Le basi immunologiche dell'alloreattività nel trapianto di organi solidi

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Punti della presentazione

- Fattori coinvolti nel «rigetto»: non solo immunità specifica
- I recettori della risposta allogenica (anticorpi, T cell receptor e sistema maggiore di istocompatibilità)
- Presentazione antigenica
- Diversità
- Sbilanciamento della risposta immunitaria verso la alloreattività

Factors involved in transplant «rejection»

Immunological factors

- Cells (natural and specific immunity)
- Antibodies
- Complement

Non immunological factors

- coagulation
- ischemia/riperfusion
- Infection
- [Hypertension]
- [Dyslipidemia]

Types of Immunity

	INNATE	**ACQUIRED** (adaptive, specific)
Specificity	Against microbes	Against any type of antigen
Diversity	Limited	Very large
Memory	No	Yes
Reactivity against self	No	No
Physical and chemical barriers	Skin, mucosa	Lympocytes and antibodies
Blood proteins	Complement	Antibodies
Cells involved	МΦ, NK	Lymphocytes

The innate immunity sets the scene for rejection

Ligands from damaged cells (DAMPS)

[damage-associated molecular patterns]

- Hsp70
- Polysaccharide fragments of heparan sulphate
- HMGB1/Rage
- Fibrinogen

Pattern recognition receptors (PRRs)

- Cell surface – TLRs, CLRs and CD14
- Intracellular – NOD, NLRs, RLRs, inflammsomes
- Secreted - MBL; serum amyloid

Innate immune system

Inflammation
Complement activation
Leukocyte recruitment
Clearance and killing
Adaptive Immunity

Activation of the innate immune system in the early phase posttransplant is largely, a non-specific response to tissue damage and will occur, irrespective of whether there is a genetic difference between the donor and recipient

[Wood et al, Transplant 2012]

Risposta allogenica

È la capacità di riconoscere e rigettare cellule, tessuti o organi tra individui della stessa specie [dalle spugne all'uomo]

La risposta immunitaria è diretta contro il "non-self" espresso dall'organo trapiantato

[Lakkis et al, 2014]

I tre recettori coinvolti nella risposta allogenica specifica

- Immunoglobuline
- Recettore della cellula T (TCR)
- Molecole del Sistema Maggiore di Istocompatibilita (MHC)

[Lakkis et al, 2014]

I tre recettori coinvolti nella risposta allogenica specifica

	Antibody	TCR (αβ)	MHC Molecule
Antigen binding site	3 CDR in VL+ 3 CDR in VH	3 CDR in Vα+ 3 CDR in Vβ	Peptide cleft (α1+α2 [cl I] (α1+β1[cl II]
Nature of Ag bound	Macromolecules + chemicals	Peptide-MHC complexes	peptides
Ag Determinant recognized	Linear + conformational	Linear determinants of peptide	Linear determinants of peptide
Diversity	~10^{11}	~10^{16}	>12.000

Risposta allogenica specifica

La risposta immunitaria è diretta contro il “non-self” espresso dall’organo trapiantato

Sistema Maggiore di Istocompatibilità (MHC o HLA nell’uomo)

[esistono altri sistemi di istocompatibilità detti minori]

[Lakkis et al, 2014]

Ruolo centrale del sistema HLA tra i sistemi alloantigenici

- Altamente polimorfico (il più polimorfico!)
- Espressione ubiquitaria
- Capace di indurre una forte risposta immunologica

[Lakkis et al, 2014]

The dual role of the MHC molecules

1. They are the principal target of the immune response directed against the "non-self" expressed by transplanted organs
2. They enable antigen presentation to the T cells of the immune system

MHC as the principal target of the immune response: expression of MHC molecules

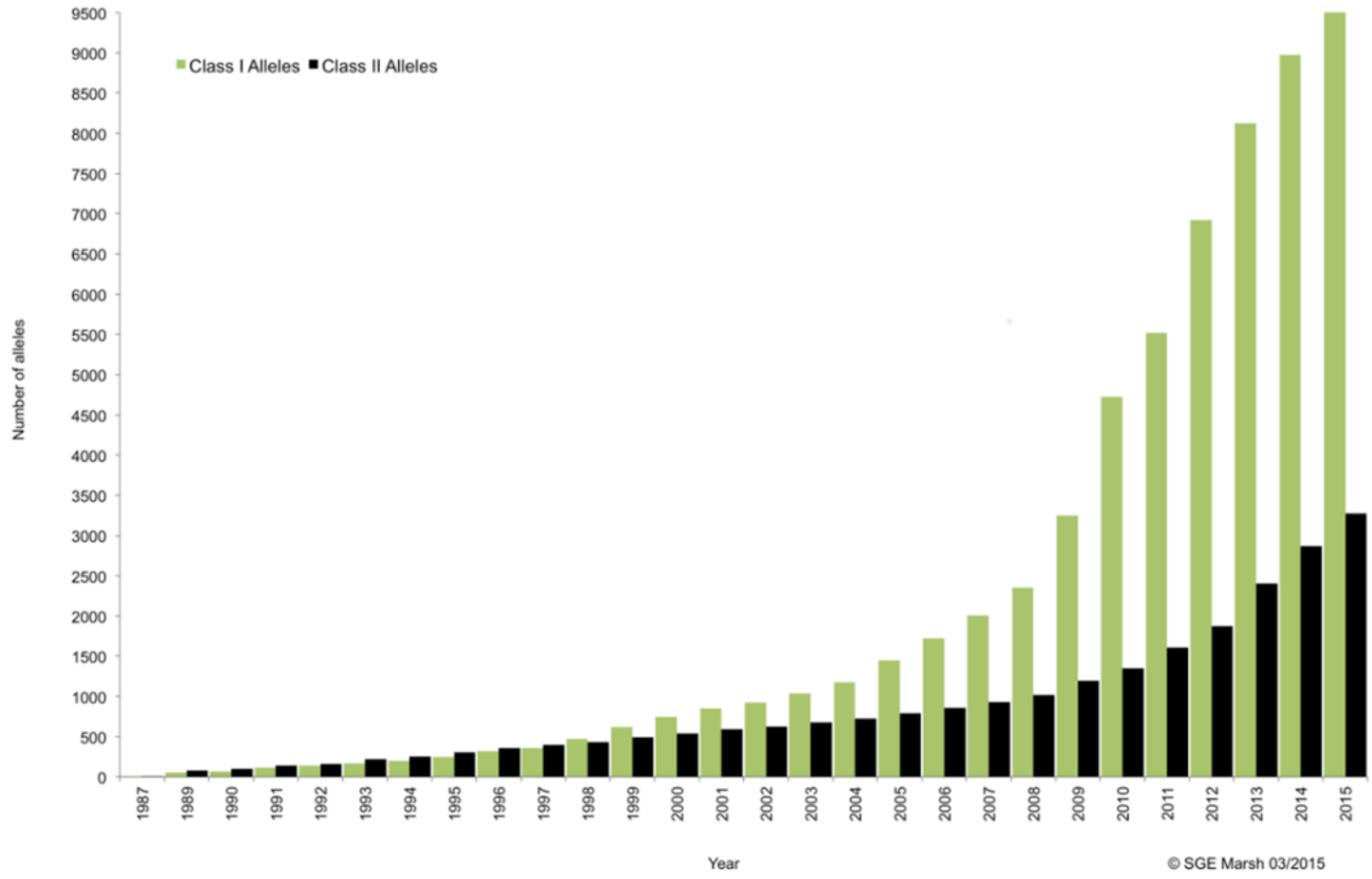
MHC I Ag: costitutively expressed on all nucleated cells

MHC II Ag: normally expressed on

- Dendritic cells ("APC")
- B lymphocytes
- Macrophages
- a few others such as endothelial cells [!]

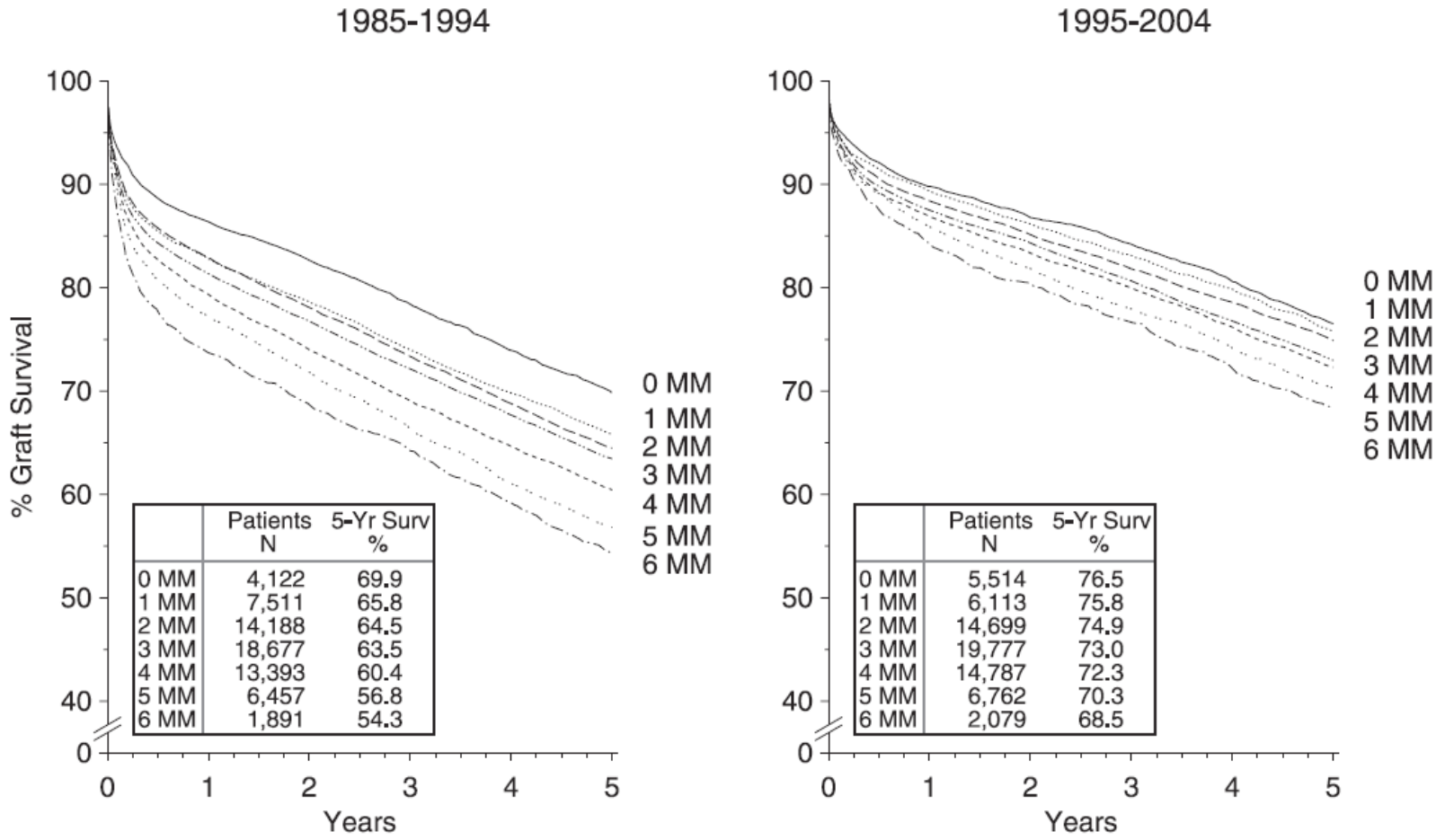
MHC expression may be enhanced by some cytokines (IFNɣ) produced during the innate or specific immune response

MHC as the principal target of the immune response: MHC I and II genes are the most polymorphic



[www.hla.alleles.org, 2015]

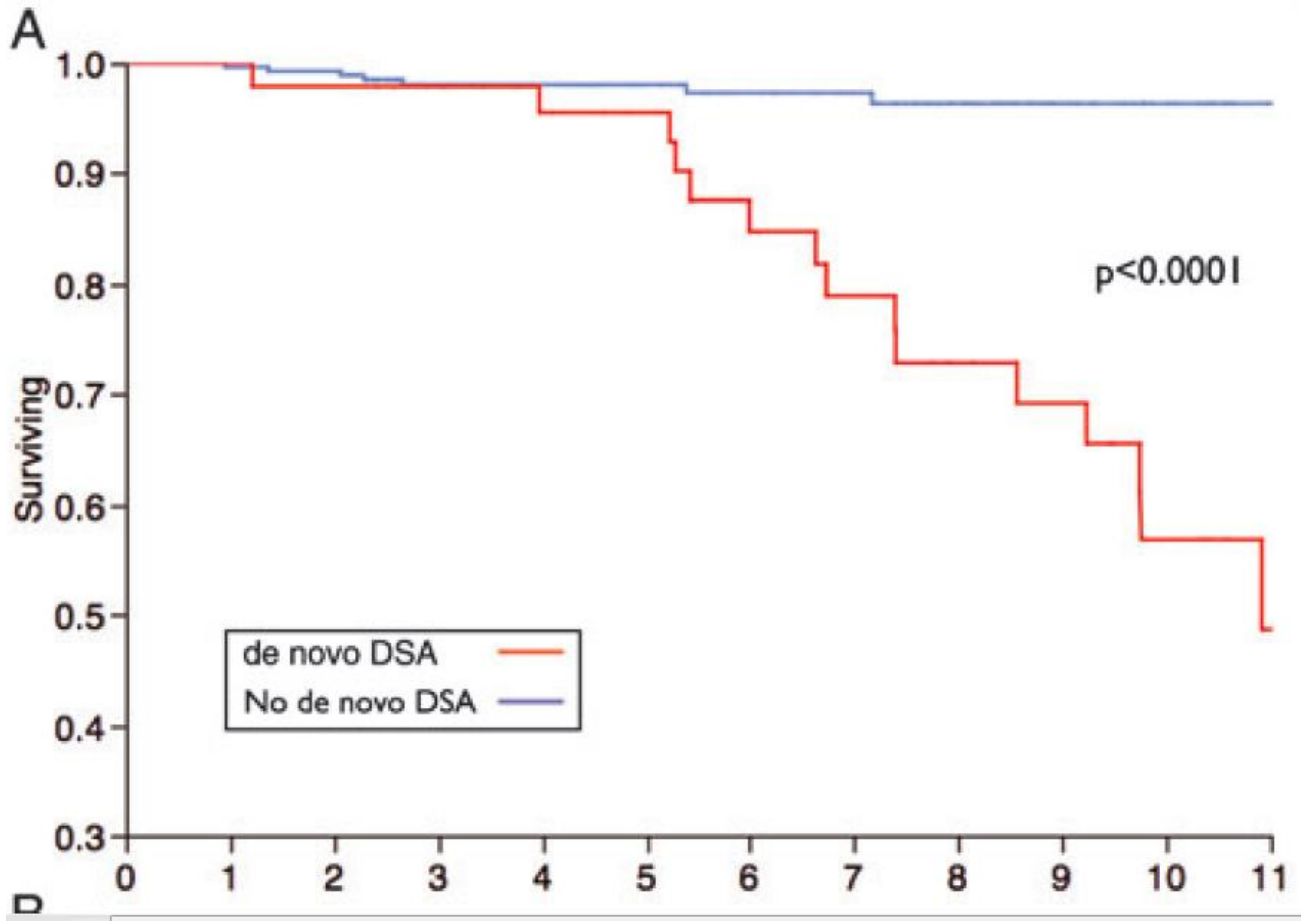
HLA as a principal target of the immune response: donor-recipient compatibility and graft survival



Better HLA matches still result in better survival

[Opeltz et al, Transplant 2007]

HLA as a principal target of the immune response: donor-specific antibodies and graft survival



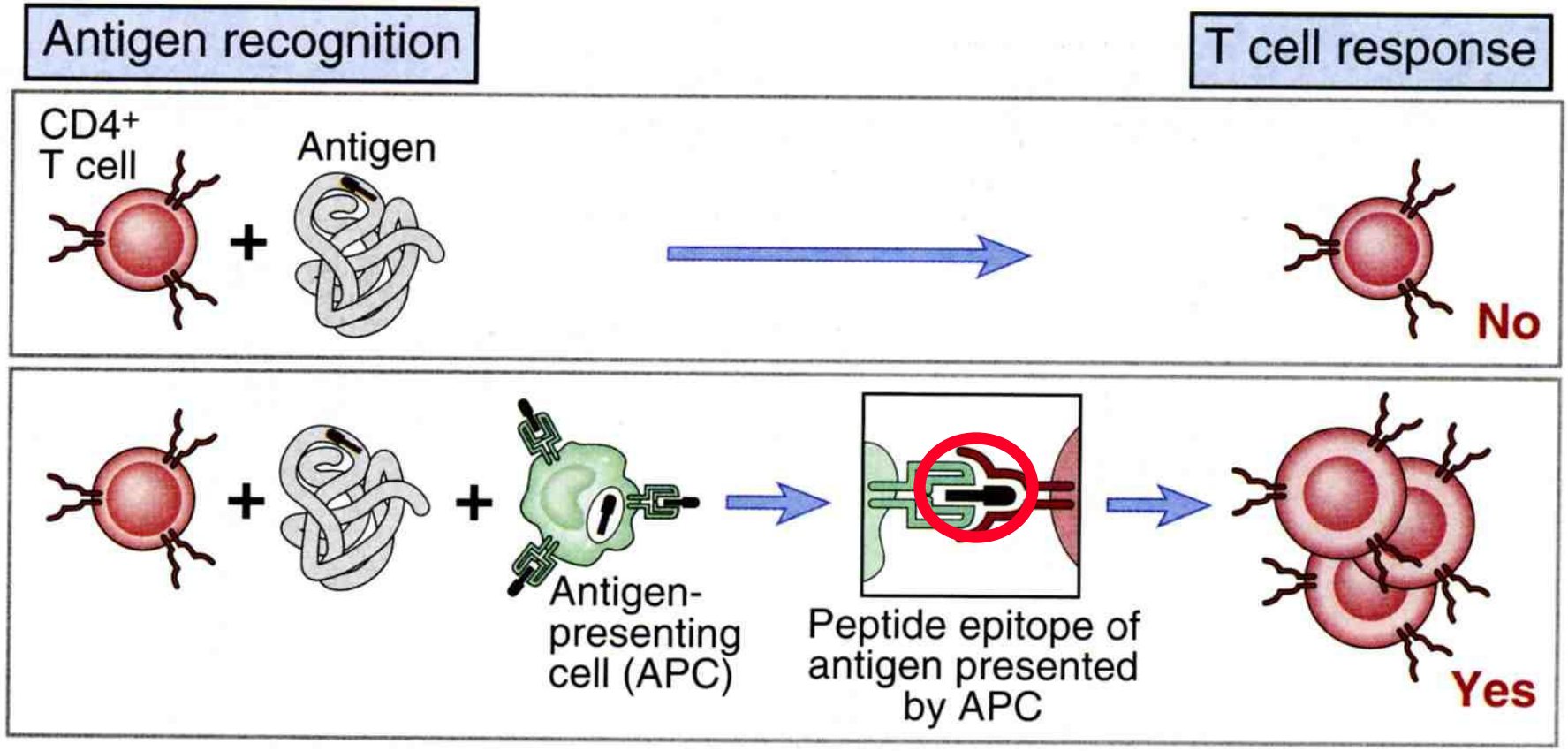
The absence of de novo anti-HLA antibodies results in better survival

[Wiebe et al, AJT 2012]

Antigen recognition by B and T cells

- B cells (antibodies) recognise Ag directly (as a microbial Ag, soluble Ag or on other cells)
- T cells can only recognize Ag if these are presented by other cells in the context of molecules belonging to the Major Histocompatibility Complex (or MHC antigens)

HLA molecules enable antigen presentation to the T cells of the immune system



[Abbas et al, 2011]

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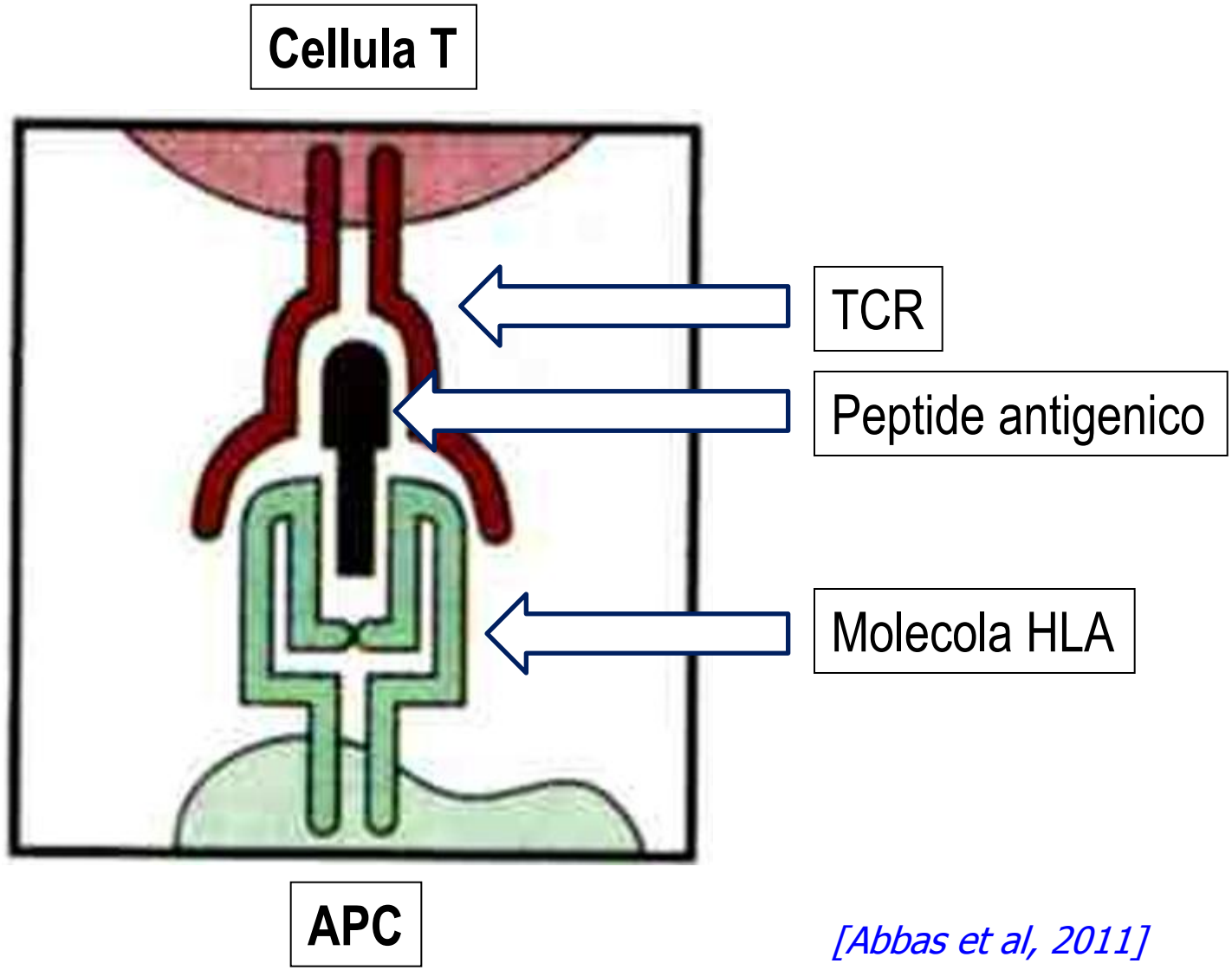
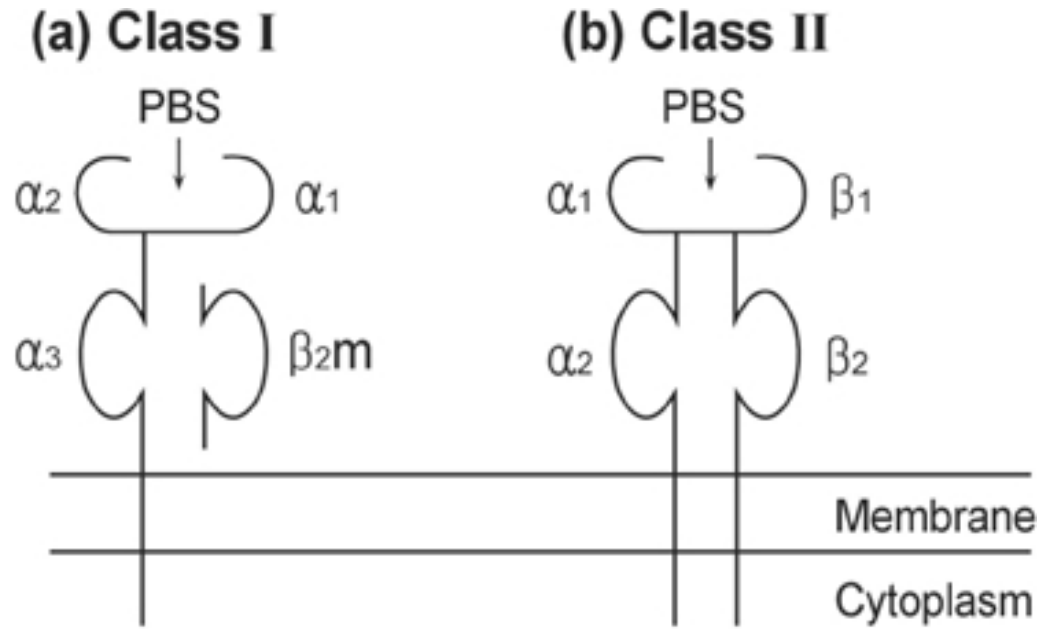


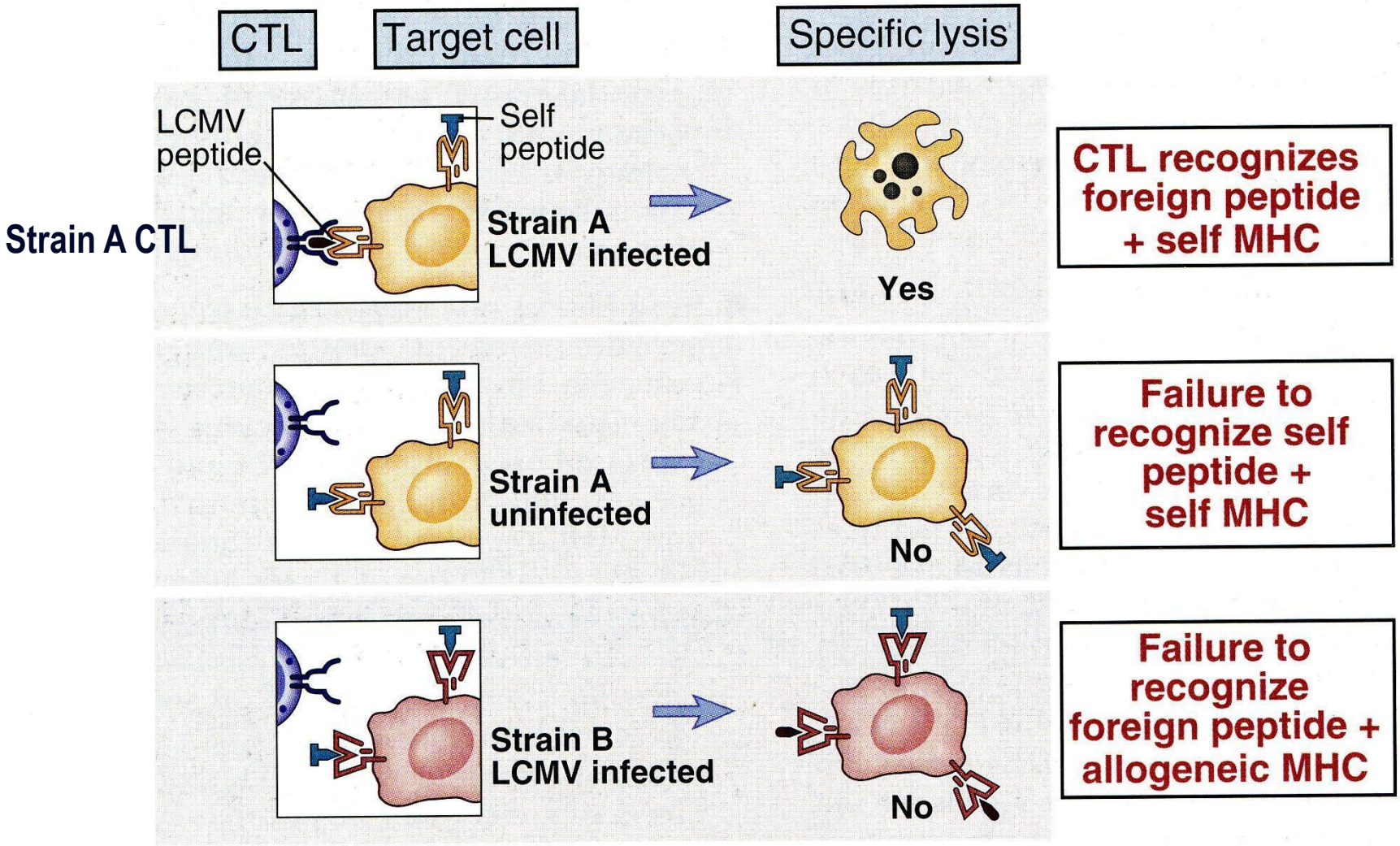
[Abbas et al, 2011]

Le molecole HLA di Classe I e II



Origin of bound peptides	Intracellular	Extra cellular
Lenght	8-10 aa	≥11 aa
Presentation to LΦ	CD8+	CD4+

Antigen presentation and T cell: MHC restriction

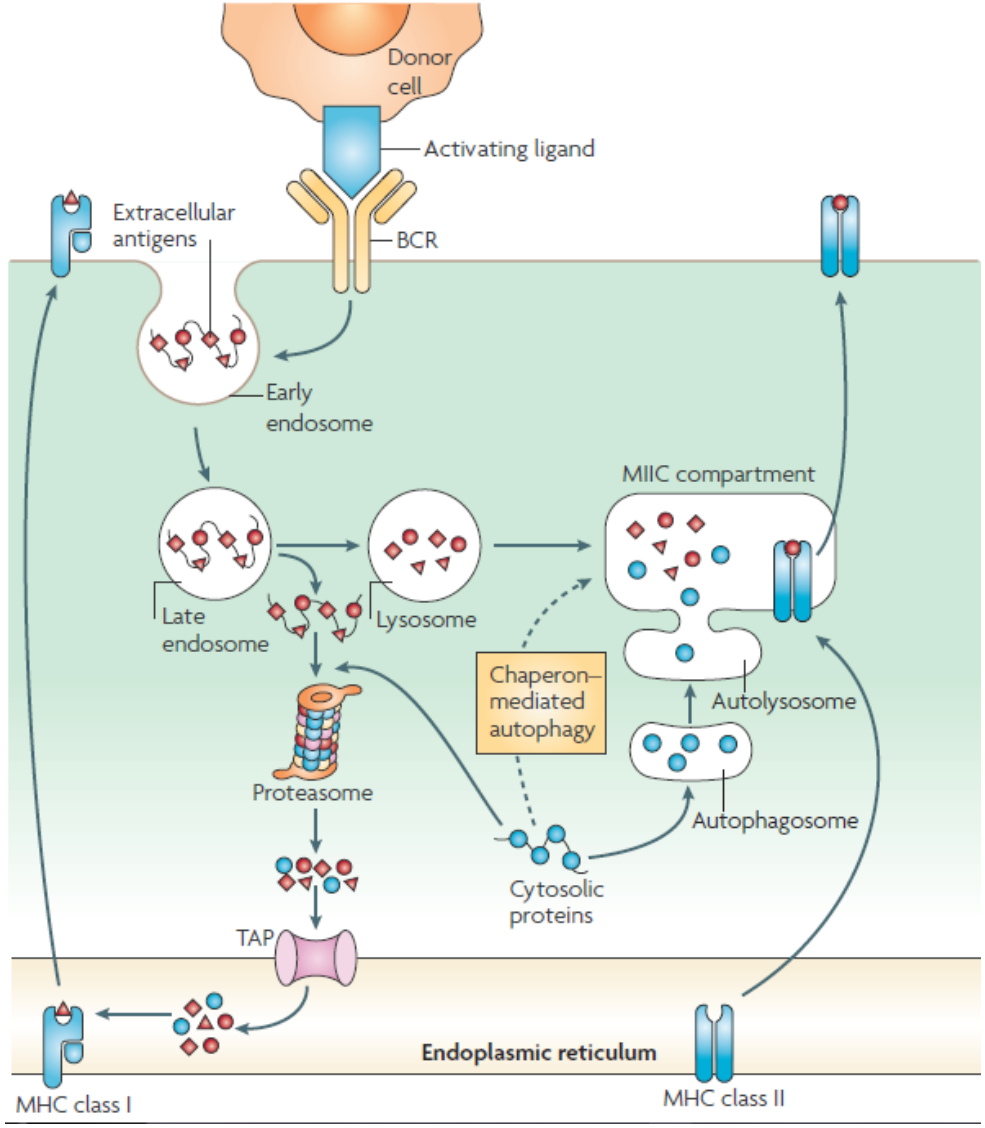


[Abbas et al, 2011]

HLA molecules and antigen presentation: antigen presenting cells in transplantation

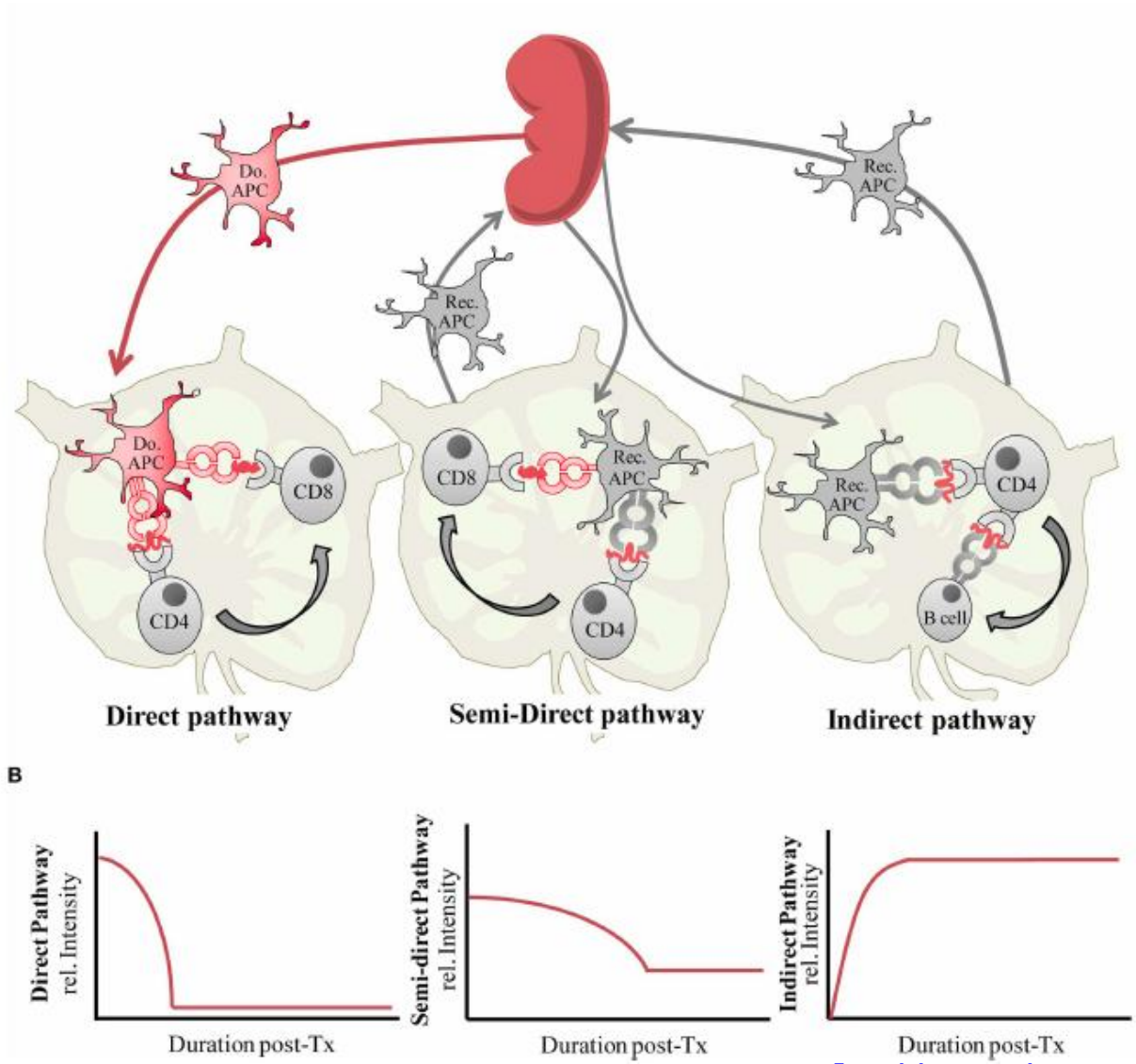
- Donor/recipient professional APC (include DC and Mϕ)
- Graft endothelial cells
- Recipient B lymphocytes

HLA molecules and antigen presentation to T lymphocytes : the key role of antigen presenting cells



[Felix et al, Nat Rev immunol, 2007]

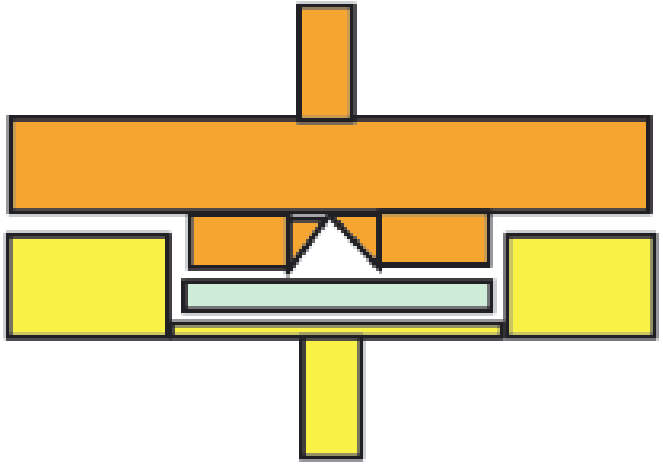
Direct, indirect and semi-direct antigen presentation to T cells



[Lechler et al, Frontiers in Immunol 2012]

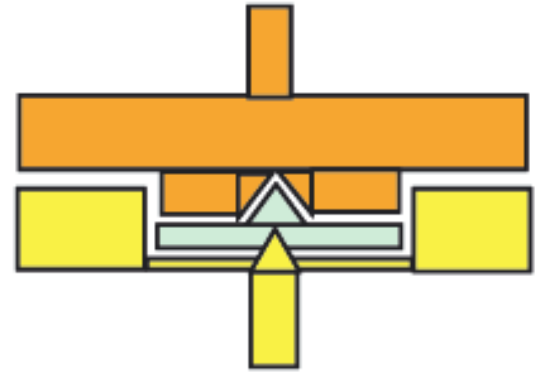
Antigen recognition by T cells in the context of MHC

Docking of TCR on self peptide-MHC

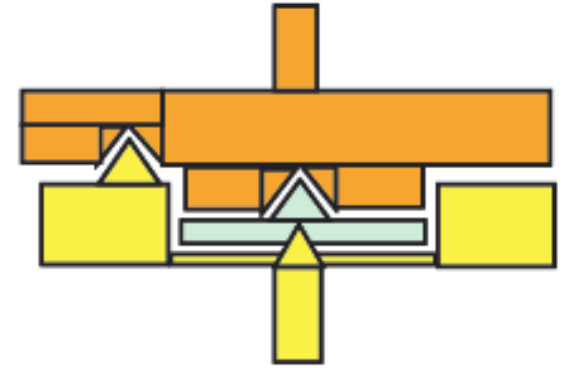


TCR with
self-peptide-MHC

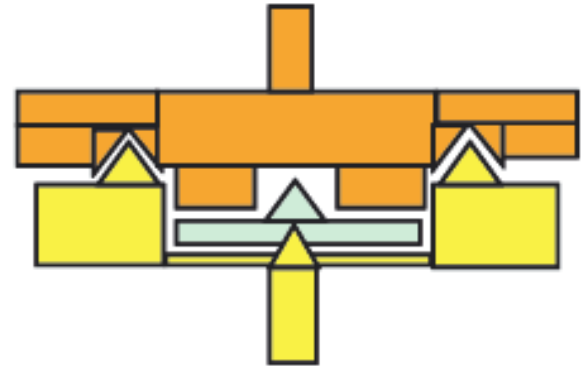
Type 1: Self restricted peptide specific



Type 2: Allorestricted peptide specific

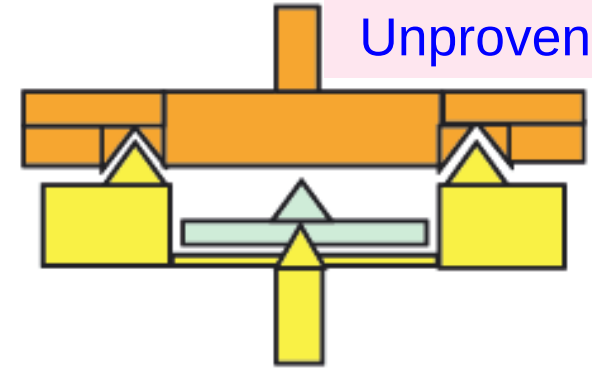


Type 3 : Alloreactive peptide dependent



Type 4: Alloreactive peptide independent

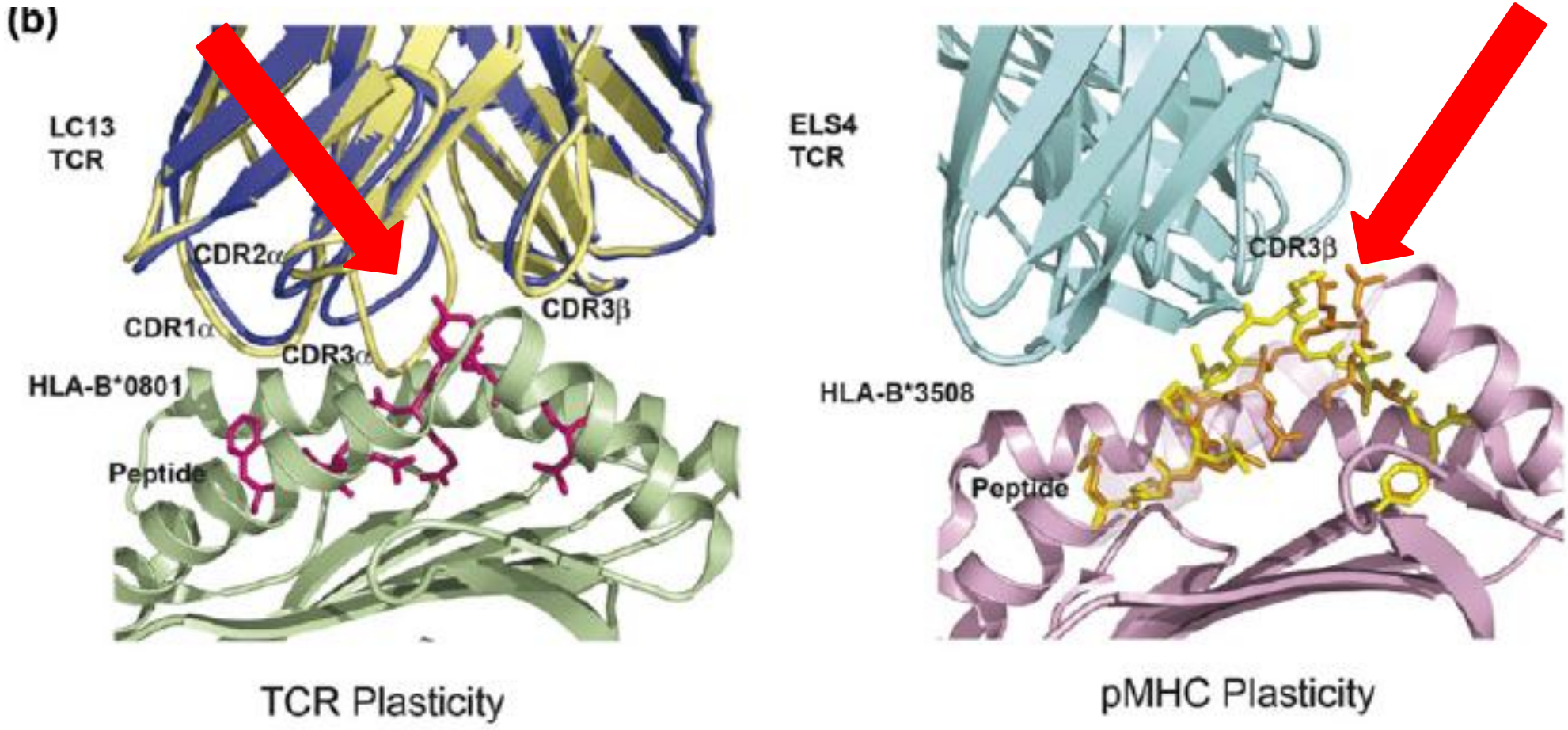
Unproven



Any alloresponse is likely to comprise a mixture of the four prototypes

[Nagy, Scand J Immunol 2012]

Plasticity of the TCR and the MHC molecule



[Archbold et al, Trends Immunol 2008]

Diversity of the antibody and TCR repertoires

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The key observation

- A limited number of genes code antibodies and TCR
- A very large number of specificities

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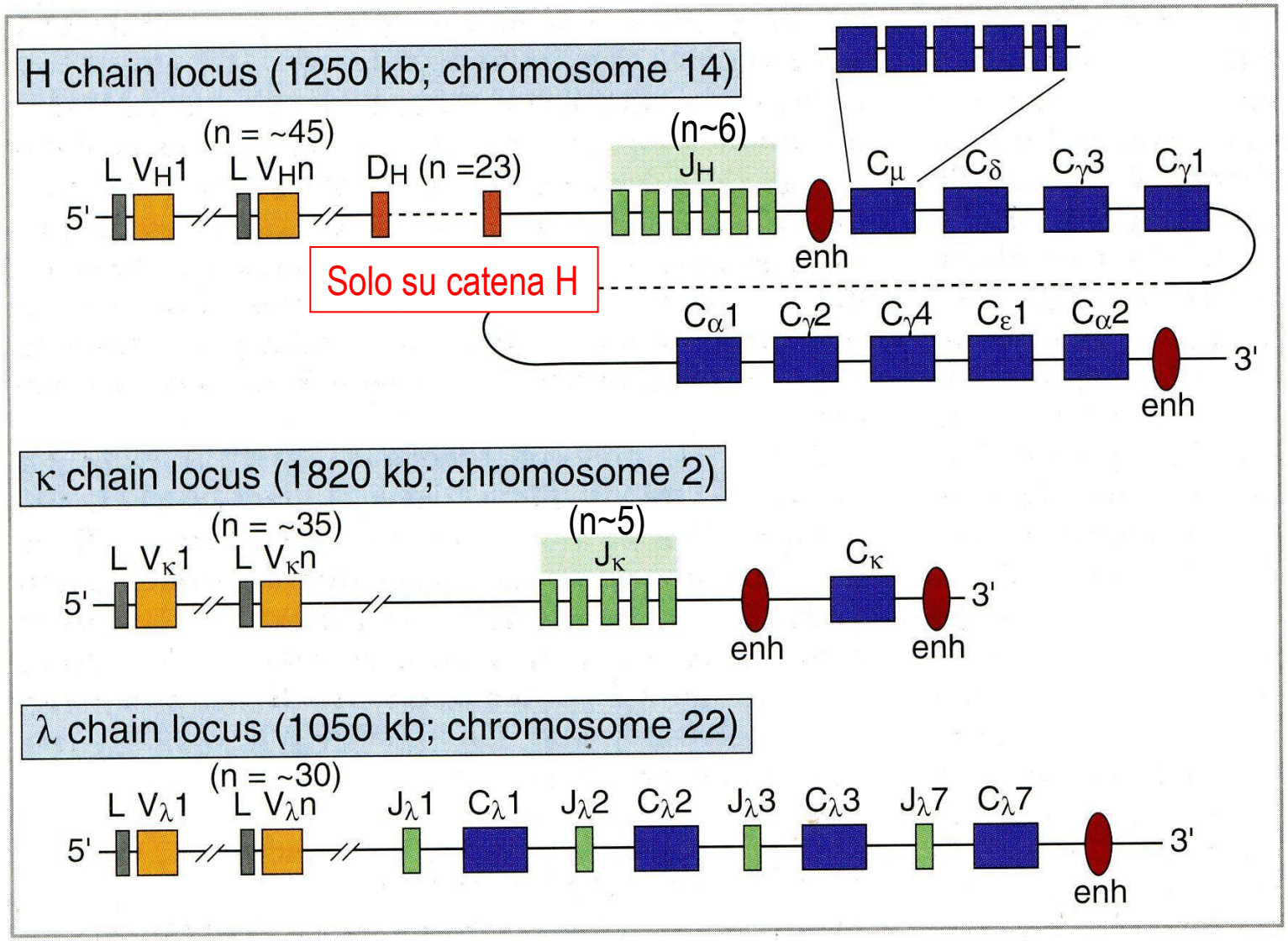
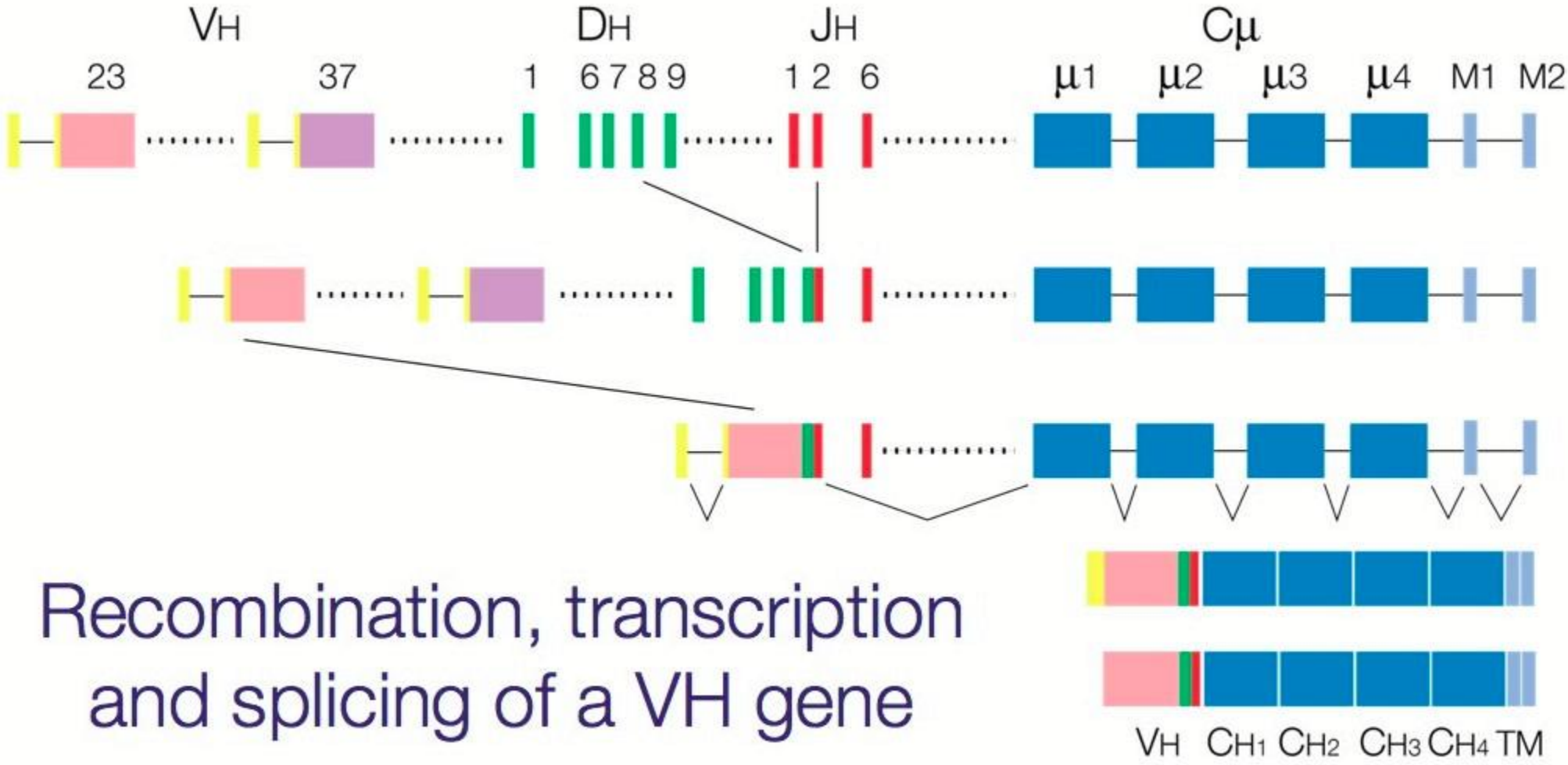
Antibodies and TCR are generated by the rearrangement of different variable (V) region gene segments with diversity (D) and joining (J) gene segments called:

V(D)J recombination enables diversity

Diversity of the antibody repertoire:
Combinatorial diversity



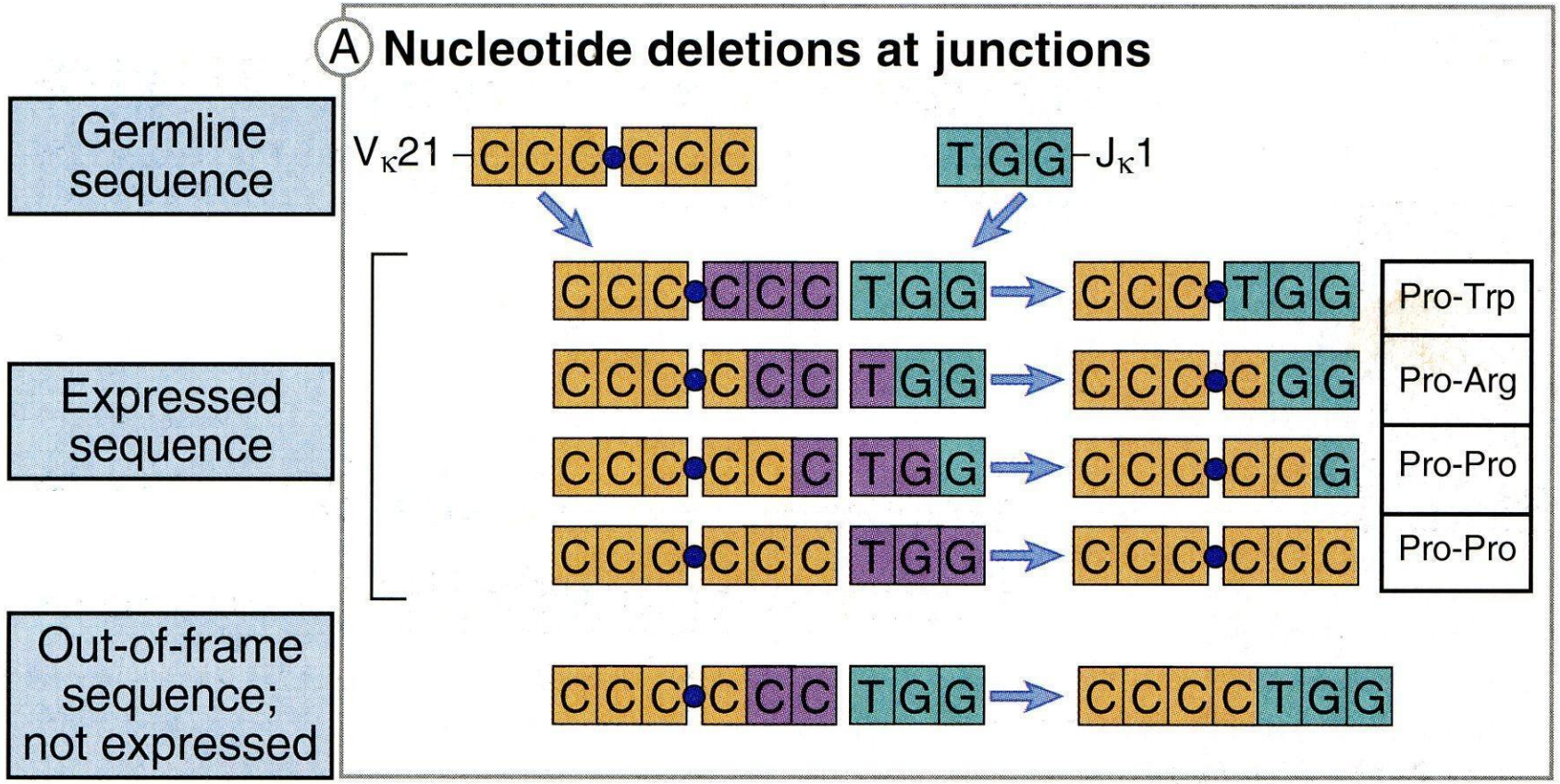
Diversity of the antibody repertoire: Combinatorial diversity



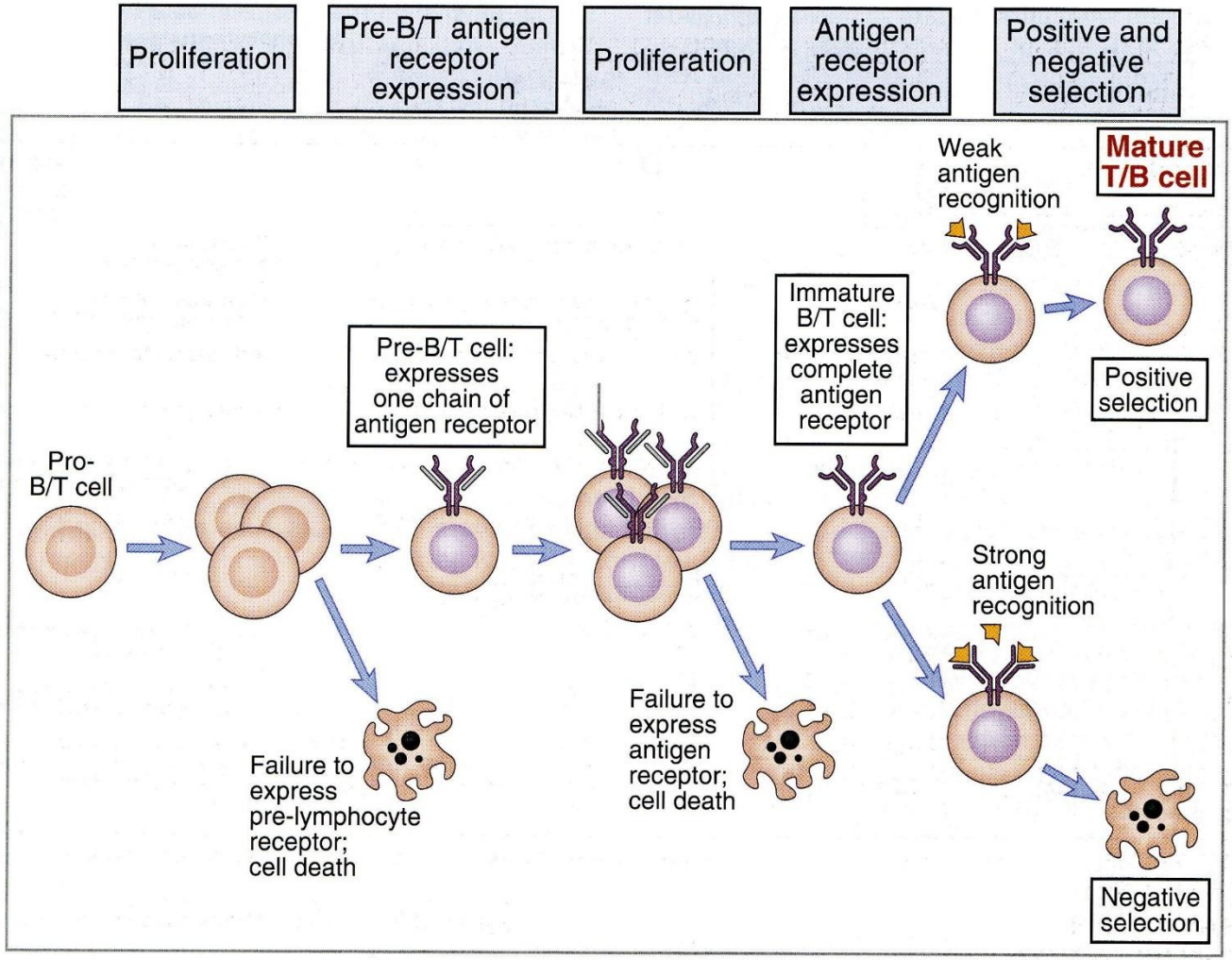
Recombination, transcription and splicing of a VH gene

Diversity of the antibody repertoire: Junctional diversity

The largest contribution to diversity is made by the removal or addition of nucleotides at the junction of the V, (D) and J segments

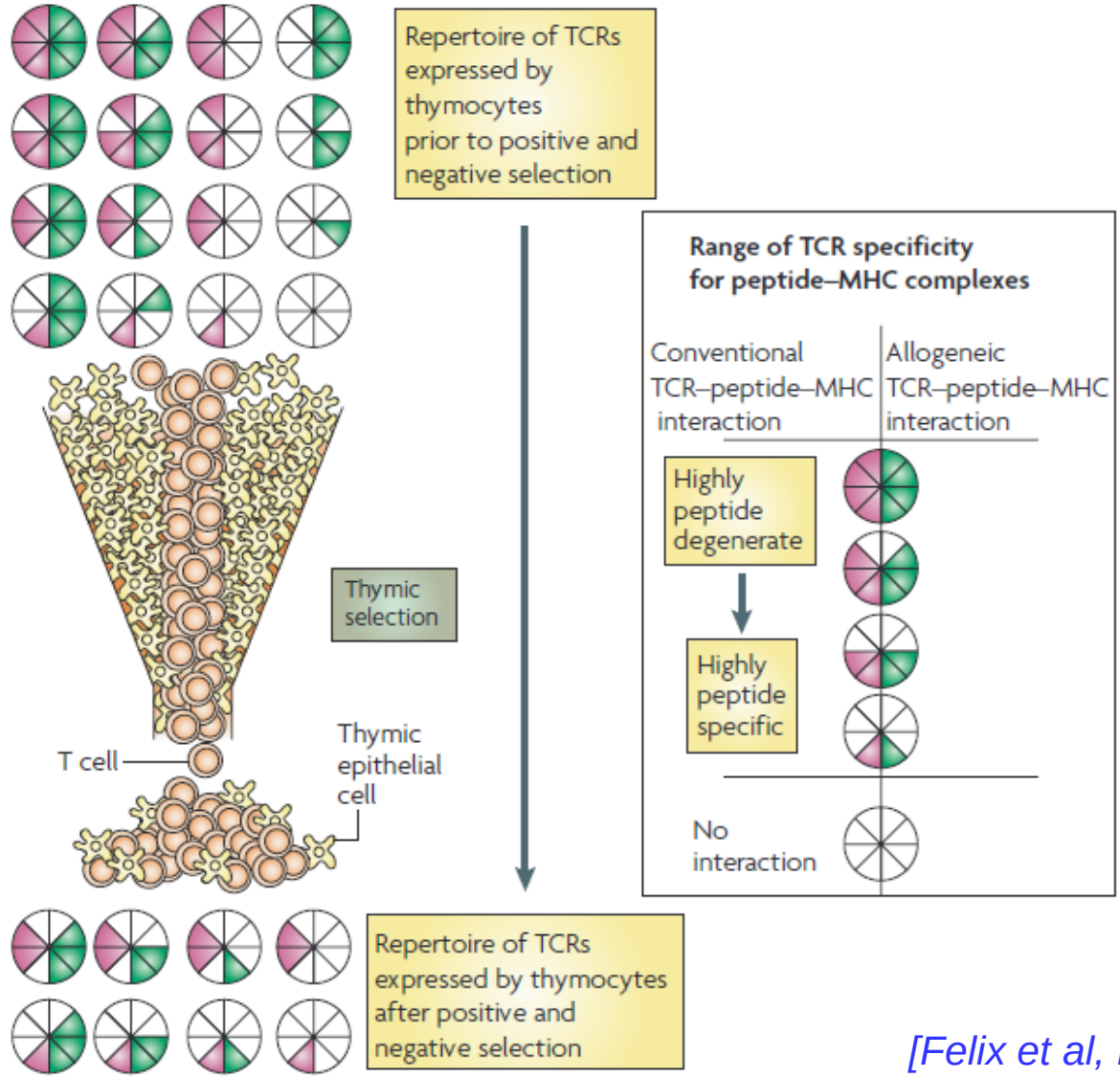


Diversity of the antibody repertoire:
Checkpoints in lymphocyte maturation



V(D)J recombination enables to go from 10^6 to ~10^{11} specificities

Diversity of the antibody repertoire: Checkpoints in lymphocyte maturation



[Felix et al, Nat Rev immunol, 2007]

Unusually high frequency of alloreactive T cells

Unusually high frequency of alloreactive T cells

- The frequency of allo-directed T cells is as high as 1-2% of all T cells.
- This is 100-1.000 times greater than that specific for any microbial peptide!

Unusually high frequency of alloreactive T cells: possible reasons

- The system is inherently biased to recognize MHC molecules
- Mimicry: the structure of an allo-MHC+peptide may mimic sef-MHC+foreign peptide
- Many peptides may combine with a single MHC molecule and further expand the number of T cells that can recognize these combinations
- All the MHC molecules on a donor MHC are foreign and recognized; in contrast, less than 1% of the MHC molecules on a self APC present microbial peptides

«Strenght» of the alloreactive response

«Strenght» of the alloreactive response: T-cell repertoire

Memory T-cells responses are faster and more vigorous than naïve T cell responses. However:

- Individuals who have not been exposed previously to alloantigens have a high frequency of memory alloreactive T cells!!
- These alloreactive memory T-cells are cross-reactive memory T-cells that possess antimicrobial specificities

[Merkenslager et al, Eur J. Immunol 1988; Macedo et al, AJT 2009]

«Strenght» of the alloreactive response: B-cell repertoire

HLA-incompatibility and pregnancy
Frequency of sensitization

	Ratio cut-off	MFI>1000	Child-specific Ab
First live birth	70%	33%	21%
Second live birth	84%	62%	37%
≥ Third live birth	92%	75%	46%

- Hierarchy of sensitization (B>A>DRB1>C)

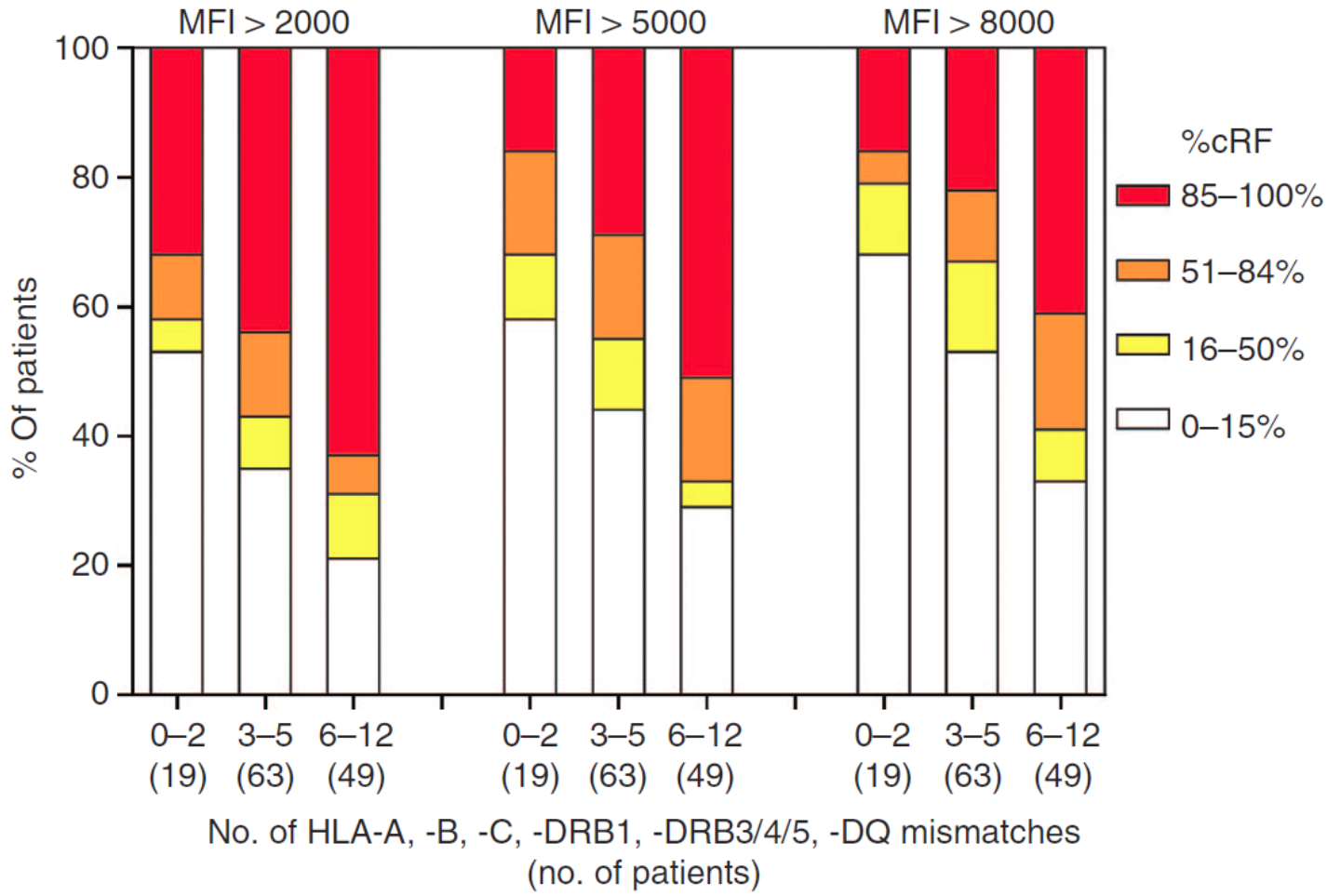
[Honger et al, AJT 2013]

HLA-incompatibility and transfusions Frequency of sensitization

Recipient	%sensitized		Titer
	CDC	Solid phase	
Transfusions alone			
Recent	10–12	10	Low
Distant		<2	
Children	35	35	Medium
Multiple	50 and up		Medium-high
Previous pregnancies alone	5	24–33	Low-medium
Plus transfusions	40	52	High
Previous transplants alone	17	72	Low-high
Plus transfusions	60–78		High

[Scornik et al, AJT 2011]

Frequency of HLA-sensitization in patients with previous transplants



[Kosmoliaptsis et al, KI 2014]

Conclusions

- Alloreactivity is only one of the multiple reasons underlying premature organ failure.
- Antibodies, TCR and MHC molecules are the key receptors involved in alloreactivity
- Whilst B cells (antibodies) recognise Ag directly, T cells require Ag presentation in the context of MHC molecules
- V(D)J recombination explains the extraordinary diversity of both antibody and TCR repertoires.
- There is an unusually high frequency of alloreactive T cells
- The alloreactive responses driven by the B and T cell repertoires are extremely vigorous and better immunosuppressive strategies are needed to improve longterm graft survival.