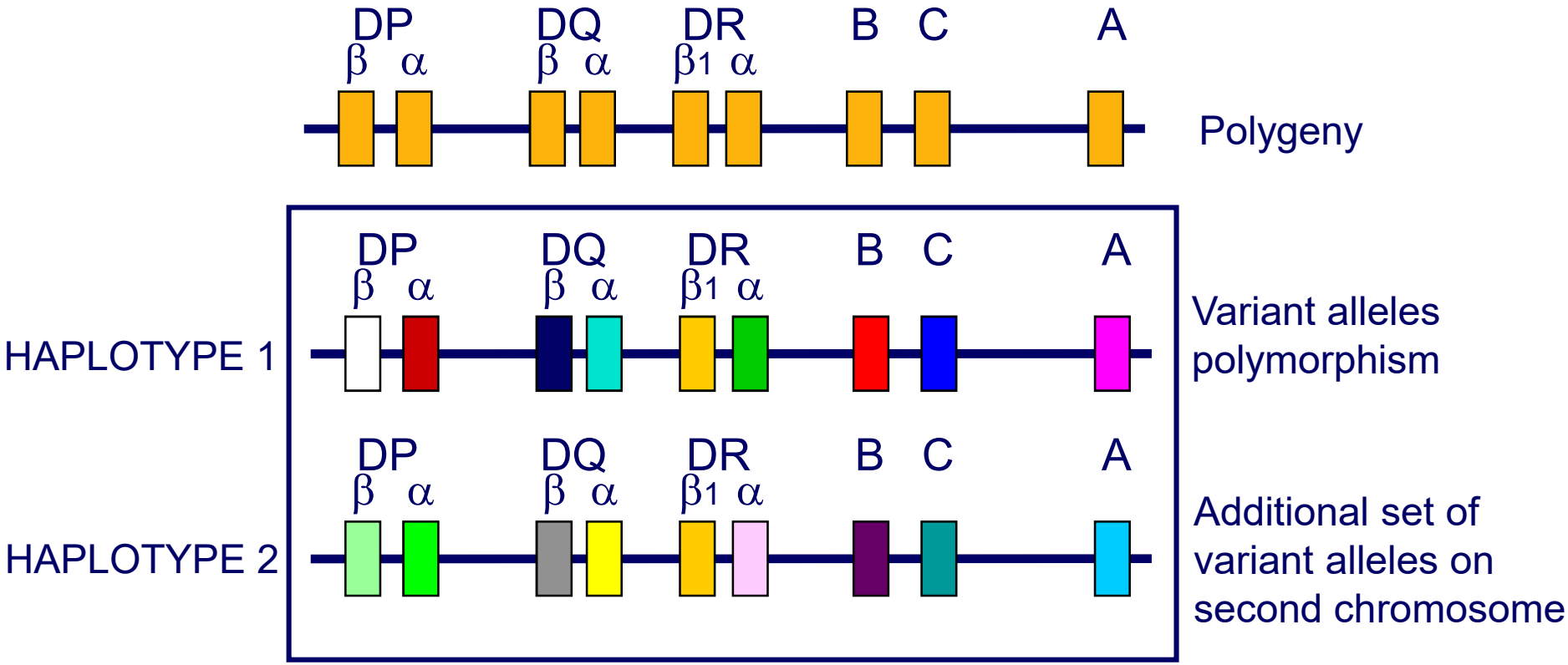
~6 x 10^{15} unique combinations

In reality MHC alleles are NOT randomly distributed in the population

Alleles segregate with lineage and race

	Frequency (%)		
Group of alleles	CAU	AFR	ASI
HLA-A1	15.18	5.72	4.48
HLA- A2	28.65	18.88	24.63
HLA- A3	13.38	8.44	2.64
HLA- A28	4.46	9.92	1.76
HLA- A36	0.02	1.88	0.01

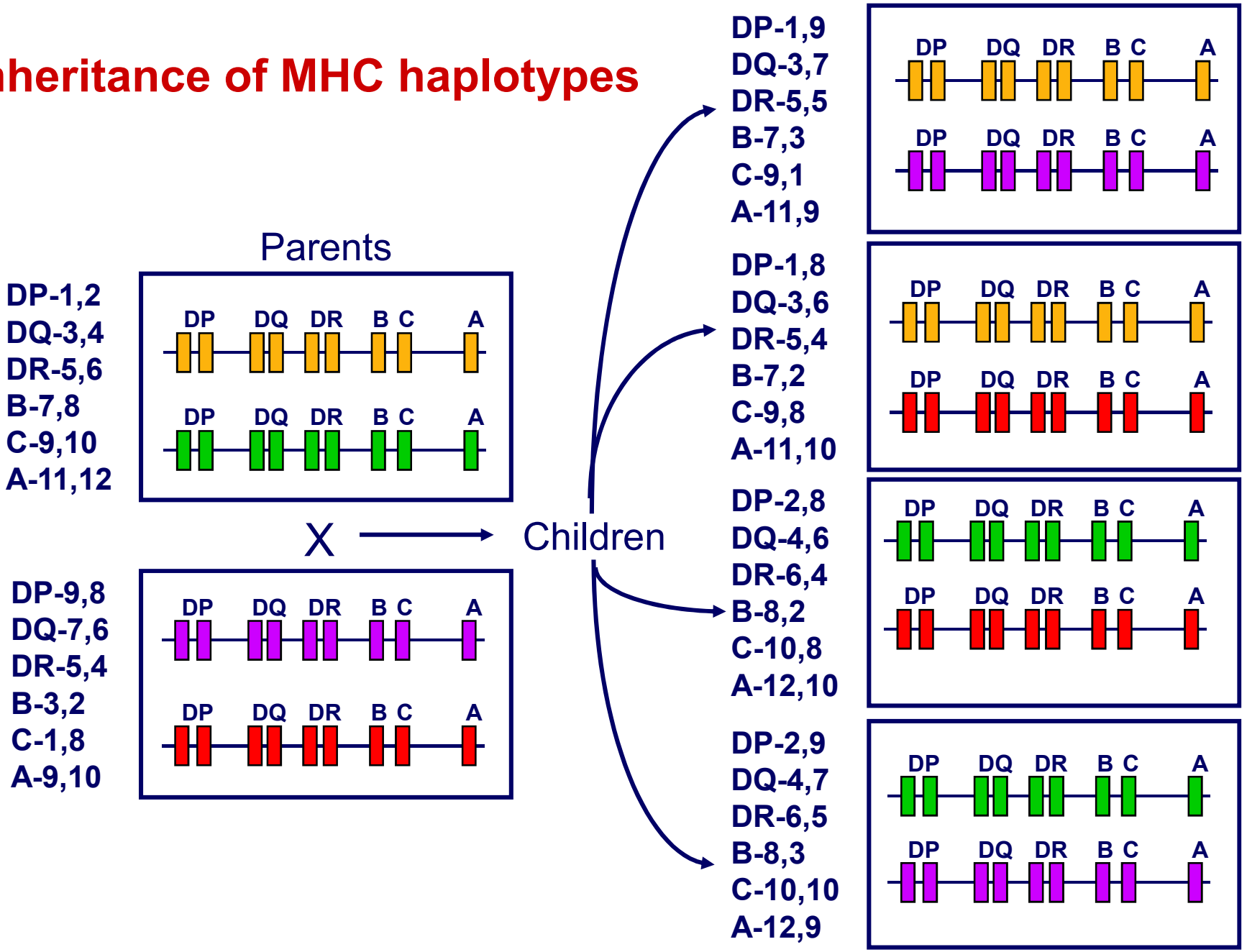
Diversity of MHC molecules in the individual



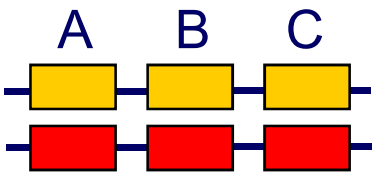
MHC molecules are CODOMINANTLY expressed
Two of each of the six types of MHC molecule are expressed

Genes in the MHC are tightly LINKED and usually inherited in a unit called an MHC HAPLOTYPE

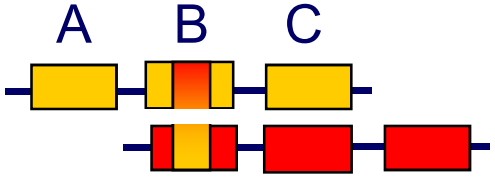
Inheritance of MHC haplotypes



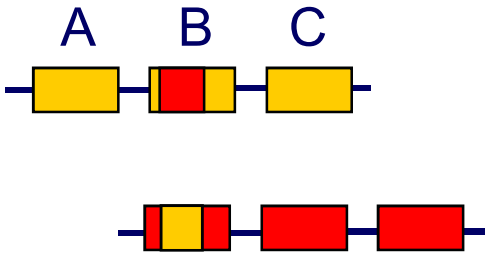
Errors in the inheritance of haplotypes generate polymorphism in the MHC by gene conversion and recombination



Multiple distinct but closely related MHC genes

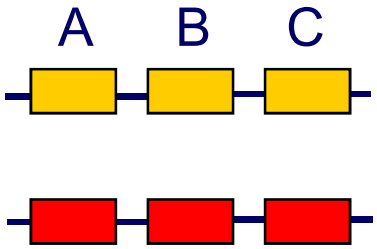


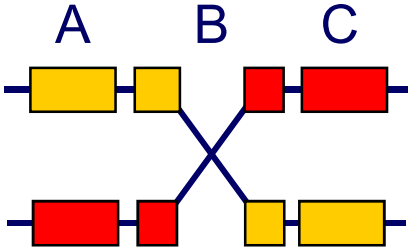
During meiosis chromosomes misalign

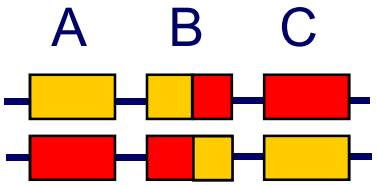


Chromosomes separate after meiosis DNA is exchanged between haplotypes

GENE CONVERSION







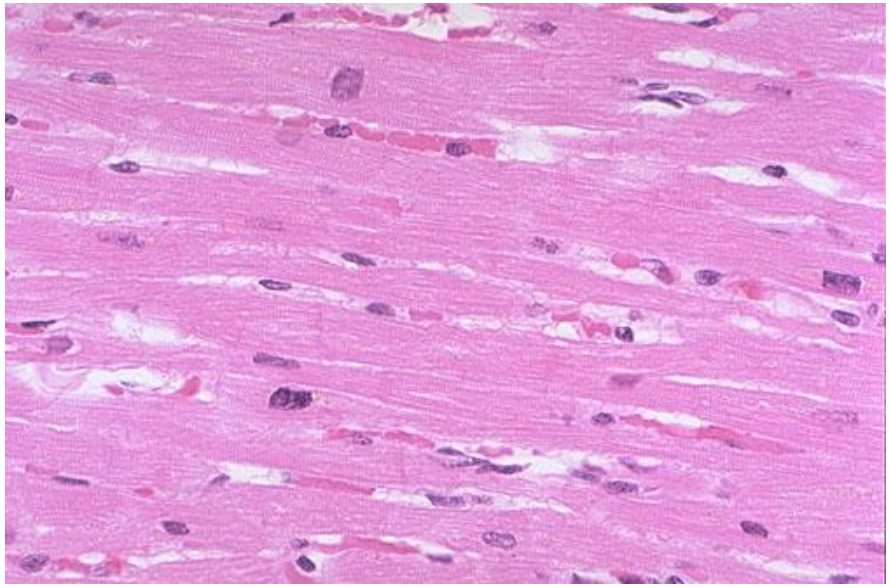
RECOMBINATION between haplotypes

In both mechanisms the **type** of MHC molecule remains the same, but a new allelic variant may be generated

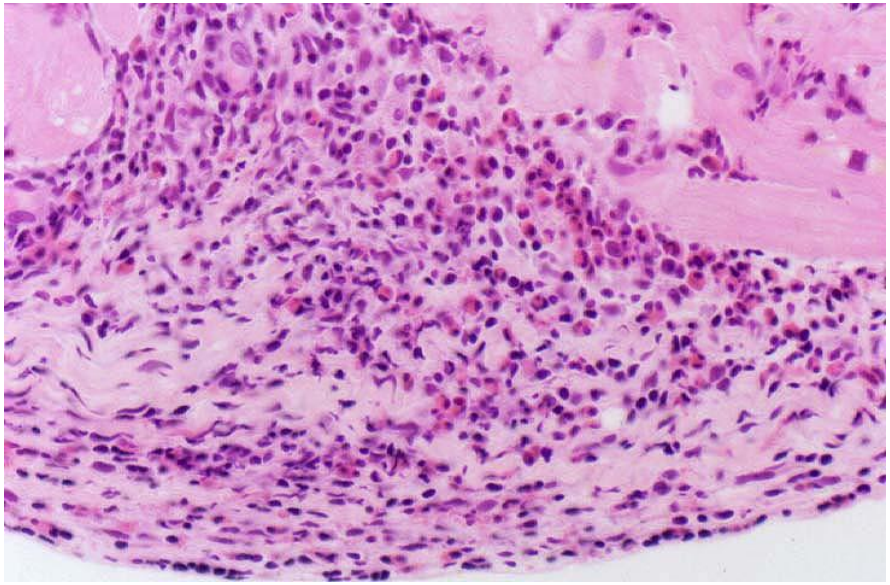
A clinically relevant application of MHC genetics: Matching of transplant donors and recipients

The biology, diversity and complexity of the MHC locus and its pattern of inheritance explains:

- The need to match the MHC of the recipient of a graft with the donor
- The difficulties faced in matching unrelated donors with recipients
- The ~20% chance of finding a match in siblings

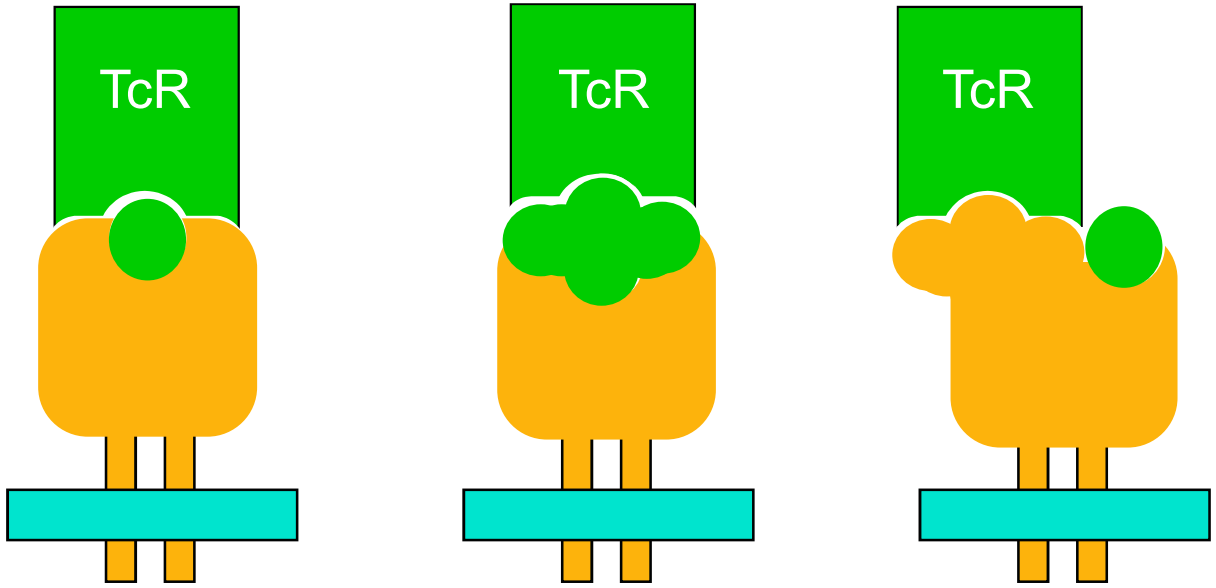


http://www-medlib.med.utah.edu/WebPath/jpeg5/CV171



http://tpis.upmc.edu/tpis/images/C00005c

Molecular basis of transplant rejection



MHC A

Normal peptide recognition

MHC B

Indirect peptide recognition

MHC C

Direct peptide recognition

Summary

- Transplant rejection occurs as a result of anti MHC immune responses
- The MHC was discovered using inbred strains of mice
- T cells recognise antigens in the context of MHC molecules
- MHC molecules bind to peptide antigens
- The structure of MHC molecules is directly related to their function in antigen presentation
- Polymorphism and polygenism in the MHC protects the population from pathogens evading the immune system