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Activation lymphocytaire T

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Activation lymphocytaire T

- **Le rapprochement cellulaire**
- **La synapse immunologique**
- **Les molécules de l'activation**
  - TCR: CMH-Ag
  - Co-récepteur CD4, CD8
  - Mol co-stimulation
  - Cytokines

- **Aspects quantitatifs et qualitatifs de l'activation**

  - Affinité de l'interaction
  - Avidité de l'interaction
  - Dynamique des interactions moléculaires

- **et ses conséquences sur le développement de la réponse immunitaire**

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Le rapprochement cellulaire

La synapse immunologique

Les molécules de l'activation

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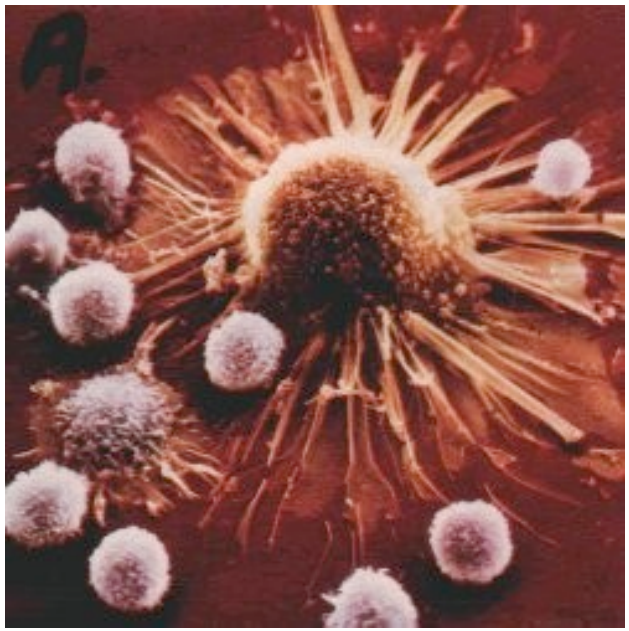
Etablissement du contact cellulaire:

- **Contact entre cellules T et APC**
- **Etapes:**
  - Migration cellulaire:
    - Stochastique
    - Chimiokines
  - Adéquation de l'architecture des organes lymphoïdes
  - Rapprochement cellulaire:
    - molécules d'adhésion : intégrines, sélectin/adressine
  - Interactions cellulaires:
    - Contacts de surface:
      - TCR/CMH
      - Co-stimulation
    - Signalisation « paracrine»:
      - cytokines
  - Activation cellulaire



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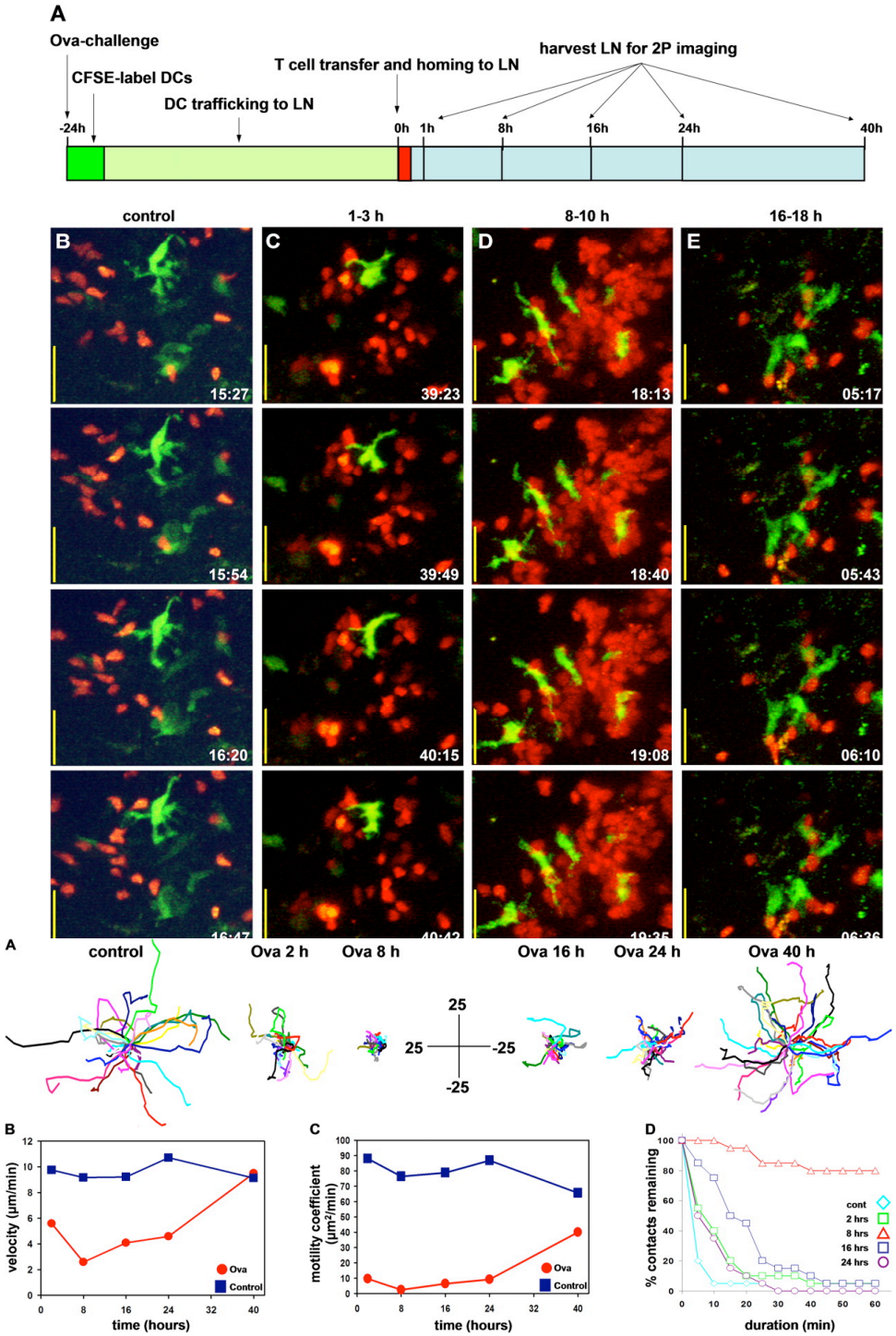
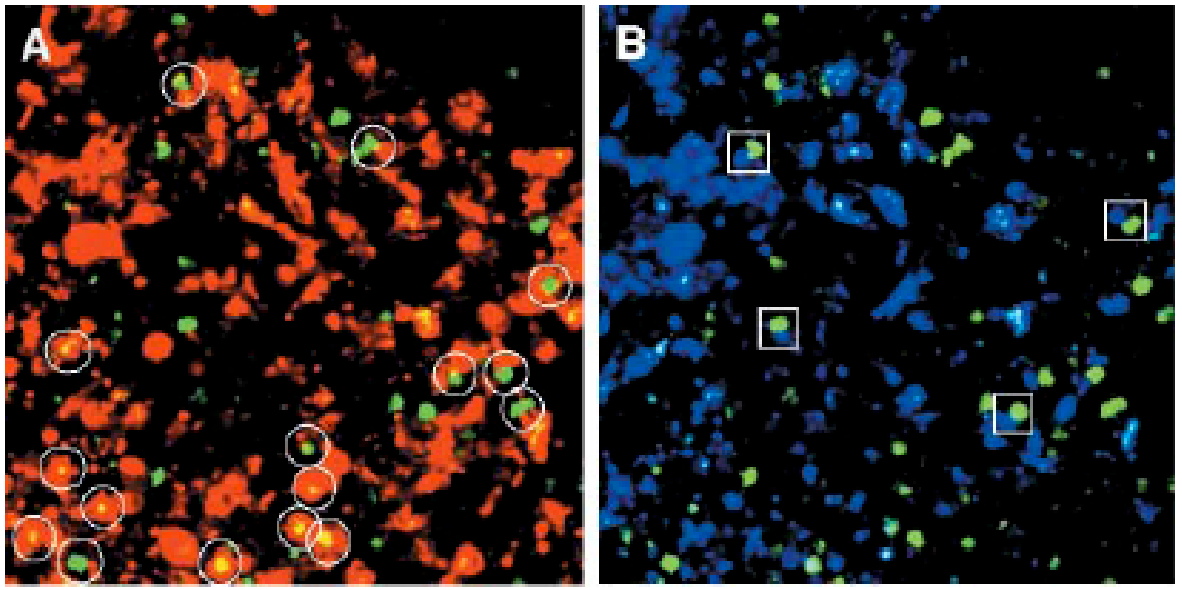
Miller, JEM 2004, **Imaging the Single Cell Dynamics of CD4+ T Cell Activation by Dendritic Cells in Lymph Nodes**

Objective: to describe cell behavior and interactions in native tissues during T cell priming, from initial contact with antigen-bearing DCs to cell division.
Method: two-photon microscopy to image OVA-specific CD4+ T cells with endogenous DCs in intact lymph nodes.
Fig1
We imaged T cells and DCs in lymph nodes during continuous periods of 30 min–2.5 h at different times after T cell transfer (time zero).
Fig2
Analysis of T cell motility at different stages of activation. (A) Tracks of individual T cells (different colors, normalized to their starting coordinates) showing representative motility of cells in control experiments (without OVA) and at various times as indicated in OVA-challenged mice. Bars are in micrometers. (B) Average instantaneous velocities of T cells in antigen-challenged (red) and control sham-treated mice (blue) as a function of time after adoptive transfer. (C) Corresponding measurements of motility coefficients, derived from plots of mean displacement against square root of time. (D) T cell–DC contact durations in control and OVA-immunized mice at various times (indicated by differently colored symbols) after adoptive transfer. Cumulative plots show the percentage of T cells that remained in contact for any given duration.



Contact antigène-dépendant



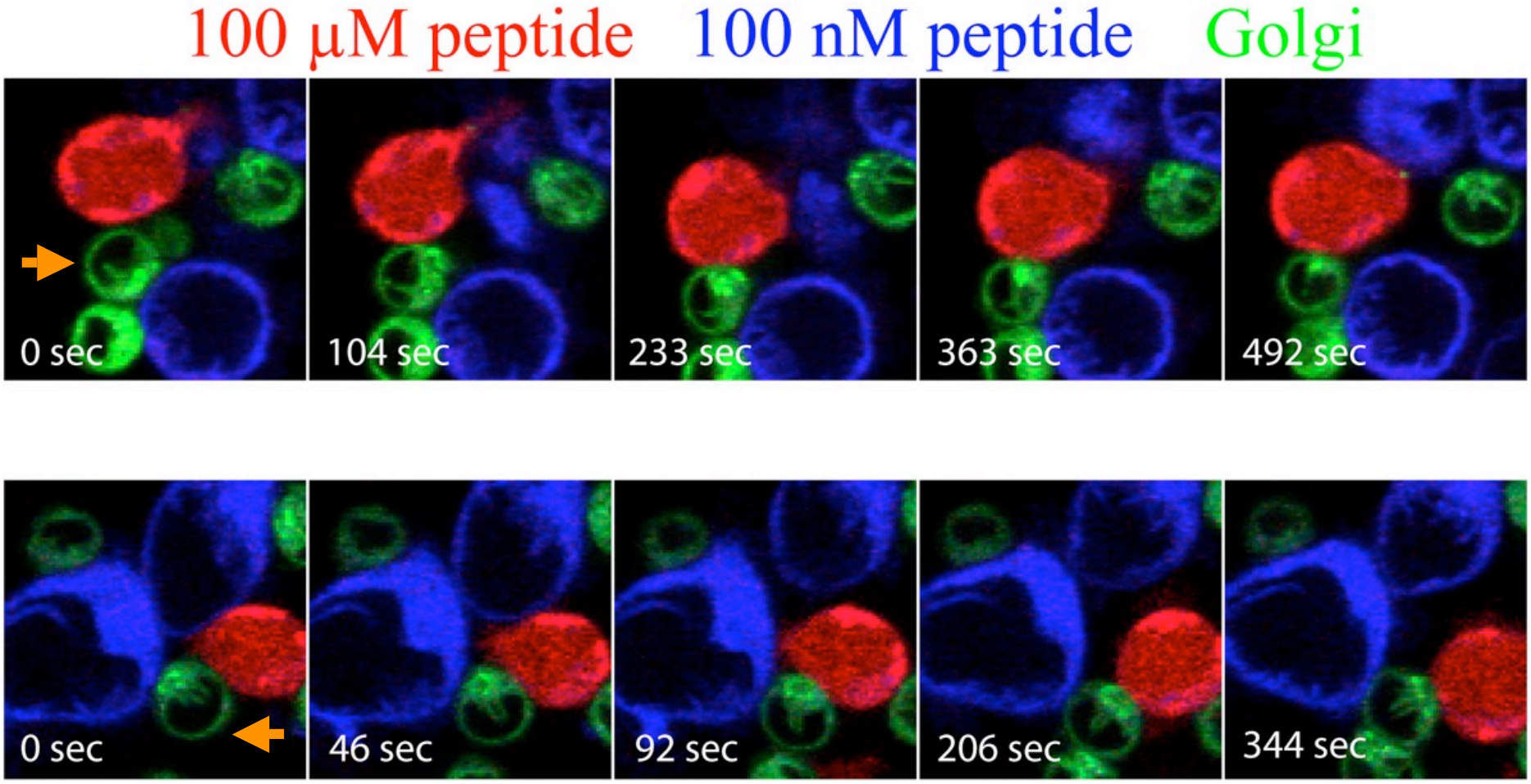
DC Ag+
DC Ag-
T cell

Fig. 3. Effect of the presence of antigen on T–DC interactions in situ. DiI (red)-labeled antigen-bearing DCs (A) and DiD (blue)-stained antigen-free DCs (B) were coinjected subcutaneously into a single hind footpad. Six hours later, CFSE-loaded (green) T lymphocytes were injected intravenously. Popliteal lymph nodes were imaged 20 h later. T cells preferentially establish stable contact with antigen-bearing rather than antigen-free DCs when both APCs are present in the same lymphoid organ. T–DC conjugates are highlighted with white open circles (A, pulsed) or white open squares (B, unpulsed). Green T cells superimposed directly over an associated red DC appear yellow.

J Delon Imm Review 2002

Contact antigène-dépendant

Influence de la concentration antigénique



J Delon Imm Review 2002

Organisation spatiale du contact cellulaire

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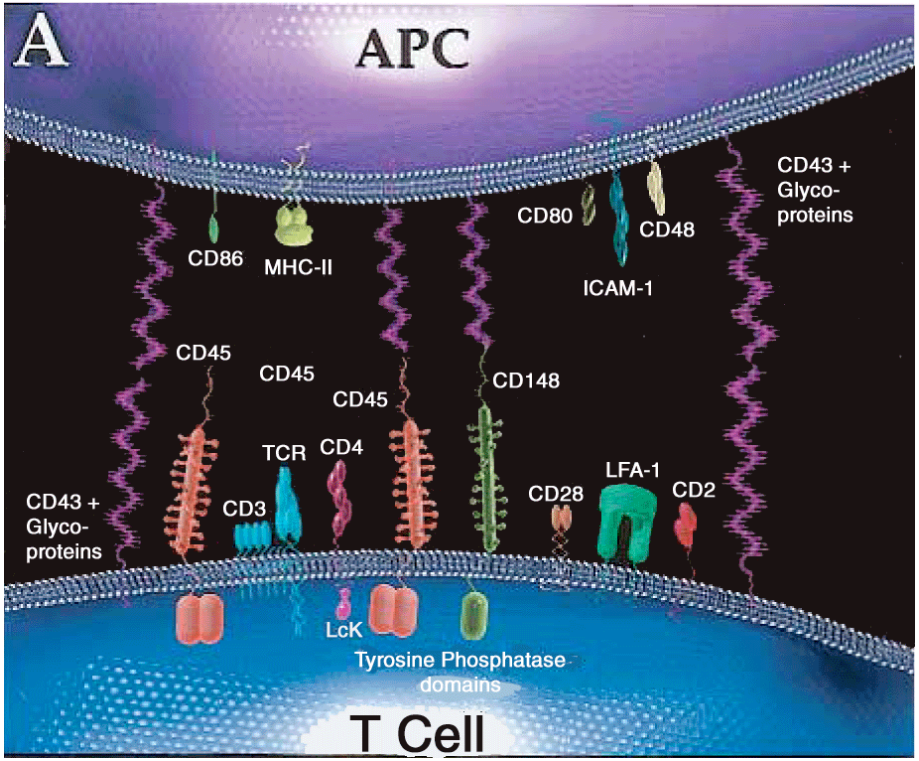

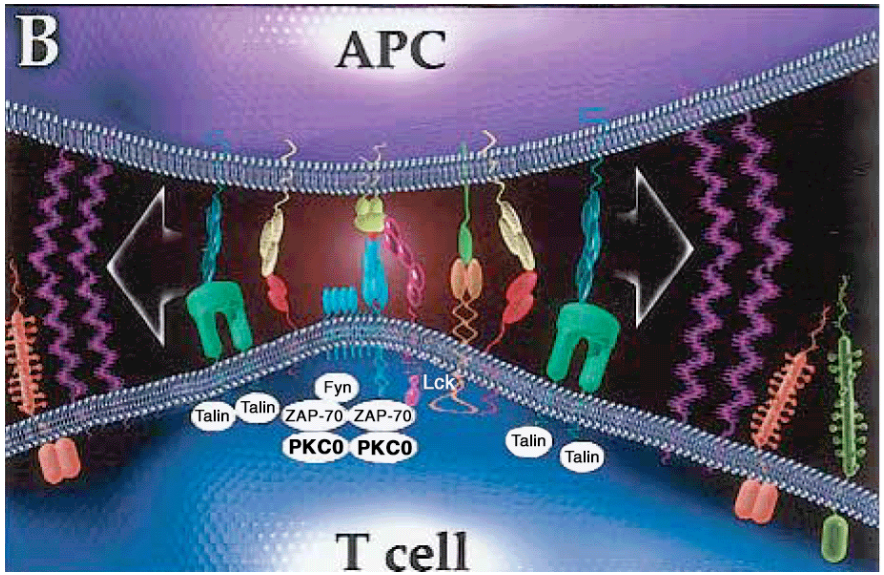

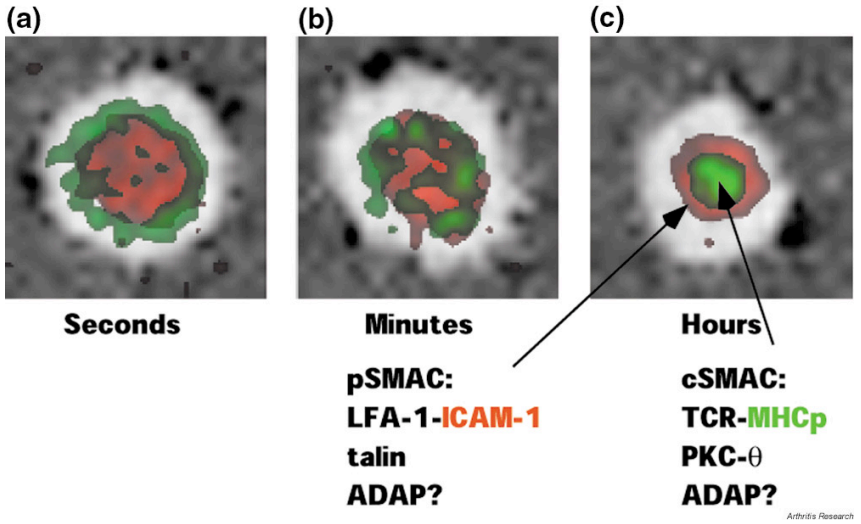

Les domaines extracellulaires des molécules de signalisation (TCR, CMH, CD28, B7) sont courtes (7nm) et sont entourées de grandes molécules comme CD43 (45nm) CD45 (30-50nm) exerçant une fonction dé-activatrice. L'adhésion via ICAM-1/3 (Superlg) (40 nm chacun) puis LFA-1 (; intégrine21 nm) va amorcer le regroupement des TCR/ CD28

Les premières sollicitations de LFA-1 puis du TCR induisent une activation du cytosquelette permettant la constitution des SMACs et de la synapse immune.

SMAC: Supra Molecular Activation Cluster

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Supra Molecular Activation Cluster

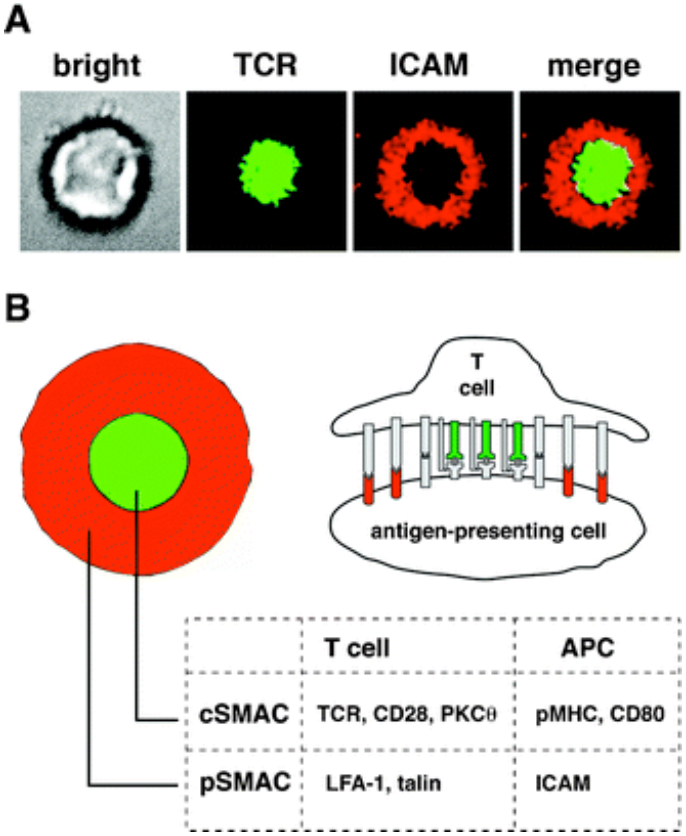
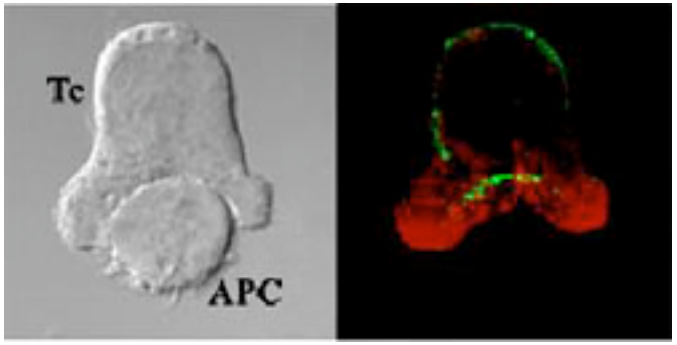


Fig. 1 The immunological synapse. (A) Brightfield and fluorescence images of a T cell forming a synapse with a supported membrane containing GPI-linked pMHC and ICAM. (B) Schematic and cross-section of the mature synapse showing segregation of signaling molecules in the cSMAC and adhesion molecules in the pSMAC.



Interaction of a T lymphocyte (Tc) with a B lymphocyte presenting a bacterial superantigen (APC) observed by confocal microscopy. The **actin cytoskeleton** associated protein ezrin (red) and the T cell antigen receptor (green) are polarized towards the antigen presenting cell (immunofluorescence, right). On the left, the morphology of the two cells is observed by differential interference contrast.

Unit: Lymphocyte Cell Biology
Director: Andres ALCOVER

Séquence d'interactions et redistribution des molécules de surface

Rafts lipidiques

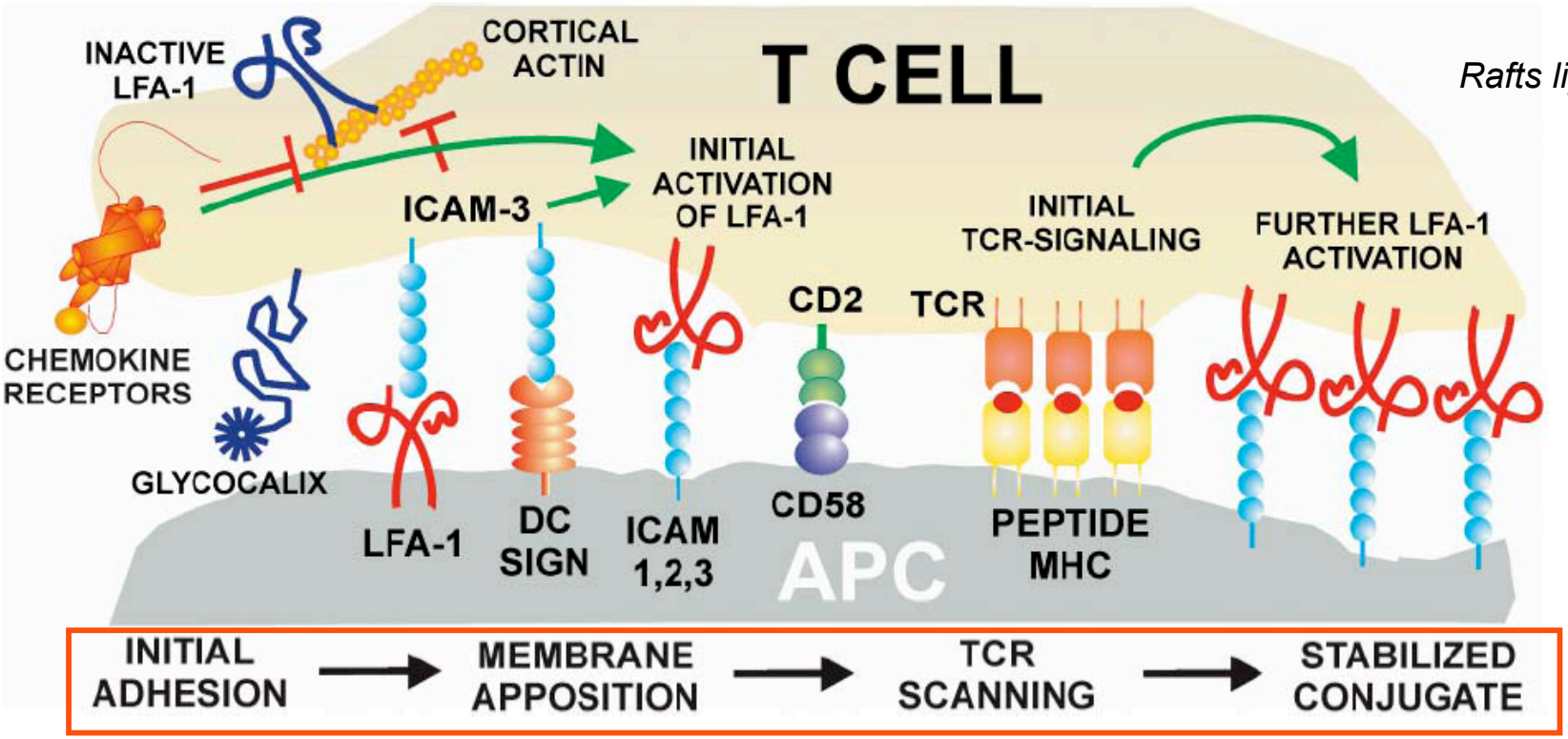
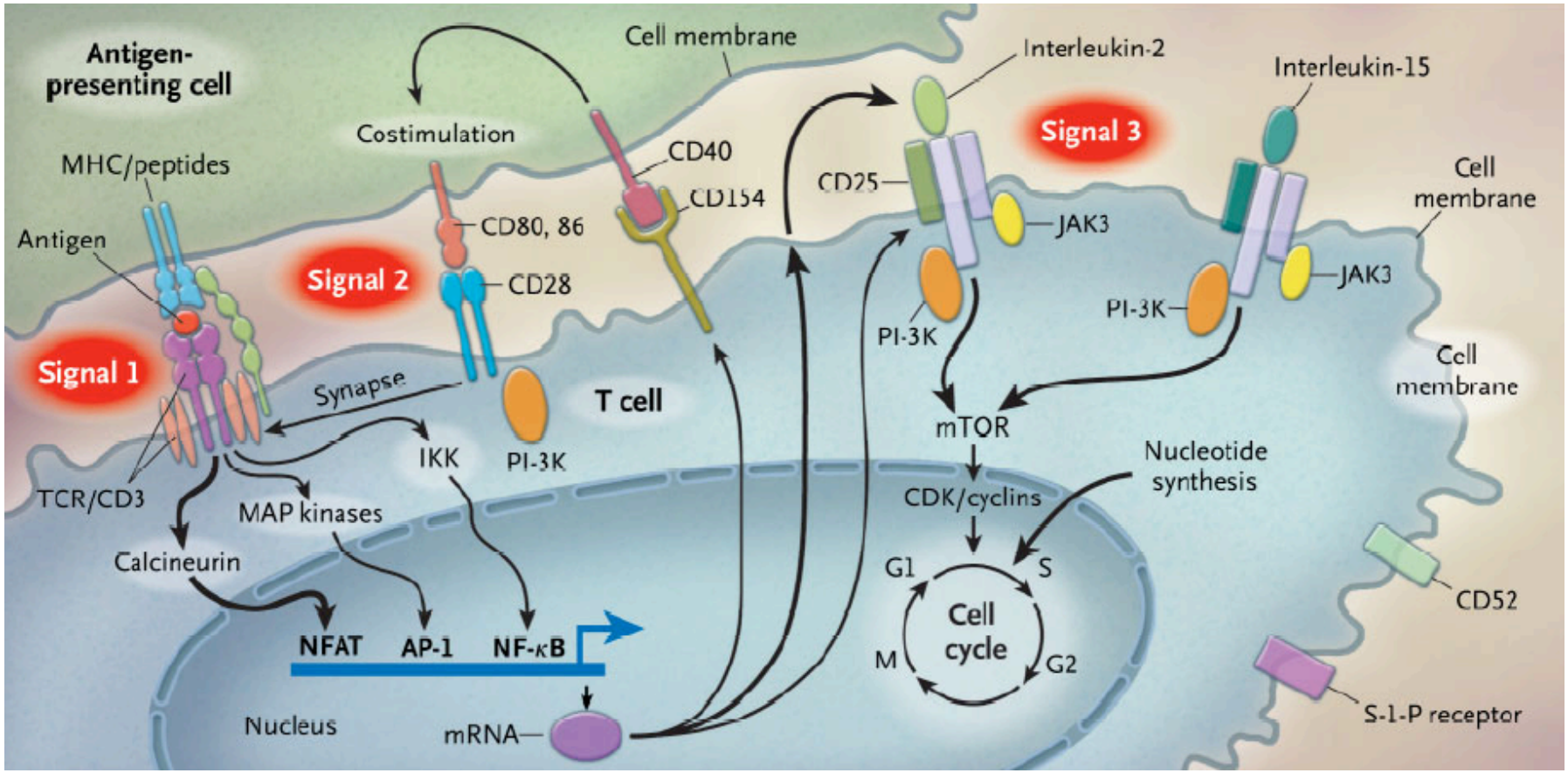


Fig. 1. Sequence of interaction and redistribution of cell surface molecules during initial immune adhesive contact. The initial adhesive contact is mediated by ICAM-3 interaction with APC ligands (LFA-1, or DC-SIGN). ICAM-3 activation and chemokine receptor signaling induce redistribution of cortical actin and initial activation of LFA-1, leading to membrane apposition that allows CD2–CD58 interactions and TCR searching of MHC-peptide. Signaling by TCR, CD2 and ICAM-3 contributes to further LFA-1 activation that leads to the stabilization of the conjugate.

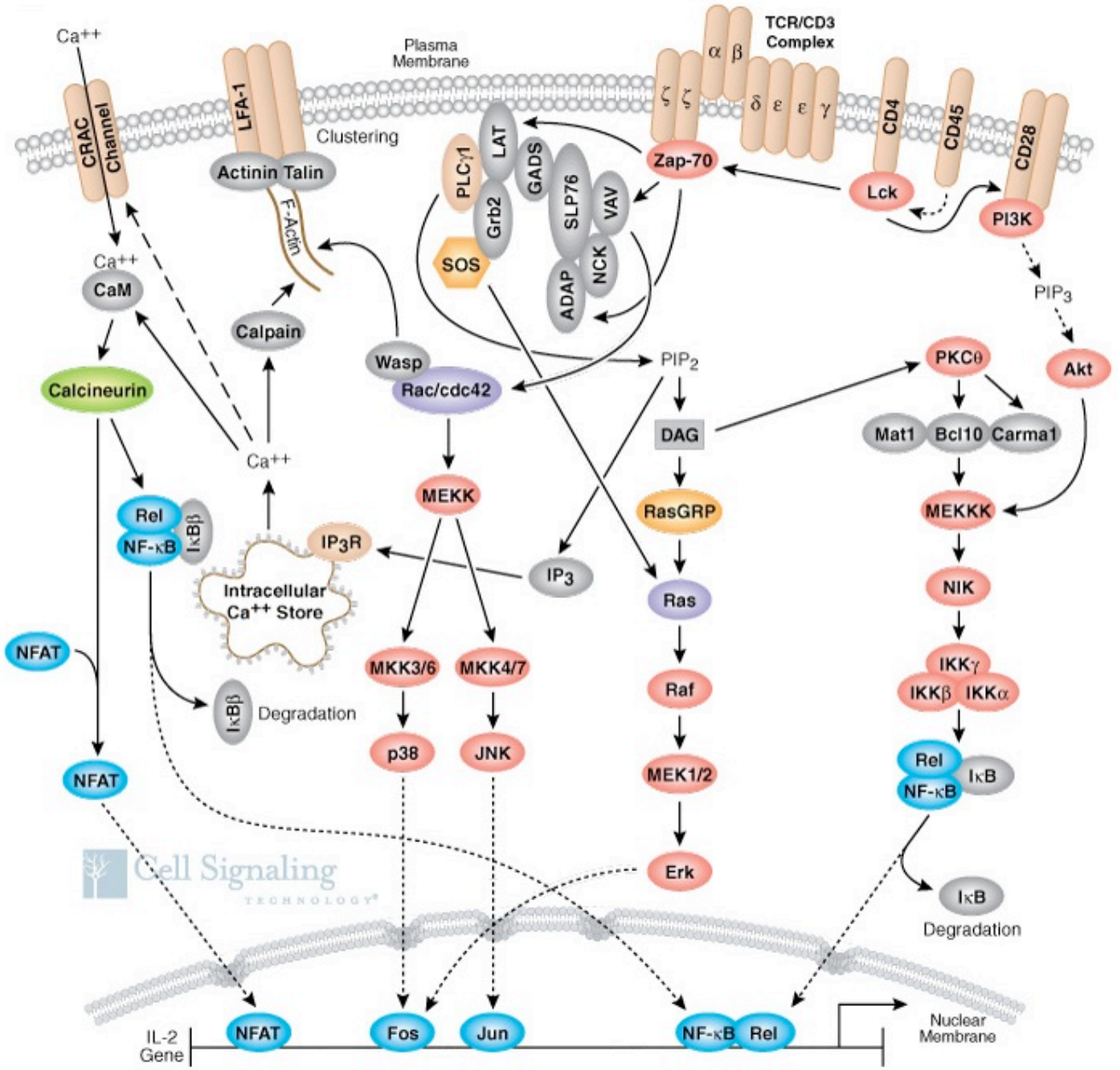
les molécules de l'activation lymphocytaire



N Engl J Med 2004, 351:2715

NFAT : nuclear factor of activated T cell
NFkB : nuclear factor kappa B

Transduction du signal



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Bases moléculaires de la reconnaissance antigénique

Influence des paramètres de l'interaction TCR/CMH-Ag

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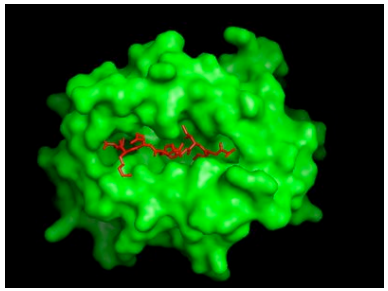
Reconnaissance antigénique

< Green = Major Histocompatibility Molecule(MHC), Red = T - cell receptor (TCR), Yellow = Antigenic peptide



> An MHC molecule (green) presenting an antigenic peptide (red) as would be "seen" from the top point of view of a T-cell....

The affinity range reported for TCRs binding to agonist pMHCs is KD ~1–50 μM. This is considerably weaker than most other protein–protein interactions of biological consequence

Mice: TCR/pCMH– I = 13 uM
Humans: TCR/pCMH–I =52 uM

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TCR/CMH-I vs TCR/CMH-II

Human TCR-Binding Affinity Is Governed by MHC Class Restriction[1]

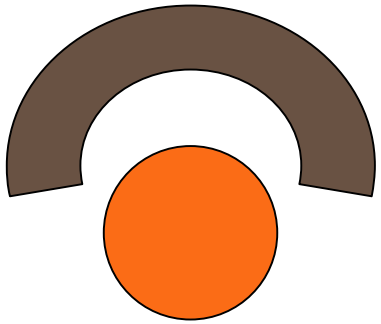
David K. Cole,*‡ Nicholas J. Pumphrey,† Jonathan M. Boulter,‡ Malkit Sami,† John I. Bell,* Emma Gostick,* David A. Price,* George F. Gao,*§ Andrew K. Sewell,*‡ and Bent K. Jakobsen[2†]

J. I. 2007

Table IV. *Kinetic data at 25°C*[a]

	K_{on} $M^{-1}s^{-1}$	K_{off} s^{-1}	K_D μM (K_{off}/K_{on})
TCR-I/pMHC-I			
gp100 TCR/A2 gp100	3.1×10^4	0.23	7
TEL TCR/A2 Tel	3.5×10^3	0.14	40
LC13 TCR/B8 EBNA	8.7×10^4	0.63	7
AM3 TCR/A24 EBV	7.3×10^3	0.21	28
JM22 TCR/A2 Flu	3.2×10^4	0.15	5
A6 TCR/A2 Tax	2.9×10^4	0.10	4
GRB TCR/B27 Flu	3.9×10^4	0.09	3
MEL TCR/A2 Mel	$>1 \times 10^6$	>1	nm
TCR-II/pMHC-II			
MAW13 TCR/DR3 M-HSP	4.0×10^3	0.12	30
AH1.23 TCR/DR4 C-HSP	4.4×10^3	0.16	36
1A12 TCR/DR2 MBP	2.1×10^3	0.17	81
2E11 TCR/DR2 MBP	5.9×10^3	0.73	123
HA1.7 TCR/DR1 HA	$>1 \times 10^6$	>1	nm
HA1.7 TCR/DR4 HA	$>1 \times 10^6$	>1	nm
Average TCR-I/pMHC-I	$3.3 \times 10^4 \pm 2.9 \times 10^4$	0.22 ± 0.19	13 ± 15
Average TCR-II/pMHC-II	$4.1 \times 10^3 \pm 1.3 \times 10^3$	0.30 ± 0.29	68 ± 43

[a] nm, Not measurable, i.e. the kinetics were too fast to accurately determine.



Ka= Kon/Koff
Kd= 1/Ka

Kon: Taux d'association
Koff: Taux de dissociation

- human TCRs bind to pMHC-I with approximately **five times greater** affinity than to pMHC-II.
- **faster on-rates** for TCR-I, compared to TCR-II, are indicative of a higher degree of conformational flexibility and higher entropic cost upon binding for the TCR-II interactions.
- Koff is a measure of the stability of a protein-protein interaction. no significant difference between the average **Koff** value for TCR binding to pMHC-I or pMHC-II. This observation is supported by TCR/pMHC complex crystal structures, which show that the **number of molecular contacts is relatively conserved** for TCR-I and II binding
- suggest that TCRs specific for **pathogenic Ags** such as those derived from viruses and bacteria could bind with stronger affinity compared with nonpathogenic

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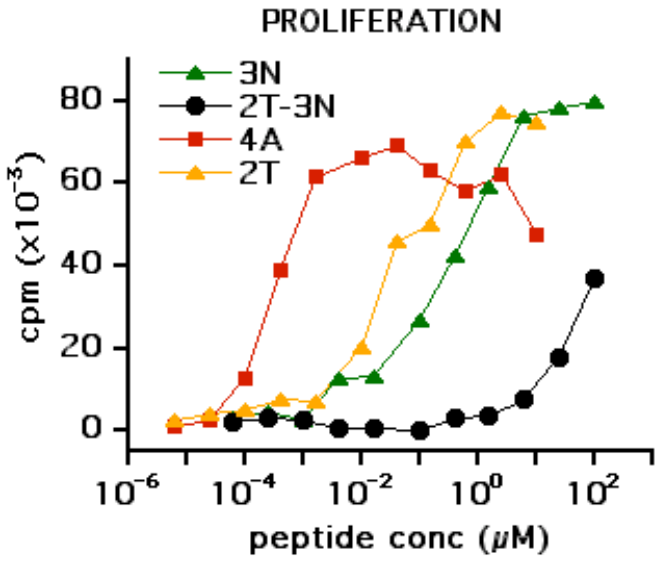
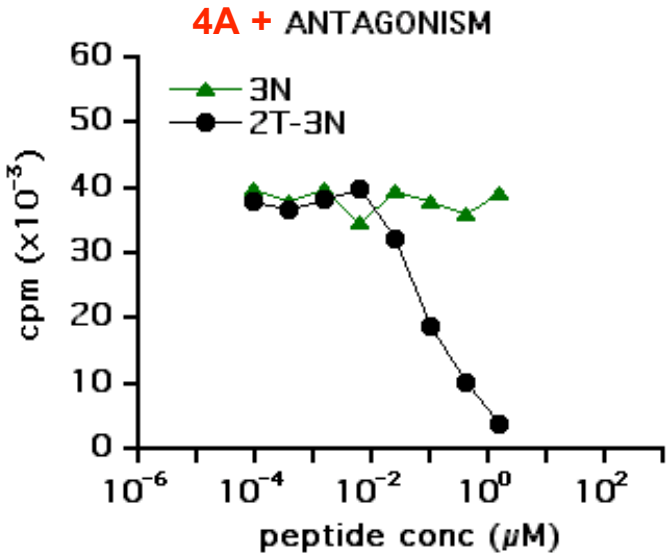
Engagement du TCR: Rôle du peptide Agoniste, Agonsite Partiel, Antagoniste





Altered MBP 1-11 Peptides are **Partial Agonists**

Agonist = 4A

Substitution of the Gln-3 residue, a TCR contact, with Asn (3N) diminishes the ability of the peptide to stimulate T-cell proliferation (top). Substitution of the Ser-2 residue, an MHC contact, with Thr (2T) also diminishes the ability of the peptide to stimulate T-cell proliferation (top). When combined, these two mutations (2T-3N), profoundly diminishes the peptide activity. Molecular models suggested that the 2T substitution would alter the side-chain conformation of the 3N residue. **Although the 2T-3N peptide does stimulate T-cell proliferation at high concentrations (top), at lower concentrations it blocks proliferation induced by the agonist 4A peptide.** These partial agonist peptides have the ability to modulate T-cell responses and may be useful in the treatment of autoimmune diseases.

In the bottom panel, antigen presenting cells were pre-pulsed with the A4 peptide and then added to T cells with altered peptide.
Proliferation was measured as the incorporation of tritiated thymidine.

>> Cas de l'échappement viral

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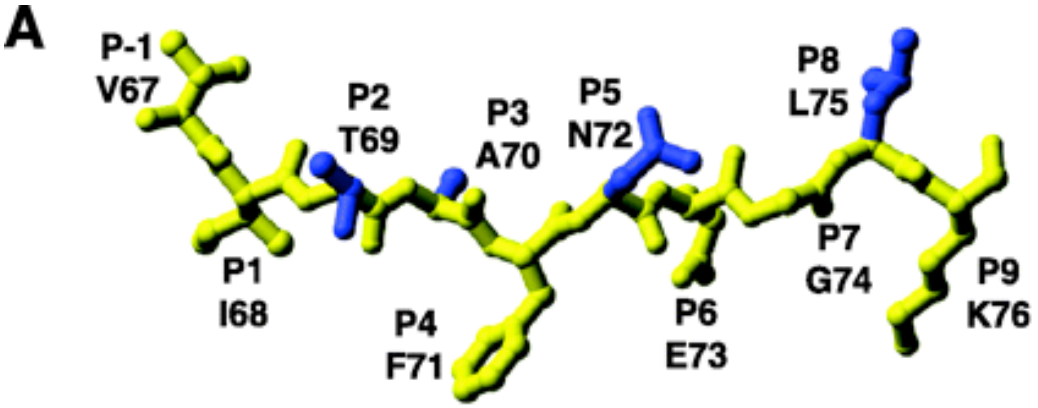
Engagement du TCR: Rôle du CDR
Définit l'affinité pour pCMH

Hb peptide



B

TCR mutants

T cell	CDR mutations β:1 2 3 α:1 2 3	K_D (k_{off}/k_{on}) *nM*	Half-Life *s*	k_{on} $M^{-1}s^{-1}$	k_{off} s^{-1}
M1	○○○ ○○○	19,860 ± 3,630	6.8 ± 0.7	5,220 ± 410	0.1023 ± 0.0107
M4	○○○ ○○●	580 ± 185	28.5 ± 0.8	42,670 ± 17,430	0.0243 ± 0.0007
M14	○○● ○○●	81 ± 2	48.9 ± 2.4	173,500 ± 3,500	0.0141 ± 0.0007
M15	○○● ●○●	25 ± 5	104.4 ± 9.1	281,500 ± 34,800	0.0068 ± 0.0006

Aff >
KD <
Koff <
Kon >

Hb peptide residues and location of CDR mutations in high-affinity 3.L2 TCR mutants. A, Structure of Hb peptide (ITAFNEGLK) showing in blue the location of the four surface-exposed residues P2(T), P3(A), P5(N), and P8(L). B, 3.L2 M1 (unmutated CDRs) and **three higher affinity mutants** (M4, M14, and M15) with the location of their CDR mutations and respective KD, t1/2, kon, and koff values (43). The CDRbeta1,2,3 and CDR{alpha}1,2,3 are designated from left to right by circles: {circ}, indicate that the CDR is not mutated; •, indicate mutations in the CDRbeta or CDR{alpha}, respectively. The amino acid mutations in these regions are the same as previously published (43).

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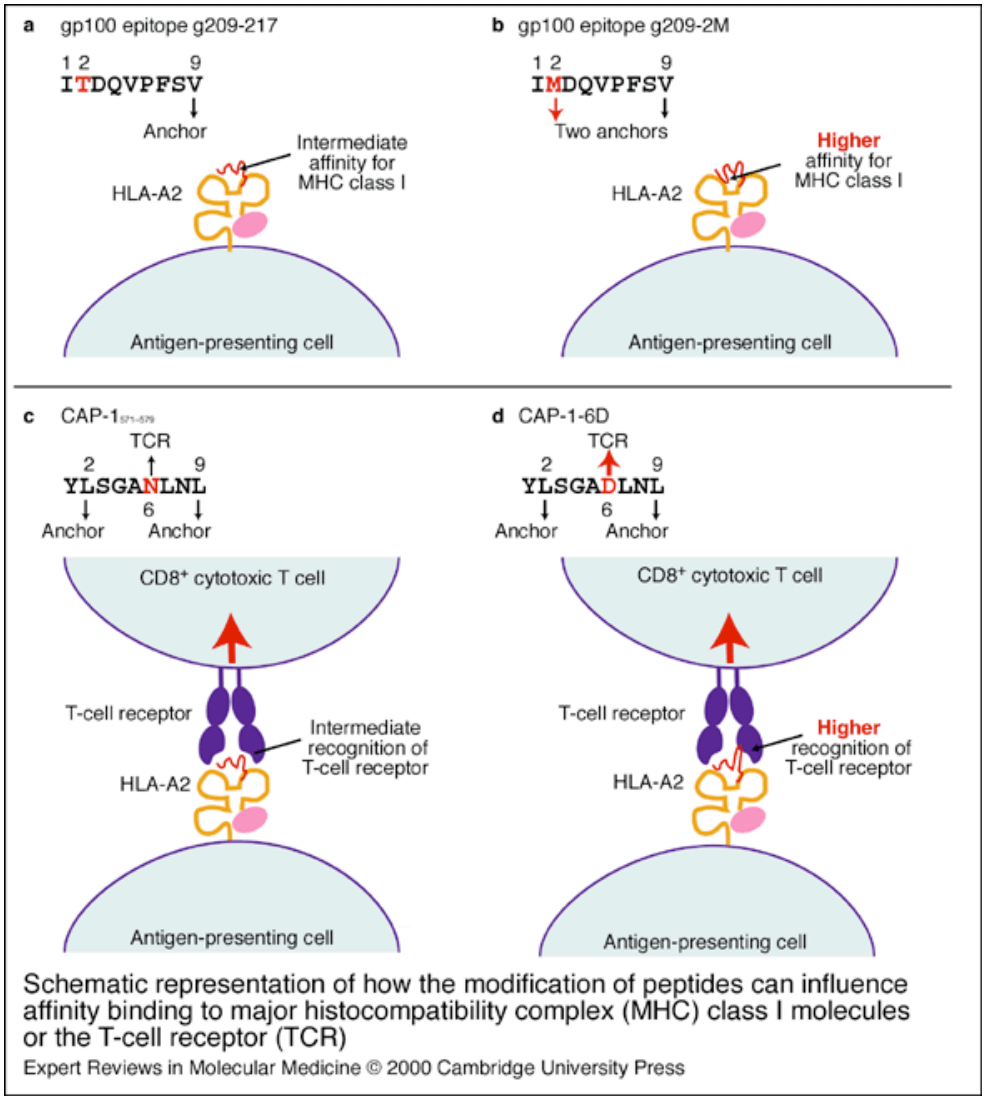
Interaction TCR/CMH-Ag: Présentation / Reconnaissance

anchor

recognition



Schematic representation of how the modification of peptides can influence affinity binding to major histocompatibility complex (MHC) class I molecules or the T-cell receptor (TCR)

Schematic representation of how the **modification of peptides can influence affinity binding to major histocompatibility complex (MHC) class I molecules or the T-cell receptor (TCR).** (a) Substitution of a (**potential anchor**) residue (the second amino acid in this case) in the gp100 epitope g209-217 (ITDQVPFSV). Replacing a threonine residue (T) with a methionine residue (M) results in the modified g209-2M peptide (IMDQVPFSV). (b) This change alters the **binding affinity of the peptide to the HLA-A2** molecule, increasing the affinity of the peptide for the MHC (because of its second anchor position for the MHC), and stabilising the complex (Refs 45, 90). This leads to an increased recognition of the MHC-peptide complex by the TCR. (c) Substitution of an amino acid residue that is not an anchor for MHC can, instead, **alter the recognition of MHC-bound peptide for the TCR**. For example, replacing an asparagine residue (N) at position 6 of the CAP-1571-579 peptide (YLSGANLNL) with an aspartic acid residue (D) results in the modified CAP-1-6D peptide (YLSGADLNL). (d) This modification does not alter the binding affinity of the peptide to the HLA-A2 molecule, but rather **increases the recognition** of the MHC-peptide complex by the TCR

Heidi Hörig; Expert Reviews in Molecular Medicine, 2000

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Importance de l'affinité du TCR pour l'activation lymphocytaire (1)

CD8$^+$ T Cell Activation Is Governed by TCR-Peptide/MHC Affinity, Not Dissociation Rate[1]

Shaomin Tian,* Robert Maile,*† Edward J. Collins,*‡ and Jeffrey A. Frelinger[2]*

CD8+ TCR-transgénique (P14 = TCR Va2 Vb8)
Ag = GP33-41 / LCMV
Or GP33-41 mutants (C9L, K1MC9M)

Table I. *Peptide variants of gp33*

Mutation Site	Sequence	Peptide Mutant
None	KAVYNFATC	gp33
P1	KAVYNFATC	K1A, K1D, K1E, K1F, K1L, K1N, K1R, K1S, K1V, K1W, K1Y
P2	KAVYNFATC	A2D, A2E, A2F, A2G, A2K, A2L, A2N, A2R, A2S, A2T, A2V, A2W, A2Y
P3	KAVYNFATC	V3A, V3D, V3E, V3F, V3G, V3K, V3L, V3N, V3R, V3S, V3T, V3W, V3Y
P4	KAVYNFATC	Y4A, Y4C
P6	KAVYNFATC	F6L
P9	KAVYNFATC	C9A, C9D, C9E, C9F, C9K, C9L, C9M, C9N, C9R, C9S, C9V, C9W
P1, P9	KAVYNFATC	K1AC9M, K1MC9M, K1SC9M
P3, P9	KAVYNFATC	V3LC9M
P4, P9	KAVYNFATC	Y4FC9M, Y4SC9M

Table II. *Effects of gp33 variants on T cell cytotoxicity, IFN-γ production, and TCR binding*

Peptide	EC_{50}^{CTL} (nM)[a]	EC_{50}^{IFN} (μM)[b]	K_D (μM)[c]	ΔG (kcal · mol^{-1})[d]	k_{on} (×10^5 M^{-1} · s^{-1})[e]	k_{off} (s^{-1})[e]	$t_{1/2}$ (s)[f]
gp33	16.8 ± 16.8	0.06 ± 0.01	12.2 ± 2.2	−6.79	1.33 ± 0.27	1.35 ± 0.68	0.51
C9F	0.53 ± 0.36	0.56 ± 0.44	9.4 ± 0.5	−6.95	1.19 ± 0.01	1.15 ± 0.01	0.60
C9L	1.36 ± 2.1	0.47 ± 0.63	8.6 ± 0.2	−7.00	0.89 ± 0.02	0.79 ± 0.02	0.88
C9M	1.59 ± 2.67	0.92 ± 0.39	9.1 ± 1.4	−6.96	1.42 ± 0.54	1.2 ± 0.46	0.58
C9V	3.20 ± 5.60	1.23 ± 0.27	10.8 ± 0.3	−6.86	1.16 ± 0.03	1.25 ± 0.03	0.55
K1MC9M	8.58 ± 5.27	0.13 ± 0.09	74.5 ± 13.6	−5.70	0.33 ± 0.08	3.53 ± 0.7	0.20
K1R	49.0 ± 22.0	7.96 ± 1.06	81.6 ± 10.6	−5.65	1.31 ± 0.47	10.6 ± 3.5	0.065
K1SC9M	>50,000	28.0 ± 4.55	267	−4.94	0.01	0.26[h]	2.67
V3LC9M	>50,000	112.8 ± 75.3	264 ± 103[g]	−4.94	ND[g]	ND[g]	ND[g]
Y4A	8,870 ± 5,550	11.57 ± 7.47	63.0 ± 14.3	−5.8	0.38 ± 0.07	2.2 ± 0.57	0.32
Y4FC9M	>50,000	38.55 ± 22.03	681[g]	−4.38	ND[g]	ND[g]	ND[g]
Y4SC9M	>50,000	264.1 ± 157.5	530[g]	−4.53	ND[g]	ND[g]	ND[g]

[a] EC_{50}^{CTL} is the effective concentration of peptide that gives 50% maximum CTL activity as determined by 4-h chromium release assay. The values represent the mean ± SD of three to five experiments.
[b] EC_{50}^{IFN} is the effective concentration of peptide that induces 50% of the maximum amount of IFN-γ as determined by ELISA. The values represent the mean ± SD of three to four experiments.
[c] Determined from steady-state SPR data with software Scrubber. The values represent the mean ± SD of two to three experiments.
[d] Determined by $\Delta G = -RT \ln K_D$, where RT at 25°C is −0.6 kcal · mol^{-1}.
[e] Determined from kinetic SPR data with software CLAMP XP. The values represent the mean ± SD of two to three experiments.
[f] Half-life of dissociation of pMHC from P14 TCR. Determined by $t_{1/2} = \ln 2/k_{off}$.
[g] From Tissot et al. (42); ND, determined.
[h] Derived from $k_{off} = K_D \times k_{on}$.

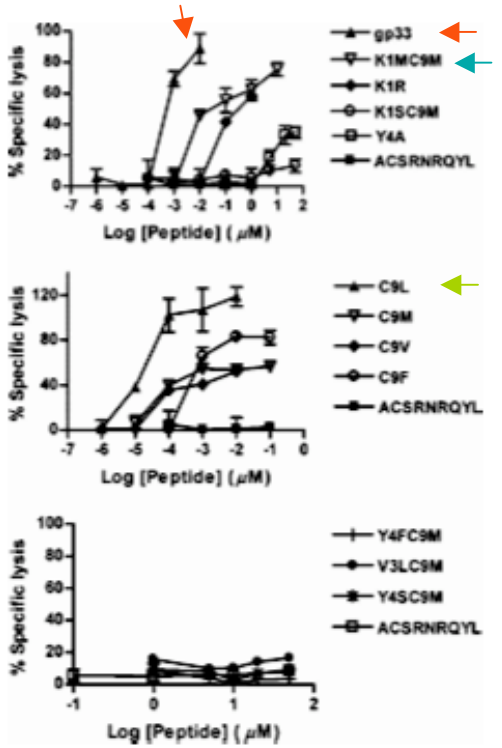
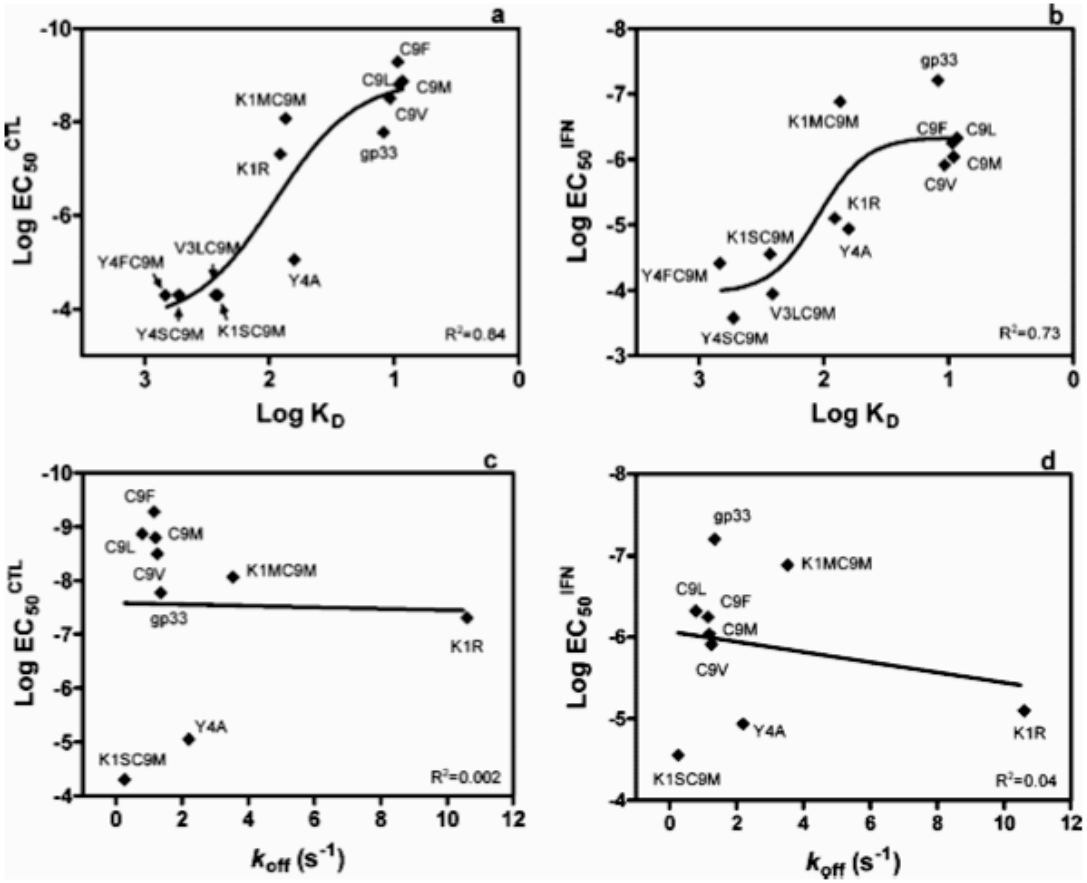
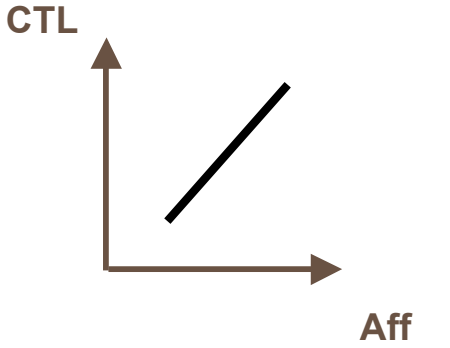


FIGURE 3. Viral epitope gp33 and APLs variably sensitize P14 T cells to kill target cells. EL4 cells labeled with ^{51}Cr and pulsed with peptides at various concentrations were incubated with activated splenocytes from P14 TCR transgenic mice (E:T ratio = 5) for 4 h. Specific lysis of EL4 cells was measured by ^{51}Cr release. Irrelevant HY(K1A) peptide (ACSRN RQYL) was used as negative control. Data are shown as mean ± SD (n = 3), and are representative of three to five independent experiments. Using GraphPad Prism, data were fit into sigmoidal dose-response model to obtain EC_{50}^{CTL} values (shown in Table II) (fitting curves are not shown).

T-

Importance de l'affinité du TCR pour l'activation lymphocytaire (2)





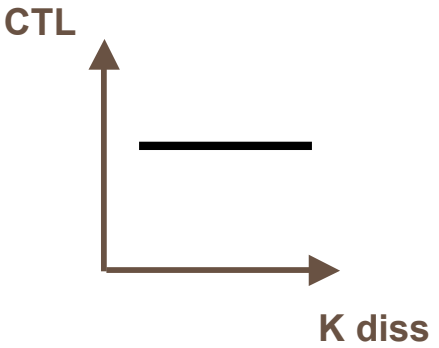


FIGURE 5. CTL response and IFN-γ production are correlated with binding affinity of peptide/D^b to P14 TCR. *a* and *b*, Correlations between log EC_{50}^{CTL} and log K_D (*a*), log EC_{50}^{IFN} and log K_D (*b*). All the data points are shown in Table II. The EC_{50}^{CTL} and EC_{50}^{IFN} values are in unit of M, and K_D in μM. The solid lines are the fittings to Boltzmann sigmoidal equation as follows: Y = bottom + (top − bottom)/(1 + exp((V50 − X)/slope)), using GraphPad Prism. The K_D values for V3LC9M, Y4FC9M, and Y4SC9M are adopted from a previous report (42). Note that the EC_{50}^{CTL} values for K1SC9M, V3LC9M, Y4FC9M, and Y4SC9M were shown on the graph as 50 μM, although the actual values could be higher than that, but are out of the measurable range with CTL assay. *c* and *d*, Correlations between log EC_{50}^{CTL} and k_{off} (*c*), log EC_{50}^{IFN} and k_{off} (*d*). The solid lines in *c* and *d* are linear fittings using GraphPad Prism 4.01. All of the data points are shown in Table II. The EC_{50}^{CTL} and EC_{50}^{IFN} values are in unit of M.

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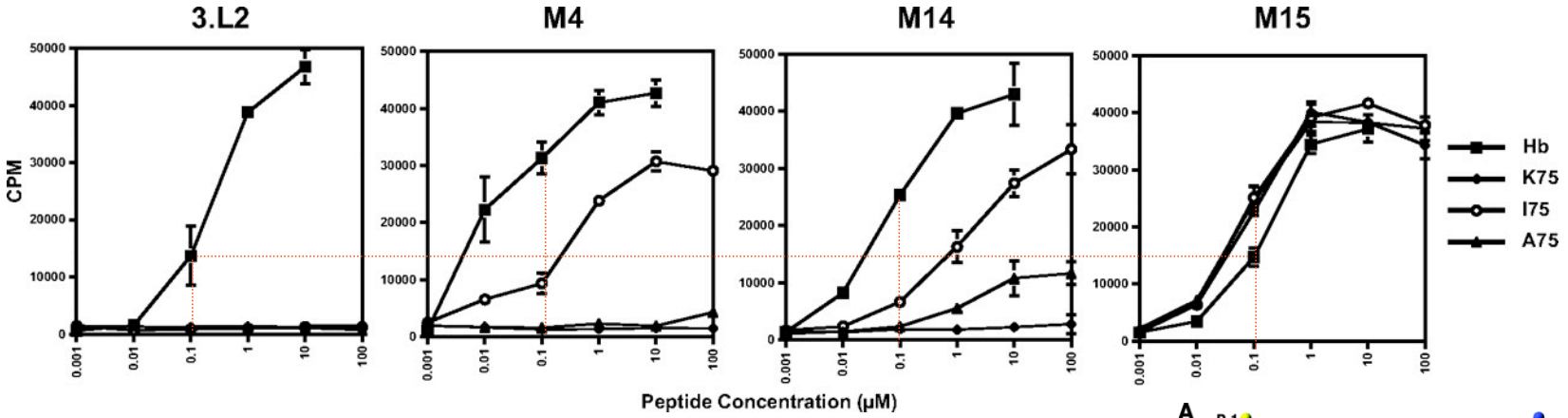
L'affinité ne prédit pas toujours de l'activation et de la réponse lymphocytaire

- **Activation:**
  - Ont été observées :
    - Une diminution de l'activation liée à une augmentation de l'affinité
    - Une augmentation de l'activation liée à une diminution de l'affinité

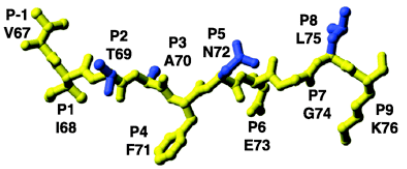
- **Spécificité**

The Study of High-Affinity TCRs Reveals Duality in T Cell Recognition of Antigen: Specificity and Degeneracy
D Donermeyer et al; Journal of Immunology, 2006,



Degeneracy in the recognition of Hb correlates with increased affinity. CD4+ T cell hybridomas expressing 3.L2, M4, M14, and M15 TCRs were stimulated with the indicated peptide concentrations of Hb or APLs of Hb at the P8 (75) position using CH27 as APC. The APLs are designated as described in Fig. 2. The level of T cell stimulation was determined using a bioassay for IL-2. The cpm values of [3H]TdR incorporation into CTLL-2 cells represent the mean ± SD of triplicate values of a representative experiment (n > 3).

A



B

T cell	CDR mutations β:1 2 3 α:1 2 3	K_D (k_{off}/k_{on}) nM	Half-Life s	k_{on} $M^{-1}s^{-1}$	k_{off} s^{-1}
M1	○○○ ○○○	19,860 ± 3,630	6.8 ± 0.7	5,220 ± 410	0.1023 ± 0.0107
M4	○○○ ○○●	580 ± 185	28.5 ± 0.8	42,670 ± 17,430	0.0243 ± 0.0007
M14	○○● ○○●	81 ± 2	48.9 ± 2.4	173,500 ± 3,500	0.0141 ± 0.0007
M15	○○● ●○●	25 ± 5	104.4 ± 9.1	281,500 ± 34,800	0.0068 ± 0.0006

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Activation T : un système dynamique

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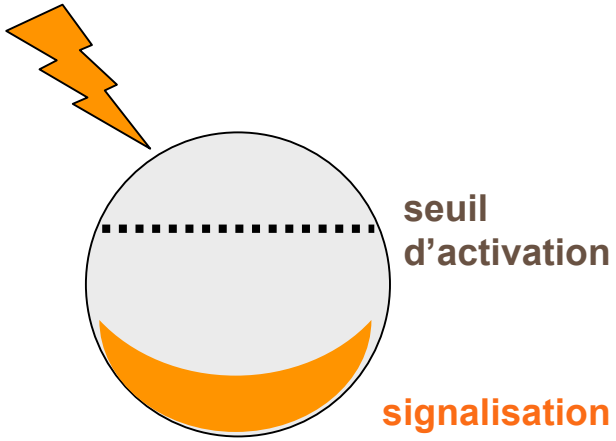
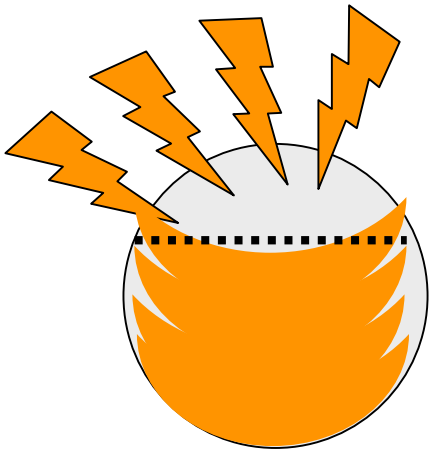
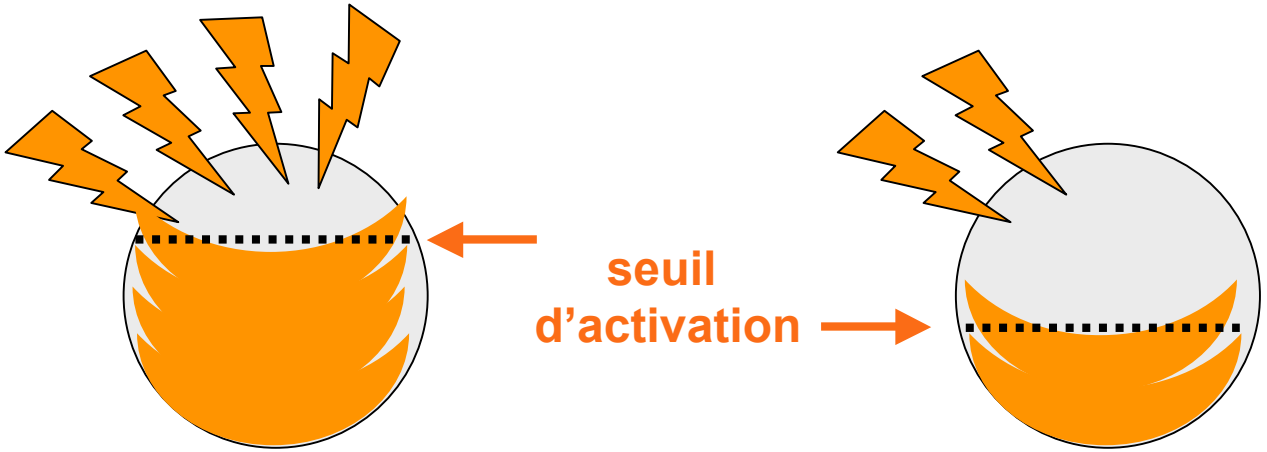
Stimulation réitérative du lymphocyte: « kinetic proofreading » model

- **Activation du lymphocyte T** si **interaction TCR/CMH-Ag suffisante (seuil d'activation)**
- **Interactions :**
  - Force d'interaction (Affinité)
  - Temps d'interaction (Koff)
  - Nombre d'interactions (x n; Avidité)
- **Activation progressive des molécules de signalisation (phosphorylation) : seuil d'activation**
- **Seuil critique pour initier une activation fonctionnelle**
- **Si interaction insuffisante, (dissociation du complexe TCR/CMH-Ag) avant initiation de l'activation: les groupements phosphates seront éliminés par les phosphatases cellulaires (McKeithan 1995)**





Seuil d'activation: T naïf vs T mémoire

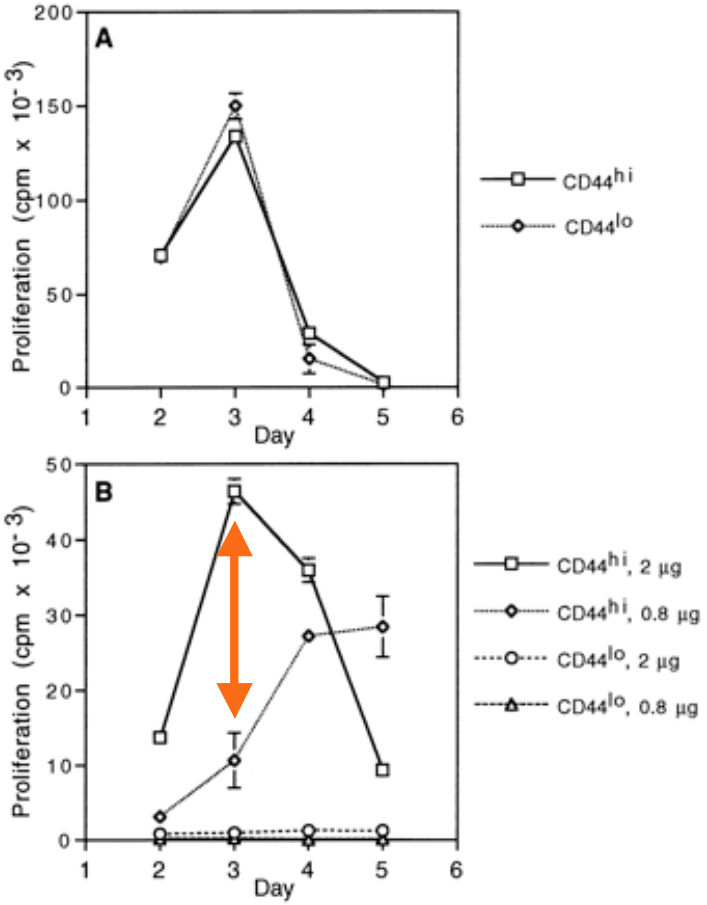


T naïf

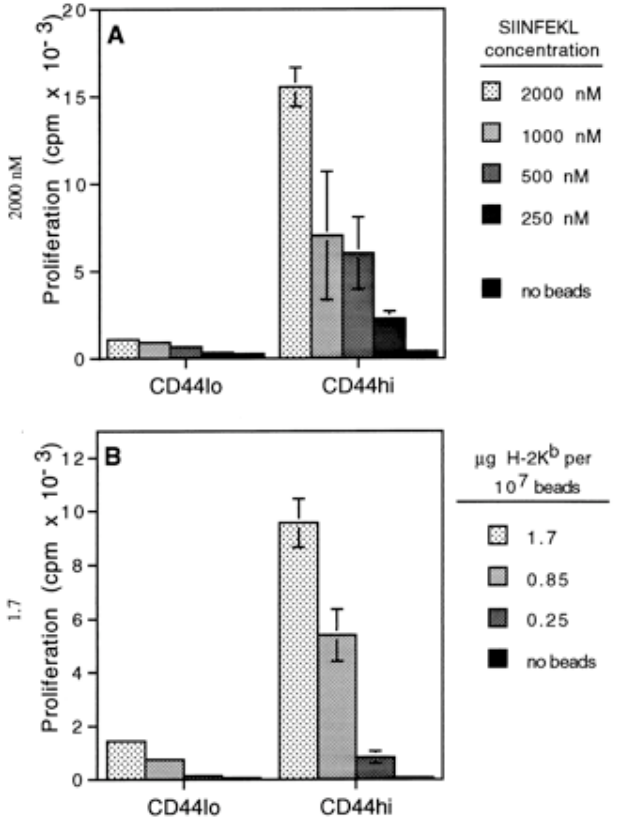
T mémoire

Seuil d'activation: T naïf vs T mémoire

Spleen cells+Ag



Beads H-2Kb+Ag



- **Proliferation of OT-1 CD8⁺ LNC in response to Kb/OVA$_{257–264}$ and rIL-2 is predominantly due to the CD44^{high} subset of cells. *A*, LNC (5 x 10^4-sorted CD44^{low}CD8⁺ and CD44^{high}CD8⁺) from OT-1 mice were cultured with 1 x 10^5 latex beads coated with H-2K^b at 1.7 *µ*g protein/10^7 beads and pulsed with OVA$_{257–264}$ peptide at the indicated concentration.**

Curtsinger, JI 1998

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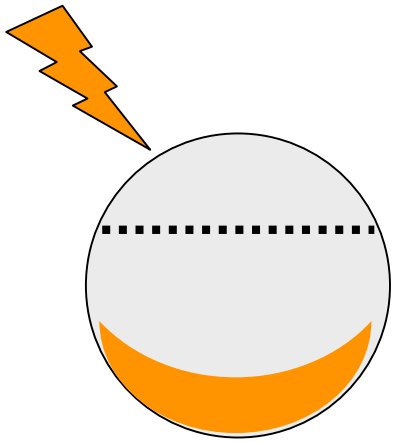
Affinité versus Avidité

force du signal

Affinité

++++



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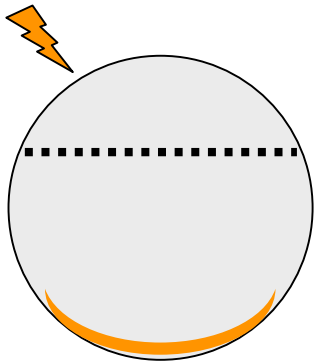
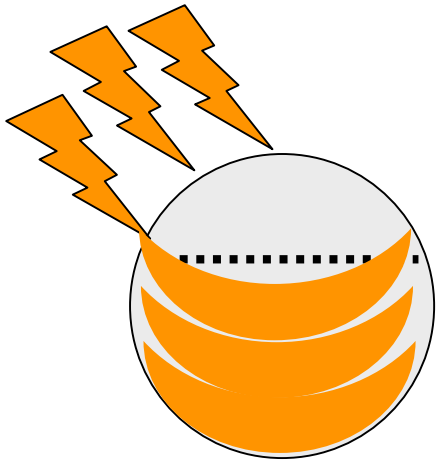
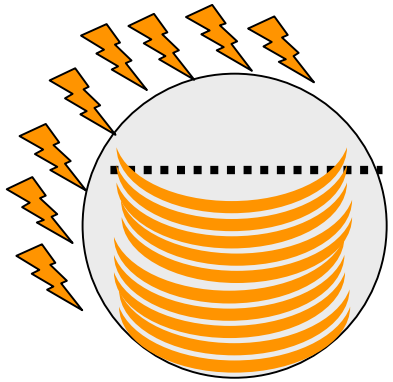


Avidité



x n

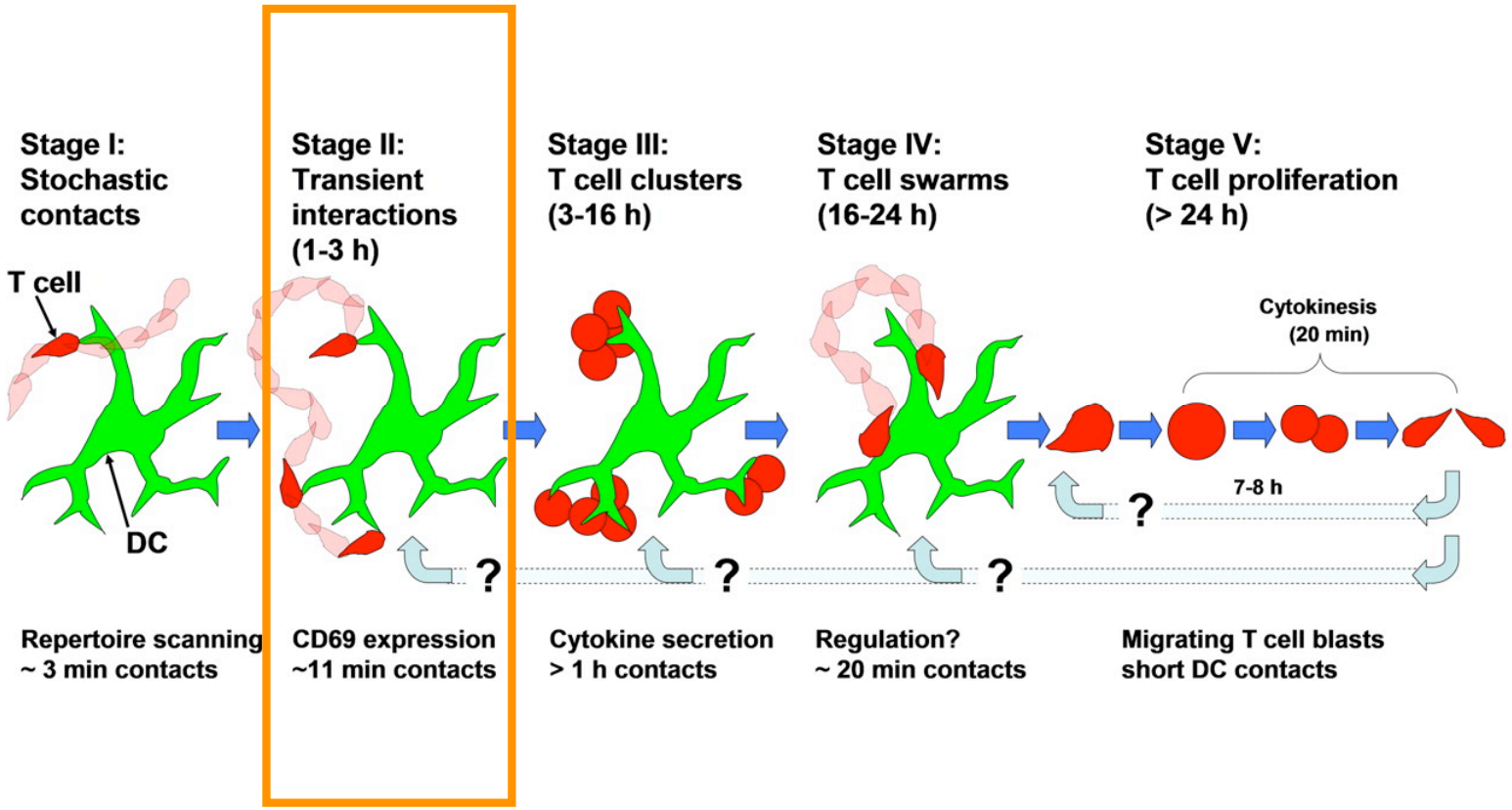
sommation du signal



x n

DC scanning

- **Contacts multiples avec complexes CMH-Ag à la surface des DC**



Nombre d'interactions TCR/CMH-Ag dépend de l'affinité du TCR

Immunity, Vol. 5, 137–146, August, 1996, Copyright ©1996 by Cell Press

Correlation Between the Number of T Cell Receptors Required for T Cell Activation and TCR–Ligand Affinity

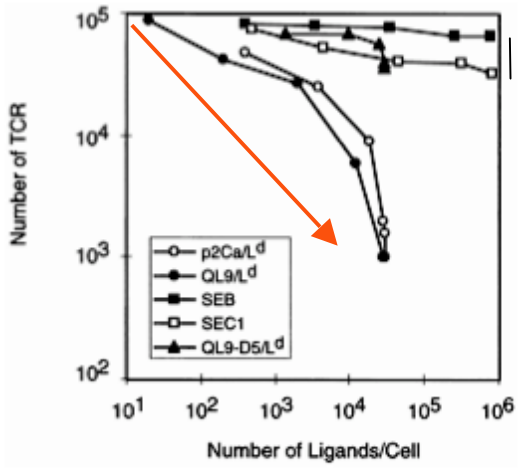


Figure 4. Relationship of Number of Ligands per Target Cell and the Number of TCRs Required for Activity

The number of 2C TCR required for recognition of various ligands was plotted as a function of the number of ligands on the target cell. The number of p2Ca–L^d, QL9–L^d, QL9-D5–L^d, SEB–class II, or SEC1–class II complexes was calculated from the K_a of the peptide or SE for MHC and the concentration of peptide or SE added in the assay. The number of TCR required for recognition was determined from the concentration of 1B2 Fab fragments required at 50% inhibition of cytolysis, using a K_a of 2×10^9 M^{-1} and 100,000 TCRs per T cell, as described in Experimental Procedures. Data are taken from Table 2.

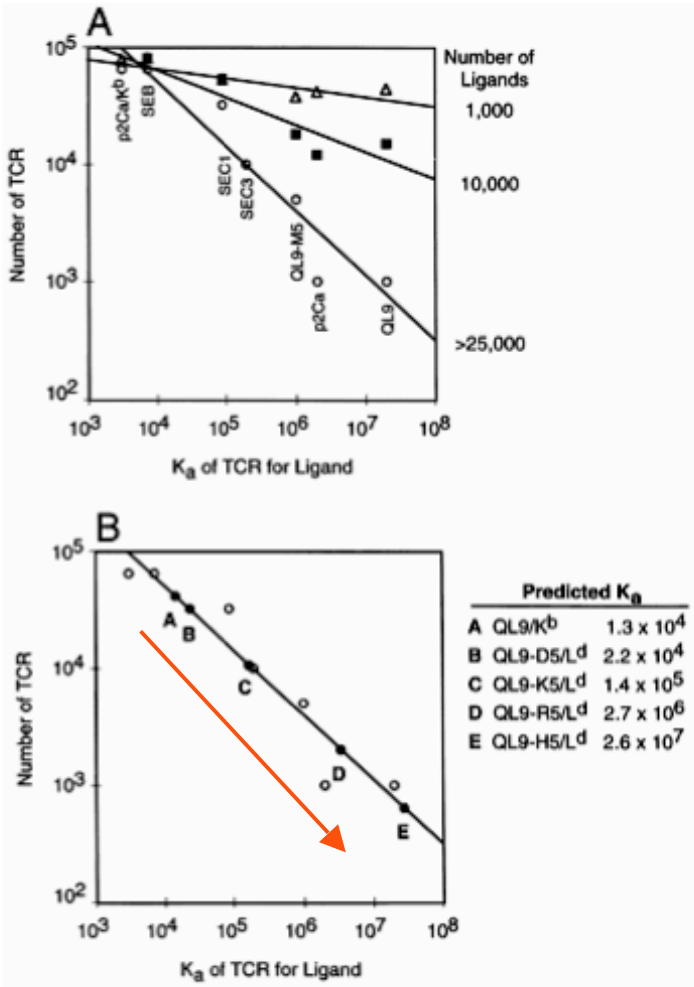


Figure 5. Correlation Between the Binding Affinity of the TCR for Its Ligand and the Number of TCRs Required for T Cell Activity

(A) K_a values from previous reports are summarized in Table 1. The number of TCR at >25,000 ligands (open circles) was determined at saturating ligand concentrations for each of the peptides or superantigens that have a published binding affinity for the 2C TCR. The number of TCRs at 1,000 (open triangles) and 10,000 (closed squares) ligands were calculated by regression analysis from the data summarized in Table 2.

(B) K_a values for the TCR interaction with the indicated peptide–class I complexes were predicted from the number of TCRs required for activity. The curve determined from known K_a values for several TCR–ligand interactions was plotted versus number of TCRs at saturating ligand concentrations (open circles) to generate a standard curve. The inhibition analysis was used to determine the number of TCRs required at 3×10^{-5} M QL9-H5, QL9-R5, QL9-H5 and 10^{-4} M QL9-D5 with T2-L^d and 10^{-4} M QL9 with T2-K^b. These values were plotted on the standard curve (closed circles) to calculate by linear regression a predicted K_a value for TCR binding to each of the peptide–class I complexes.

Modèle:

Clone 2C anti-AlloAg

Spé p2Ca/Ld; QL9/Ld

Nb ligands: Δ[peptide]

Nb TCR: [Fab 1B2], bloque TCR

Affinité: Mutants de QL9

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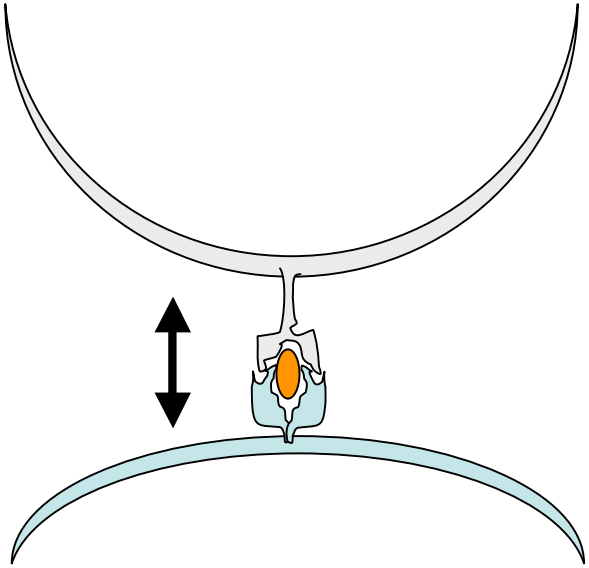
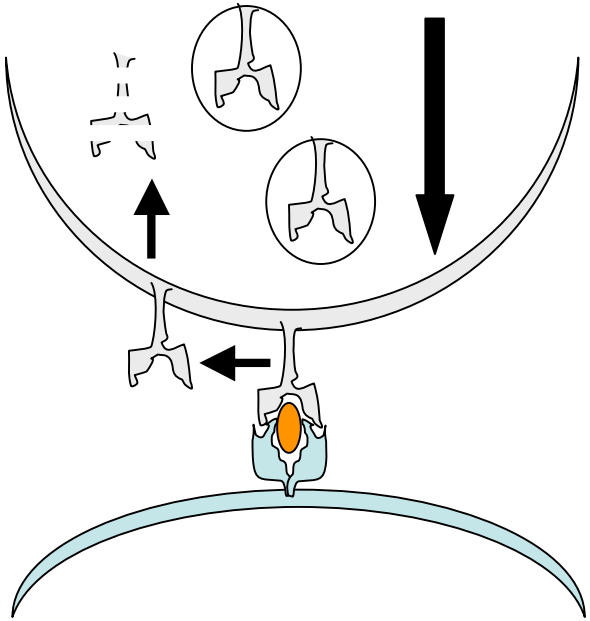
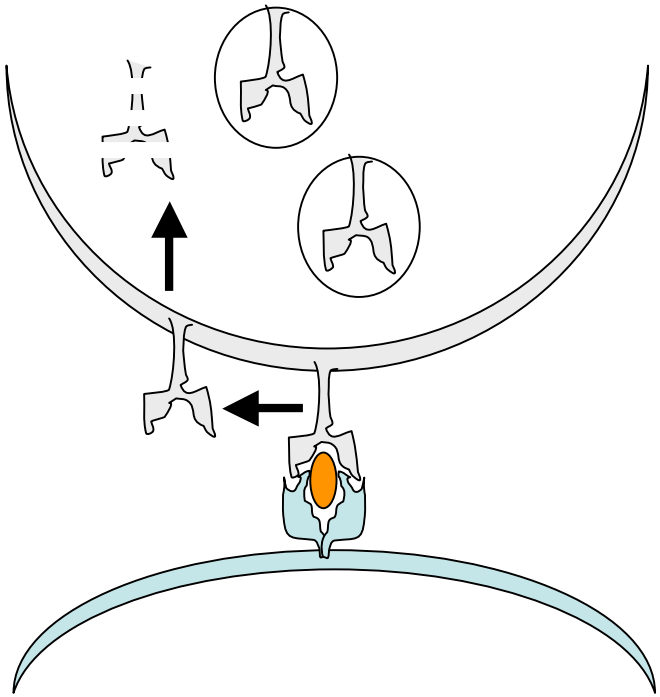
Nombre d'interactions TCR/CMH-Ag: aspects moéculaires

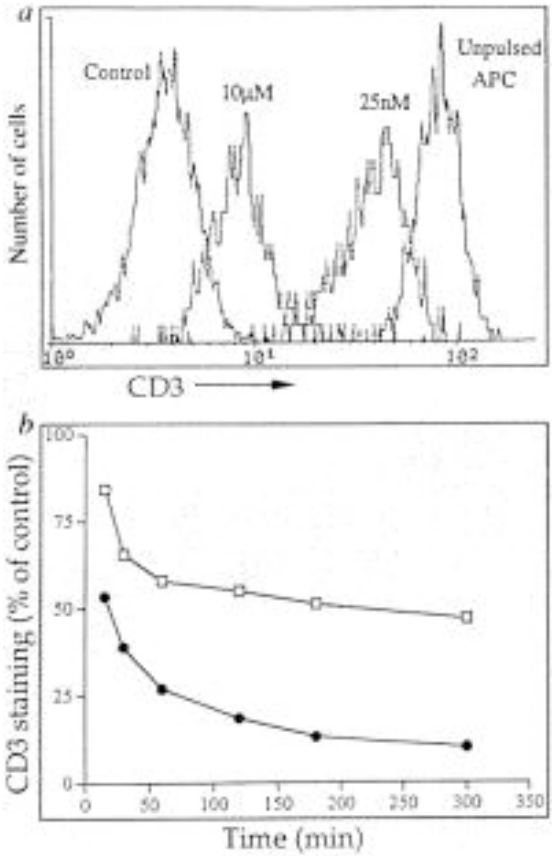
- 1 à 50 complexes CMH-Ag sur APC pour activer LyT
- Engagement multiple des TCR pour un complexe CMH-Ag
  - N= 200 contacts répétitifs (Valitutti 1995)
- Temps de contact cellulaire requis ? (Long / Court)
- Temps de contact TCR/CMH-Ag limité pour assurer des engagements répétés des TCR
- Engagement répété de la même molécule de TCR vs différentes molécules de TCR ?





Serial triggering of many T-cell receptors by a few peptide-MHC complexes
Valitutti, Nature 1995

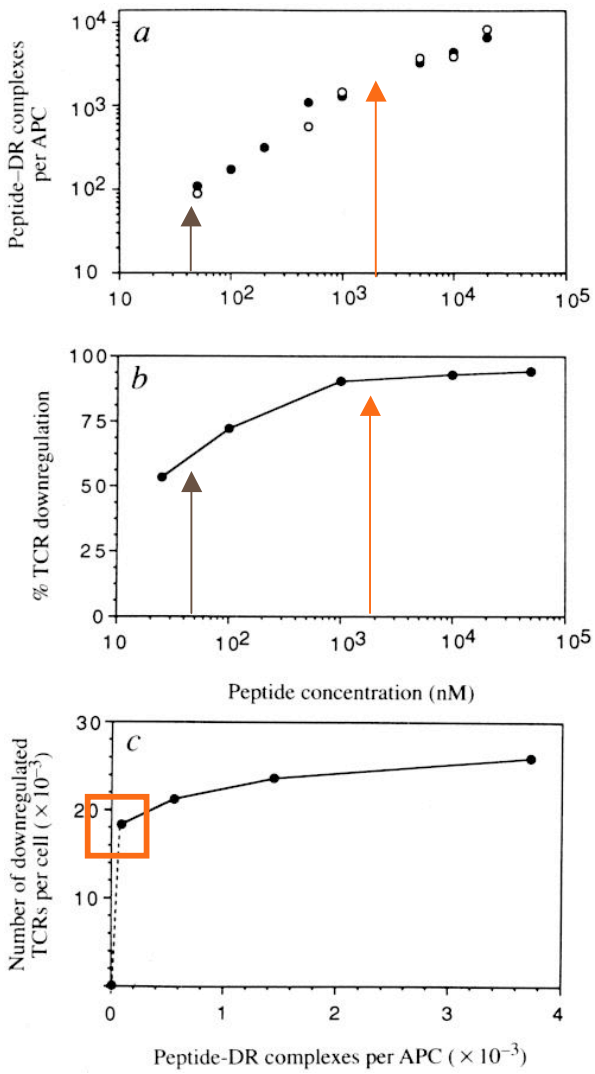




T cells conjugated with peptide-pulsed APCs undergo an antigen-dependent **downregulation of the TCR**/CD3 complex.

Nombre d'engagements

Serial triggering of many T-cell receptors by a few peptide-MHC complexes
Valitutti, Nature 1995



we measured at different antigen concentrations the number of complexes per APC and the number of TCRs downregulated after T-APC interaction. In one series of experiments, EBV-B cells were pulsed with different concentrations of **125 I-labelled peptide** and the number of peptide-DR complexes per cell was calculated at each peptide concentration Figure 3(a). In parallel experiments, the fraction of TCRs downregulated by APCs pulsed in the same conditions was measured Figure 3(b). From comparison of the two curves it is estimated that **APCs pulsed with high peptide concentrations (20 micromolar) display approximately 7,500 complexes and induce downregulation of 93 percent of TCRs.** Strikingly, **APCs pulsed with a low peptide concentration (50 nM) display only approximately 100 peptide-DR complexes, yet they downregulate 62 percent of TCRs.** The relationship between the number of peptide-DR complexes per APC and the number of TCRs downregulated per T cell is shown in Figure 3(c). This plot clearly shows that each peptide-DR complex must engage a large number of TCRs in successive rounds. This effect is dramatic at low complex density, where approximately **100 complexes can trigger up to 18,000 TCRs**, but is less marked at high complex density, indicating that **a single peptide-DR must be able to trigger 180 TCRs in successive rounds.** This figure may increase at lower complex density and could be an underestimate as it is unlikely that all complexes present on an APC may be available to the responding T cell.

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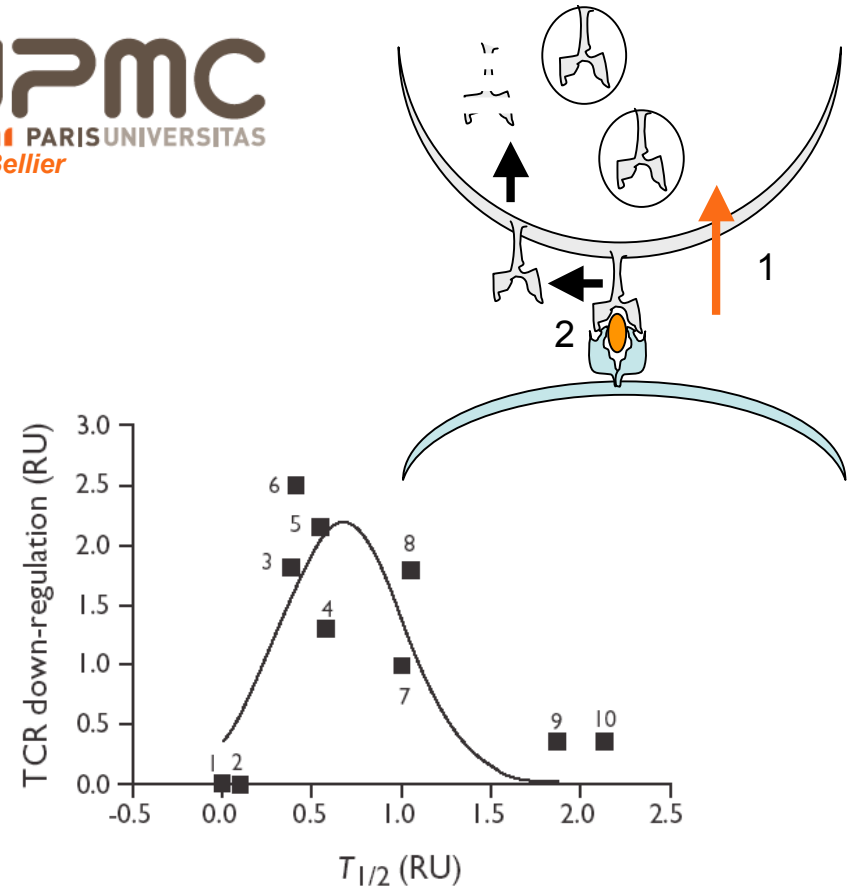
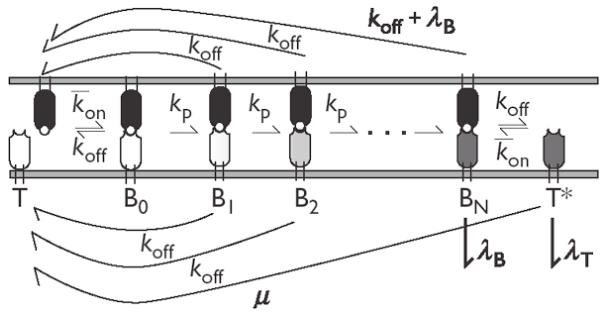
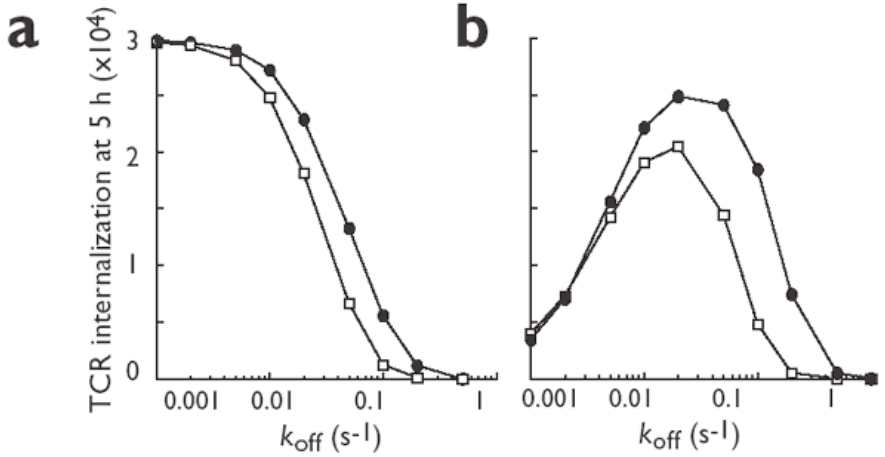
Internalisation du TCR

Coombs et al 2002



TCR down-regulation as a function of TCR-pMHC half-life.
Data are derived from 5-h TCR down-regulation experiments done with a panel of TCR mutants derived from a Kb-VSV-specific TCR (N30.7). Each point represents the indicated TCR-pMHC pair: 1, V98D- Kb-VSV; 2, V98L-Kb-VSV; 3, N30.7-KbA158T-VSV; 4, G99A-KbA158T-VSV; 5, G97/99A-KbA158T-VSV; 6, G97A-KbA158T-VSV; 7, N30.7- Kb-VSV; 8, G97A-Kb-VSV; 9, G97/99A- Kb-VSV; 10, G99A-Kb-VSV. Half-lives are shown as relative units (RU) normalized to the half-life for the N30.7-Kb-VSV (number 7) interaction4. TCR down-regulation data are shown as RU normalized to TCR down-regulation measured for N30.7 hybridomas that were interacting with 10-4 - 10-5 M VSV peptide.





TCR internalization as a function of the dissociation rate constant. Results are shown for the monomer model (closed circles) and the dimer model (open squares). (a) Only activated bound TCRs are internalized, lambdaB = lambdaD = 0.003 s-1, lambdaT = 0 s-1. (b) **Only unbound activated TCRs are internalized**, lambdaB = 0 s-1, lambdaT = 0.003 s-1.

> Internalisation du TCR dépend:

- –De la constante de dissociation
- –De temps de contact

> Après libération du CMH-Ag = Modèle 2

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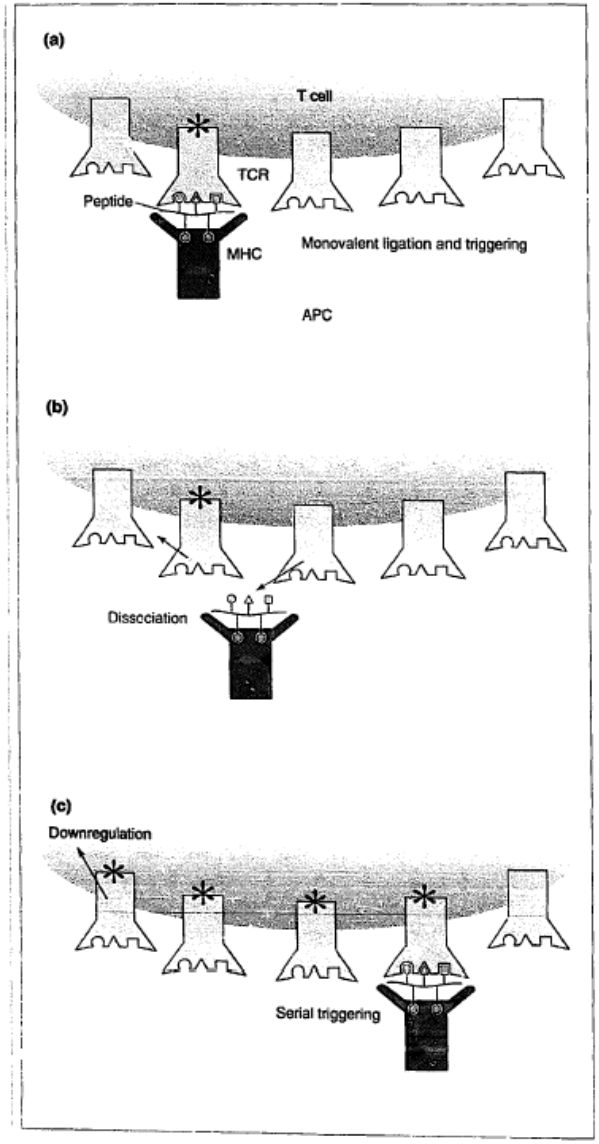
Interactions en série



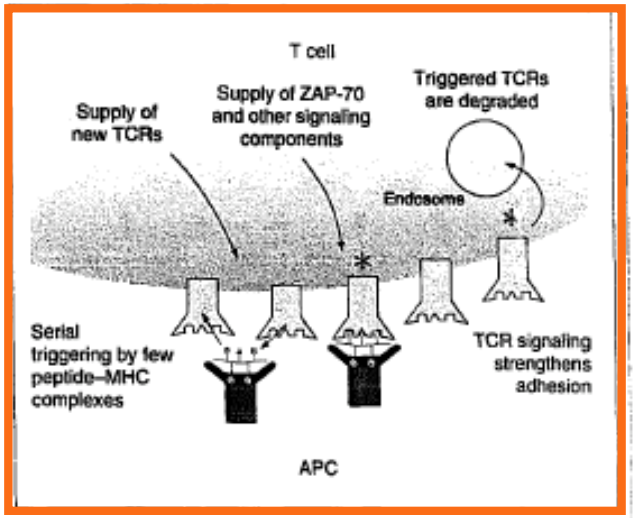


Fig. 3. *Parameters that influence TCR triggering and signaling at the level of the T-cell–APC interaction. Sustaining the process requires a continuous supply of signal transduction components from the cytosol, as well as new TCRs to substitute for those that are downregulated and degraded*[39]*. Cell–cell adhesion is boosted by TCR signaling and favors TCR engagements with peptide–MHC complexes. Abbreviations: see Fig. 1 legend.*

Serial triggering of TCRs: a basis for the sensitivity and specificity of antigen recognition

Salvatore Valitutti and Antonio Lanzavecchia

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Serial triggering of TCRs: a basis for the sensitivity and specificity of antigen recognition

Salvatore Valitutti and Antonio Lanzavecchia

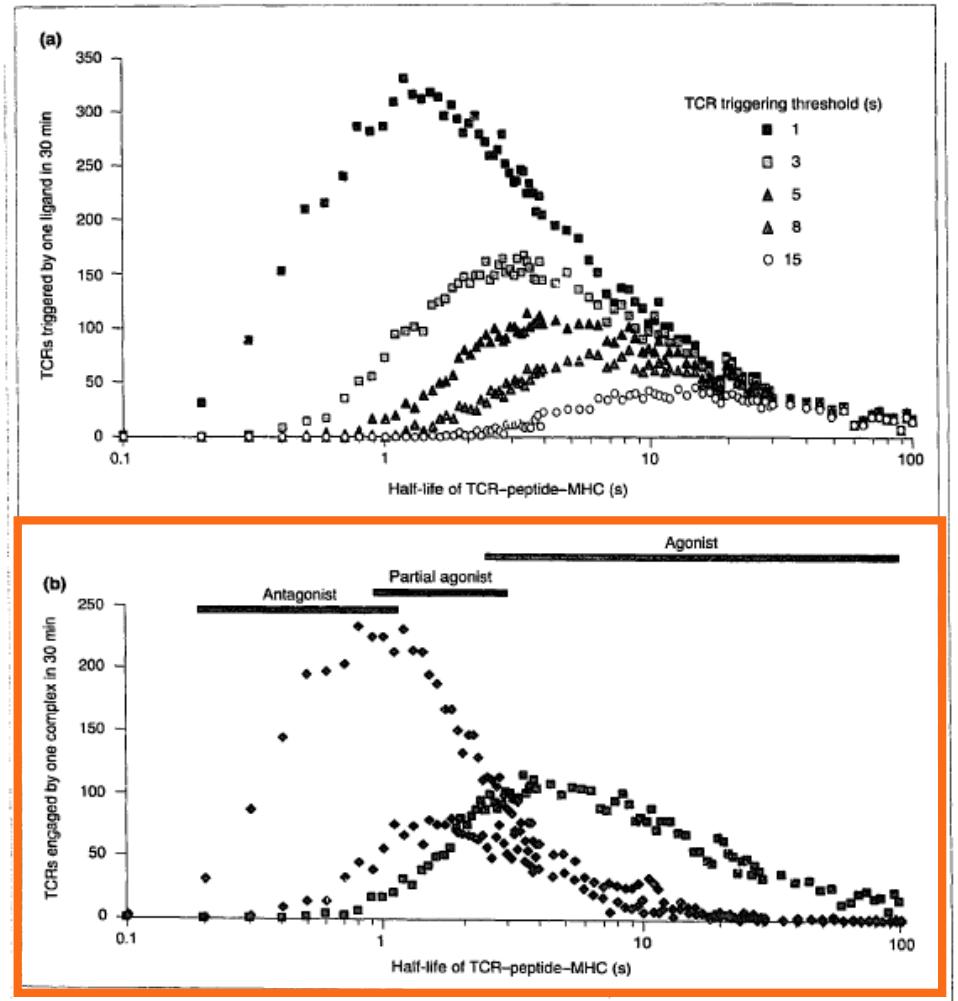
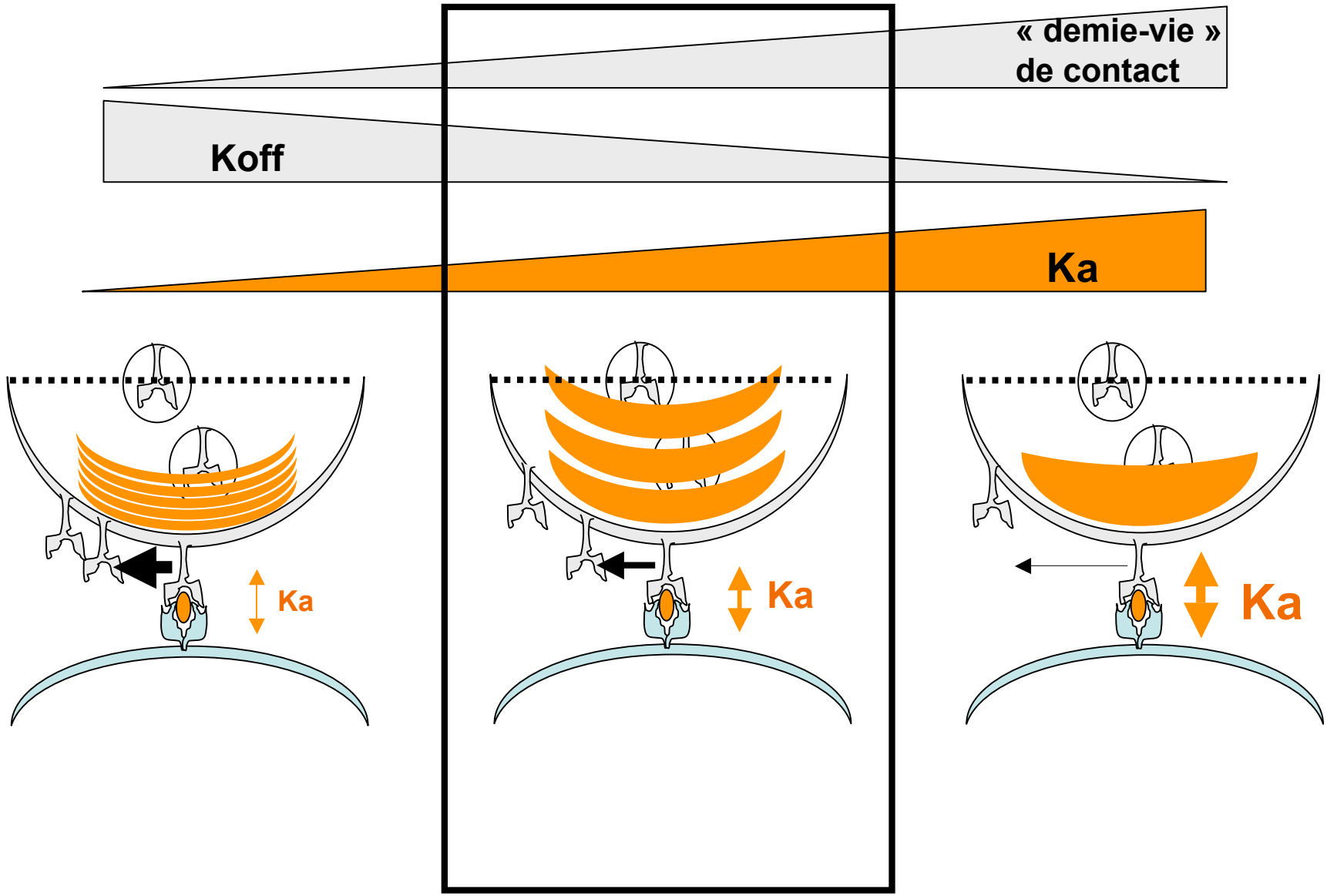


Fig. 2. *Serial TCR triggering or inactivation as a function of complex stability and time required for TCR triggering. (a) Simulation describing the number of TCRs triggered in 30 min as a function of the half-life of TCR–peptide–MHC interactions. Each curve is calculated for times required for TCR triggering ranging 1–15 s. (b) Simulation describing the number of TCRs fully triggered (green), partially triggered (blue) or inactivated (red) as a function of the half-life of TCR–peptide–MHC interaction. It is assumed that ligation for >5 s results in full TCR triggering, ligation for 3–5 s in partial triggering and ligation for 1–3 s in TCR inactivation. Abbreviations: see Fig. 1 legend.*

	Agonist	Antagonist
T1/2	3-10	0.5-1
#TCR	100	250

Influence du temps de contact



Influence de la « demie-vie » de contact

L.J. Carreño et al. / Immunobiology 211 (2006) 47–64

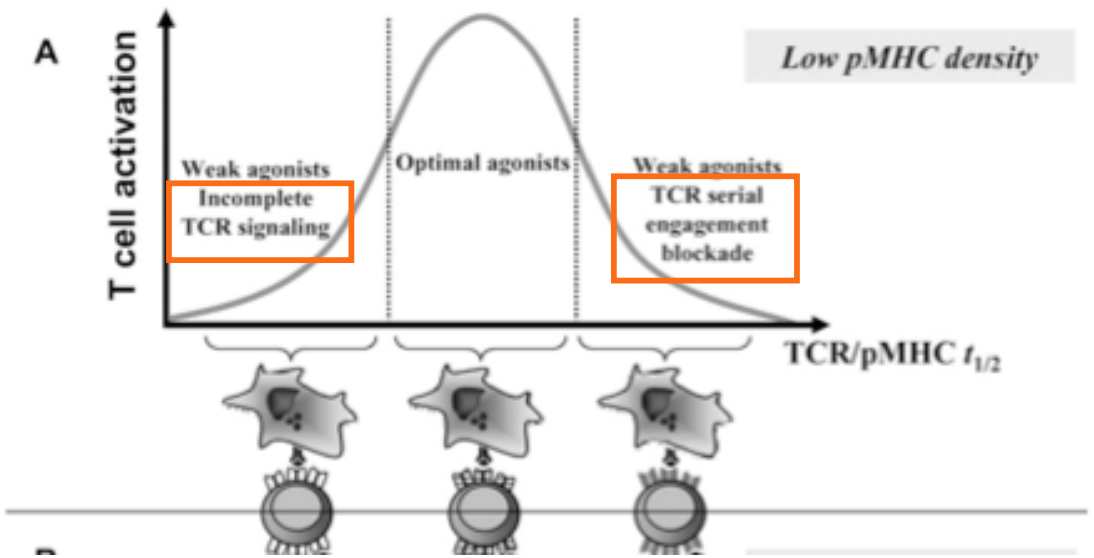
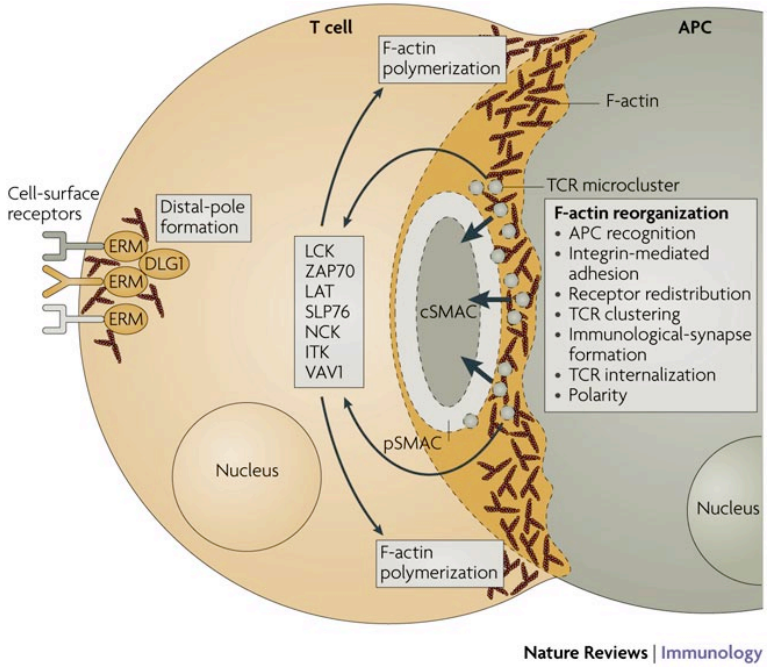


Fig. 2. T cell activation depends on TCR/pMHC interaction half-life and pMHC density. (A) When T cells interact with cognate pMHC at low density on the APC, efficient activation takes place within an optimal range of TCR/pMHC interaction half-life. Interactions with short half-lives cannot complete necessary intracellular signals for T cell activation, due to impairment on TCR kinetic proofreading. TCR/pMHC interactions with excessively long half-lives impair T cell activation due to TCR serial engagement blockade. At low pMHC density, the plot of T cell activation versus TCR/pMHC half-life results in a Gaussian distribution, in which only pMHCs that interact with intermediate half-lives with the TCR behave as agonists (Kalergis et al., 2001; Coombs et al., 2002). (B) When T cells interact with cognate pMHC at high density on the APC, T cell activation can take place when the half-life of the TCR/pMHC interaction is intermediate or high, because serial engagement requirement no longer applies (Gonzalez et al., 2005). At high pMHC density, the plot of T cell activation versus TCR/pMHC half-life results in a sigmoid distribution, in which pMHCs that interact with intermediate and long half-lives with the TCR behave as agonists.

B. Bellier

Importance de la synapse immunologique pour le maintien du contact

- **Favorise interactions multiples TCR/CMH-Ag**
- **Rôle dans la transduction du signal ?**
  - Formation de la synapse est associée à l'activité PI3K
  - Blocage de la synapse empêche signal calcique (tardif) et la production de cytokine (IFNg) mais ne perturbe pas les événements de signalisation précoces (phosphorylation ERK)
- **Lien étroit entre activation T et maintien de la structure « synapse »**
  - De la meme façon qu'il existe des paramètres d'interaction du TCR optimum, la formation des suprastructures cSMAC/pSMAC dépend de la demi-vie d'intéraction du TCR



Recruitment and activation of proximal signalling intermediates — such as lymphocyte-specific protein tyrosine kinase (LCK), ZAP70 (-chain-associated protein of 70 kDa), LAT (linker for activation of T cells), SLP76 (SRC homology-2-domain-containing leukocyte protein of 76 kDa), NCK (non-catalytic region of tyrosine kinase), ITK (interleukin-2-inducible T-cell kinase) and VAV1—is probably initiated at sites of T-cell receptor (TCR)-microcluster formation[80, 84]. Recruitment of these molecules leads to the activation of filamentous (F)-actin-regulatory proteins, which control actin polarization in T cells. Interestingly, signals that arise from TCR microclusters not only induce F-actin polymerization, but are required for the stable formation and movement of TCR microclusters, and ultimately lead to the organization of the mature immunological synapse (consisting of the central and peripheral supramolecular activating clusters; cSMAC and pSMAC, respectively)[13, 22, 29, 80]. In addition, global F-actin reorganization resulting from T-cell activation leads to lamellipodium formation and distal-pole formation, facilitating T-cell–APC (antigen-presenting cell) recognition[11]. DLG1 (discs-large homologue-1)[61], ERM (ezrin, radixin and moesin) and F-actin also polarize to the rear of the T cell to form the distal-pole complex[11, 48]. It is thought that this complex recruits some receptors to the rear of the cell, away from the immunological synapse.

Conséquences sur l'immunodominance

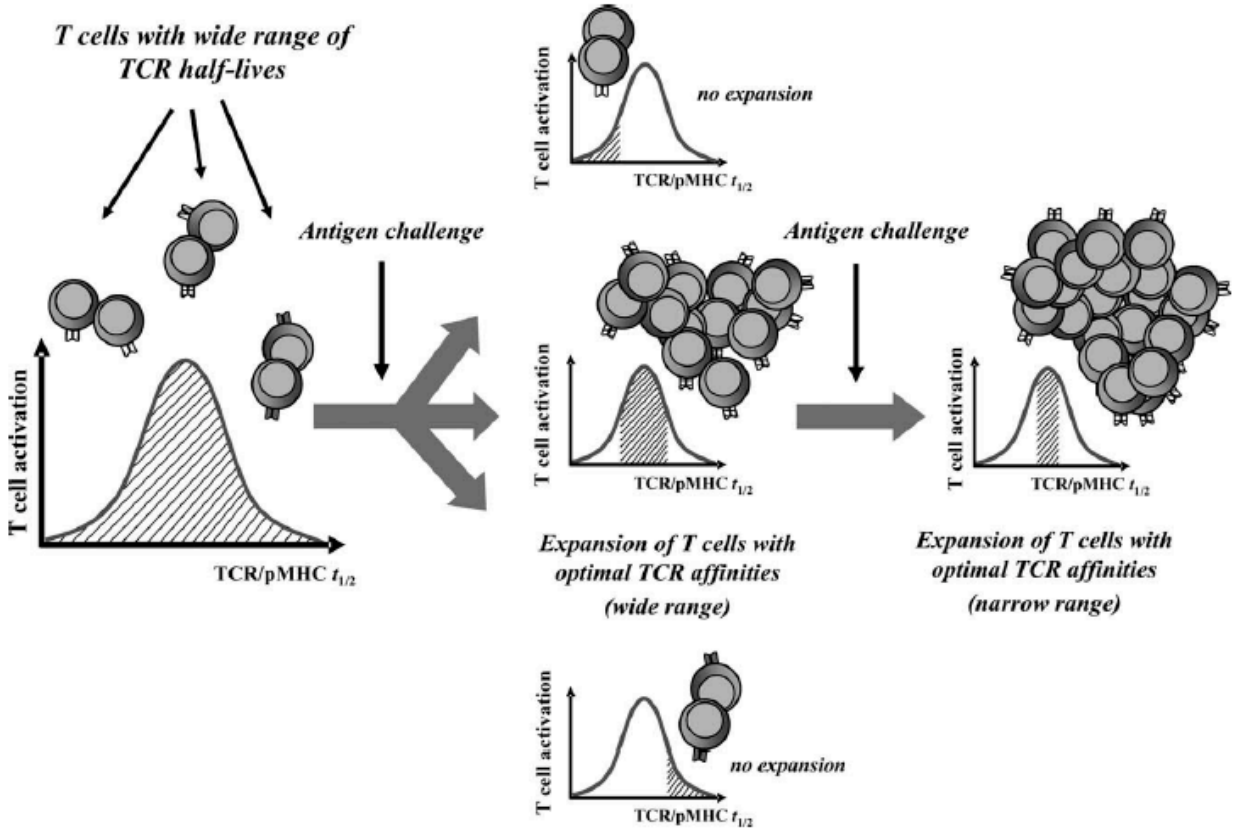
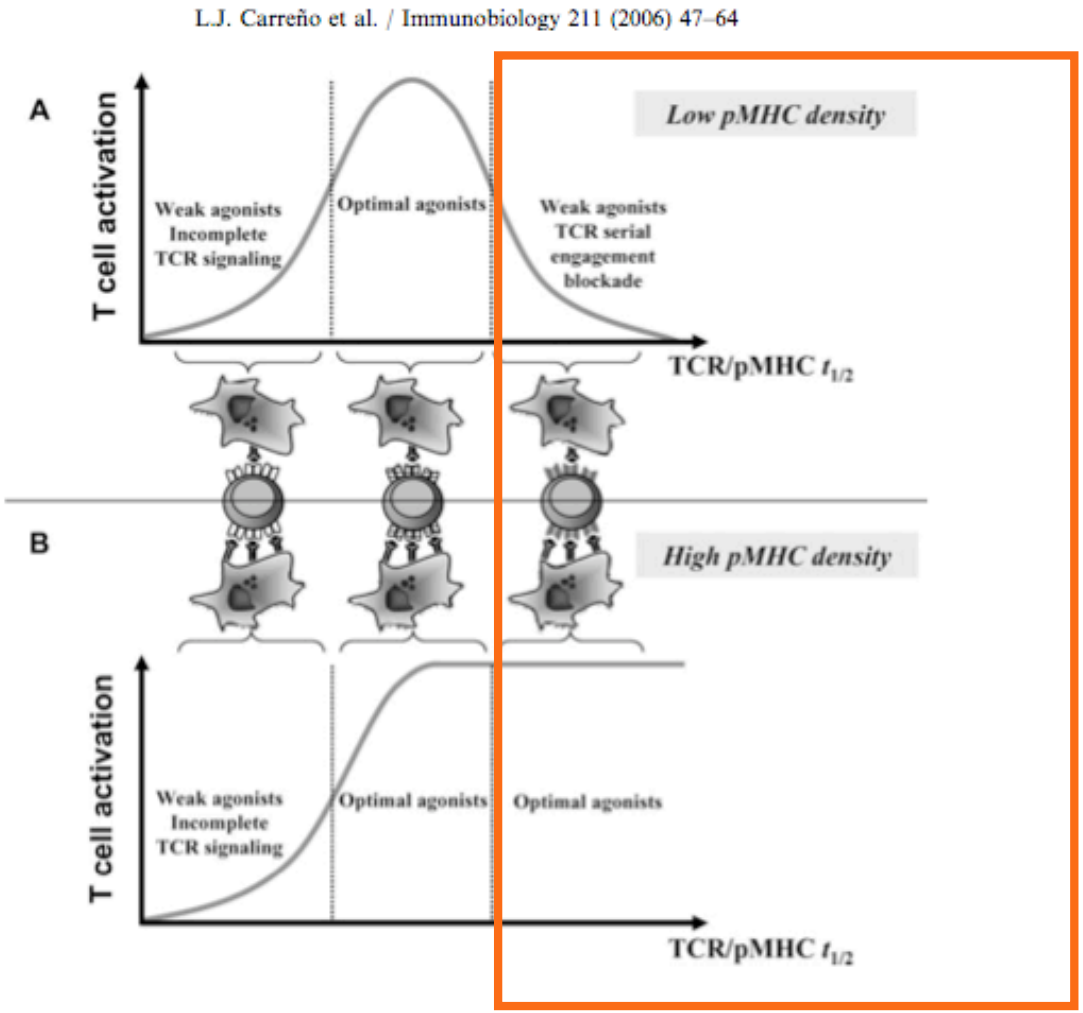


Fig. 3. Clonal expansion of T cells in response to antigenic challenge is determined by the half-life of TCR/pMHC interaction. Before antigen challenge, the T cell population shows a wide range of half-lives for the TCR/pMHC interaction. After the first antigen challenge, only T cells bearing TCRs with optimal half-lives are clonally expanded and the global TCR/pMHC half-life of the responding T cells shifts towards an optimal value. Although subsequent antigenic challenges do not lead to a significant increase in the overall TCR affinity of the T cell population, there is a selection of T cells within a narrower range of TCR/pMHC half-lives.

Influence de la densité de complexe CMH-Ag



B. Bellier

Cas de l'échappement viral

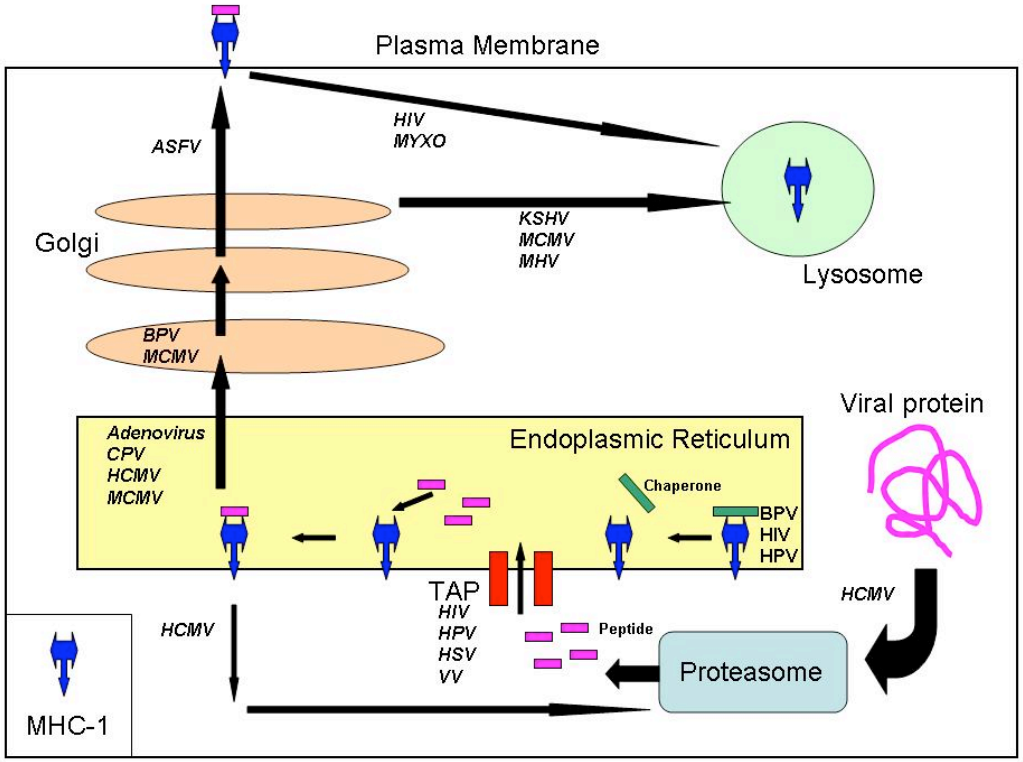
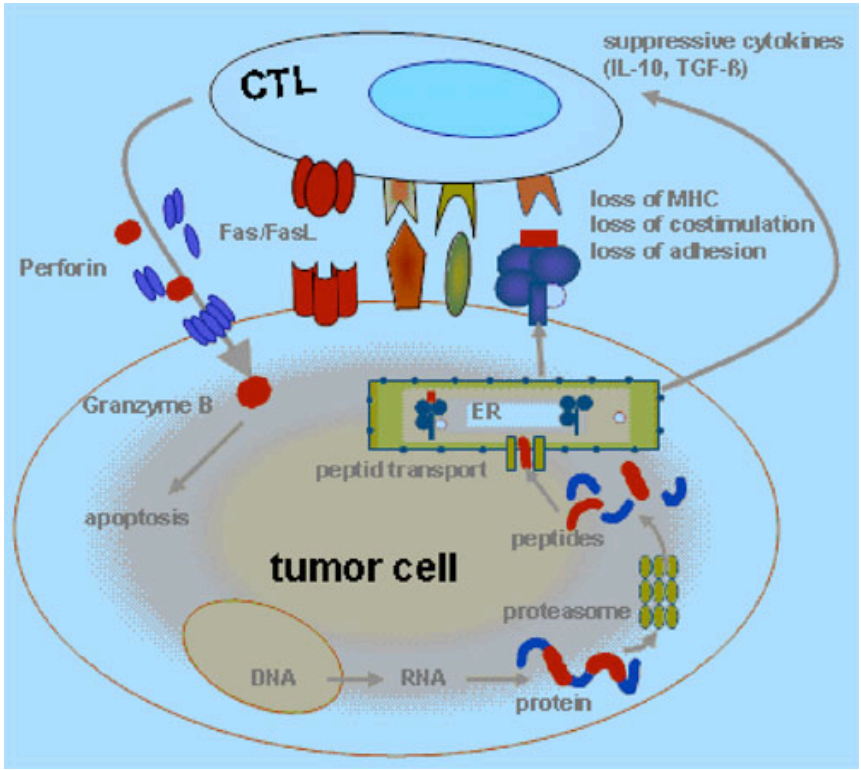


Table 5. Virus mechanisms to evade MHC-1 expression

Virus	Gene / Gene Products	Function
Adenovirus	E3/19K	Retention of MHC-1 in ER
African Swine Fever Virus	Unknown	Dispersion of trans-Golgi network, slowing of MHC-1 to plasma membrane
Bovine Papillomavirus	E5	Retention of MHC-1 in Golgi, repression of MHC-1 gene promoter
Cowpox Virus	Unknown early gene	Retention of MHC-1 in ER
Human Cytomegalovirus	pp65	HLA-DR segregation and destruction, phosphorylation of 72-kDa transcription factor
	US2	Destruction of MHC-1 heavy chains
	US3	Retention of MHC-1 in ER
	US6	Inhibition of TAP
	US11	Destruction of MHC-1 heavy chains
Human Immunodeficiency Virus	Nef	Endocytosis of MHC-1 and CD4
	Tat	Repression of MHC-1 gene promoter
	Vpu	Destabilization of newly synthesized MHC-1
	Unknown	Inhibition of TAP
Human Papilloma Virus	E7	Repression of MHC-1 gene promoter, repression of TAP gene promoter
Herpes Simplex Virus	ICP-47	Inhibition of TAP
Kaposi's Sarcoma Herpes Virus	K3 K5	Targets MHC-1 for degradation
Murine Cytomegalovirus	gp37/40	Retention of MHC in ER / Golgi
	gp48	Targets MHC for degradation
Murine Herpes Virus	mK3	Targets MHC-1 for degradation
Myxoma Virus	m153R	MHC-1 internalization and degradation
Porcine Reproductive and Respiratory Syndrome Virus	Unknown	Down regulation of MHC-1 expression
Vaccinia Virus	UL49.5	Inhibition of TAP

A review of viral strategies to evade selected components of the host immune system, Bruce Wobeser

Cas de l'échappement tumoral



Tumor escape from immune recognition: loss of HLA-A2 melanoma cell surface expression is associated with a complex rearrangement of the short arm of chromosome 6MJ Maeurer, MJ Maeurer et al; Clinical Cancer Research, 1996

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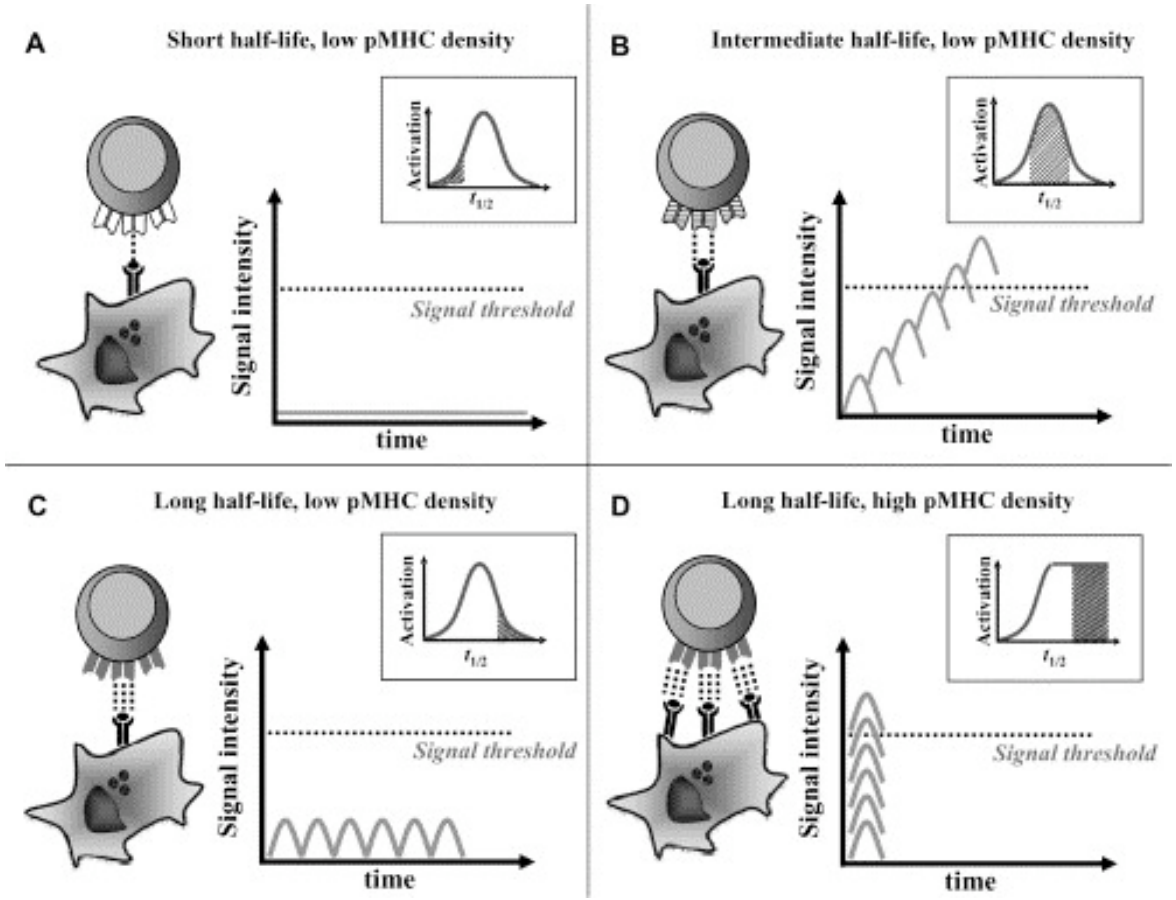
B. Bellier

Importance des paramètres de fixation du TCR en fonction de la fréquence des complexes CMH-Ag

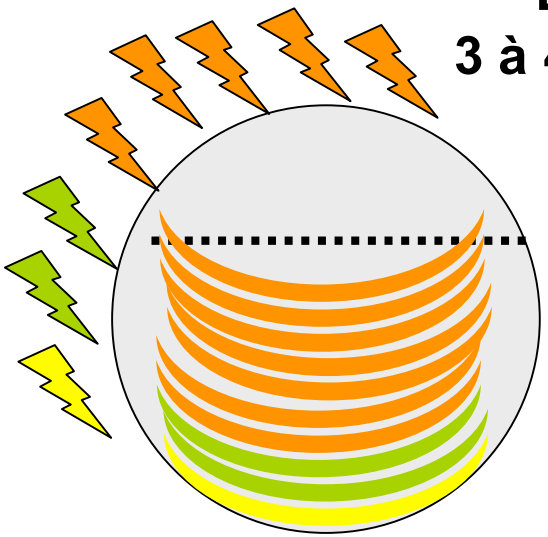


The temporal TCR signal summation model suggests the existence of an optimal TCR/pMHC half-life for T cell activation in response to low-density cognate pMHC ligand. (A) TCR/pMHC interactions with short half-lives fail to activate the T cell (inset) in response to low-density pMHC due to summation of TCR signalling not reaching the activating threshold. (B) TCR/pMHC interactions with optimal half-life lead to efficient T cell activation (inset) as a result of summation of productive TCR signalling that reaches the activating threshold. (C) TCR/pMHC interactions with prolonged half-lives fail to activate T cells (inset) in response to low-density pMHC, due to TCR signals being generated at low frequency (TCR serial engagement blockade) and fail to be added one over the other due to intrinsic signal decay. Thus, T cell activation is impaired (inset) as activating threshold is not reached. (D) However, TCR/pMHC interactions with prolonged half-life can lead to T cell activation (inset) in response to high pMHC-density on the APC, because multiple productive TCR signals are generated simultaneously. These signals can be added and the T cell activation threshold reached.

Paramètres quantitatifs de l'activation

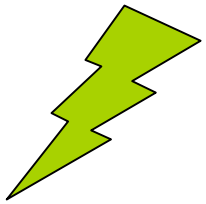
10^5 TCR / T

Engagement de
3 à 400 TCR différents

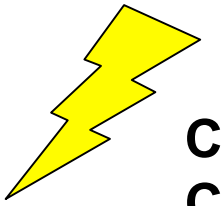


x 1- 200 / t = 2 - 30 s

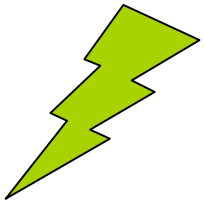
Dépende de la présence de



CD8



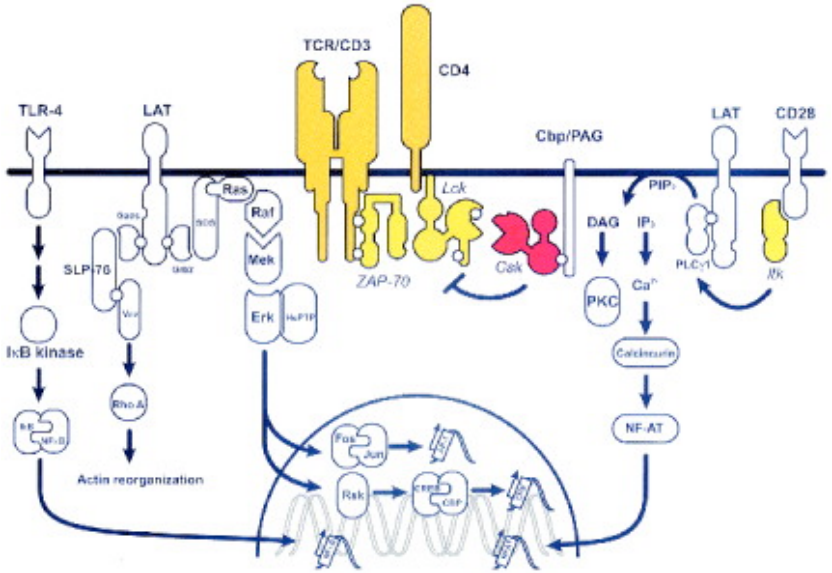
CD28
CD40L



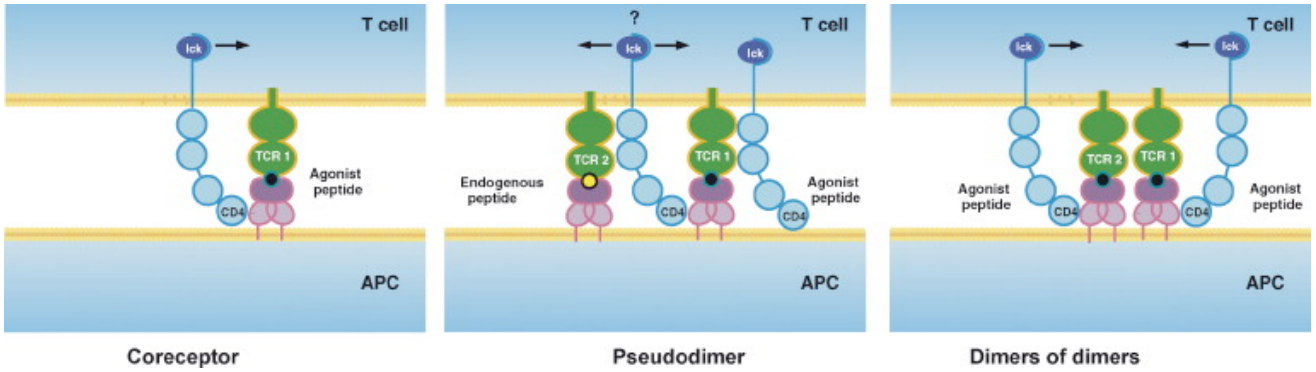
LFA-1

Rôle des molécules adaptatrices et de costimulation

- **Transduction du signal :** renforcement des voies de signalisation



- **Modèles d'initiation de l'activation**
  - **Importance des Ag du soi**



Michelle Krogsgaard, Sim Imm 2007

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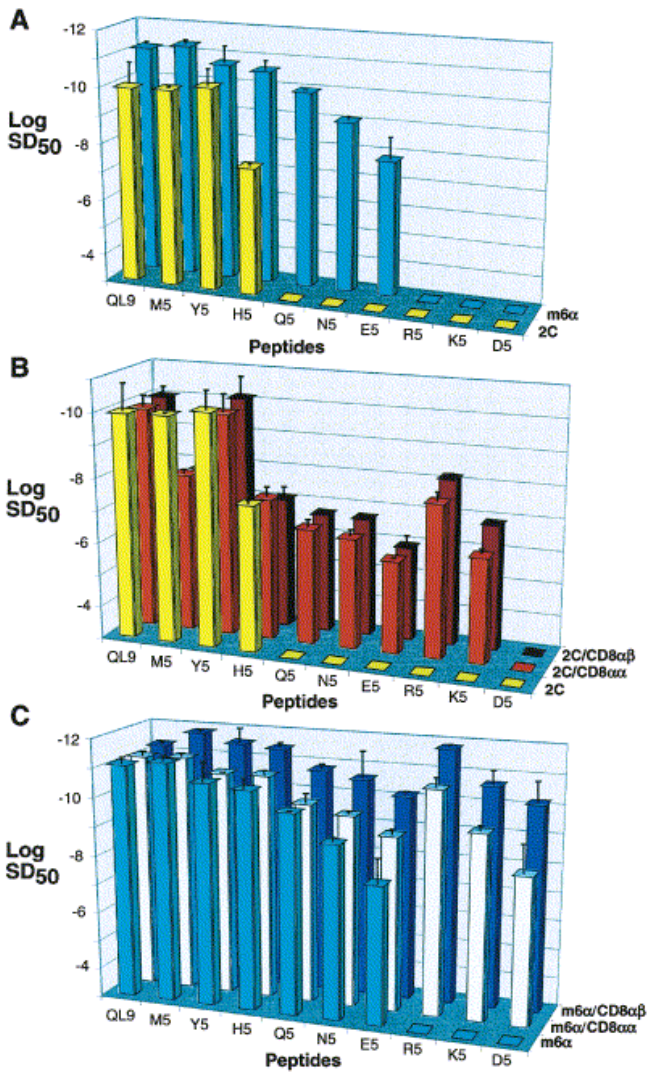
B. Bellier

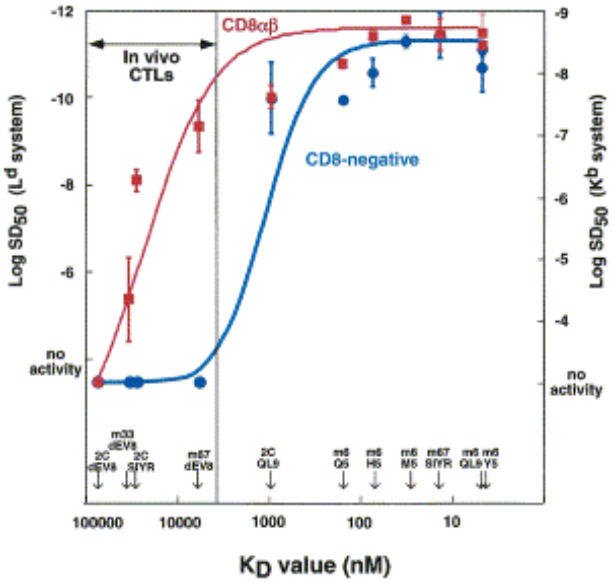
Rôle des molécules adaptatrices : CD8

Holler D , Imm 2003



The αβ-negative T cell hybridoma 58-/- was cotransfected with the 2C wt α chain gene and one of five different α chain genes from 2C and the mutants m6α m13α m33α and m67α . Stable transfectants that expressed similar levels of TCR were identified based on their staining with anti-Vα antibody KJ16

Sensitization Doses of Various QL9 Position 5 Variant PeptidesIL-2 production by transfectants stimulated with various peptides was measured as described in the Experimental Procedures. The amount of peptide that yielded 50% of the maximum IL-2 release (SD50) was calculated by linear regression of IL-2 curves (see Figure S1 at http://www.immunity.com.gate1.inist.fr/cgi/content/full/18/2/255/DC1). The log of the SD50 value was plotted for each of the peptides used to stimulate transfectants: (A) 2C and m6_ CD8-negative; (B) 2C CD8-negative, 2C/CD8__ and 2C/CD8__ (C) m6_CD8-negative, m6_CD8__ and m6_CD8__ In Figure 3, Figure 4 and Figure 5, log SD50 values represent the mean of at least two assays. Error bars indicating one standard deviation are included for each point (for some points these error bars are not apparent as they are smaller than the size of the symbol).



Relationship of Peptide Activity and TCR:pepMHC AffinitySD50 values of various peptides were plotted versus the equilibrium affinity constants (KD) of the corresponding TCR:pepMHC interaction (Table 1). In order to include values from the Ld and Kb systems, SD50 values from the two different antigen-presenting cell systems (T2-Ld and T2-Kb) are shown on the two Y-axes. The points represent data derived from CD8-negative transfectants (blue circles) and CD8__transfectants (red squares) of the corresponding TCR transfectant. The corresponding TCR/pepMHC interactions are shown at the bottom of the figure. The range of KD values that correspond to affinities measured for known TCR:syngeneic MHC interactions from in vivo CTLs are shown. This range falls exclusively in the CD8-dependent category, as determined by TCR-transfection studies (not necessarily anti-CD8 antibody inhibition studies).

The significant enhancement in recognition of the peptides could be improved by one to two orders of magnitude by the introduction of CD8 into transfectants

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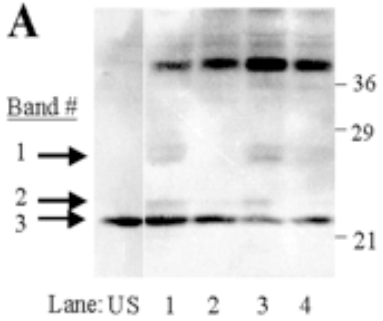
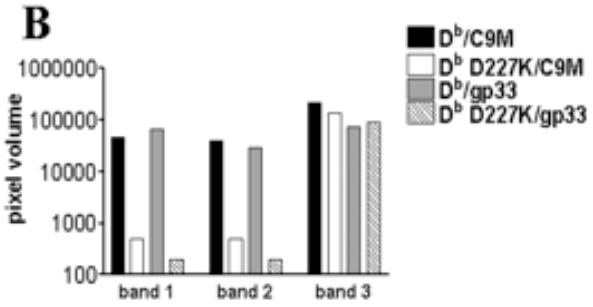
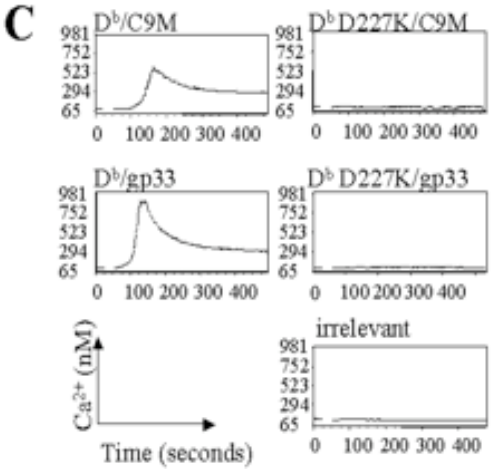
B. Bellier

Molécule adaptatrices: Transduction du signal

- **Model:**
- **TCR-transgenic CD8 (P14)**
- **GP33 (KAVYNFATC) ou C9M (KAVYNFATM; affinity >) peptides presented by**
- **H-2Db tetamers or tetramers containing the D^b D227K mutation, which has been shown to abrogate CD8 interaction with MHC**

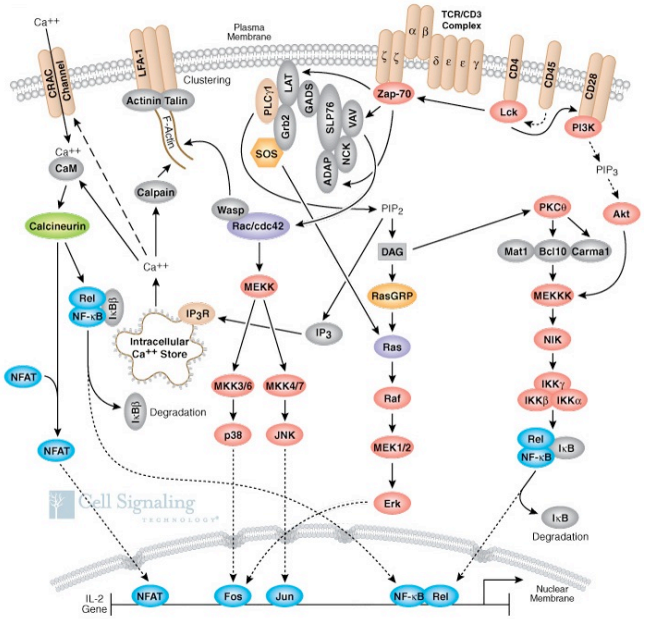






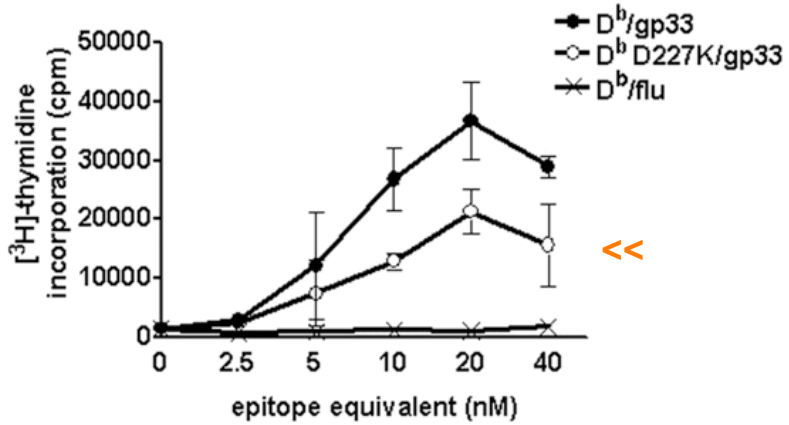
Kerry JI 2003

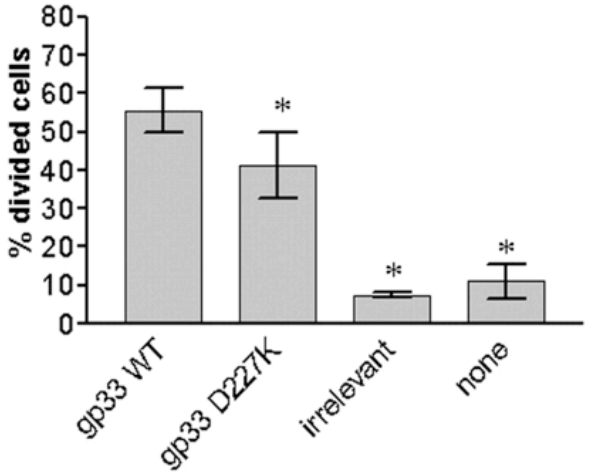
$CD8^+$ P14 splenocytes were stimulated for 3 min with tetramers at 37°C, and phosphotyrosine activity was detected by Western blot. Cells were unstimulated (US) or stimulated with D^b/gp33 (*lane 1*), D^b D227K/gp33 (*lane 2*), D^b/C9M (*lane 3*), or D^b D227K/C9M (*lane 4*). Data are representative of three independent experiments. *B*, Quantitation of band intensity of tetramer-stimulated cells. To measure the intensity of the bands, pixel volume of identical areas of the gel were determined for each lane and corrected for local area background. Two bands missing in the D^b D227K stimulated and one control band are plotted. *C*, Calcium mobilization of cells treated with indicated tetramers. Data are representative of two independent experiments.

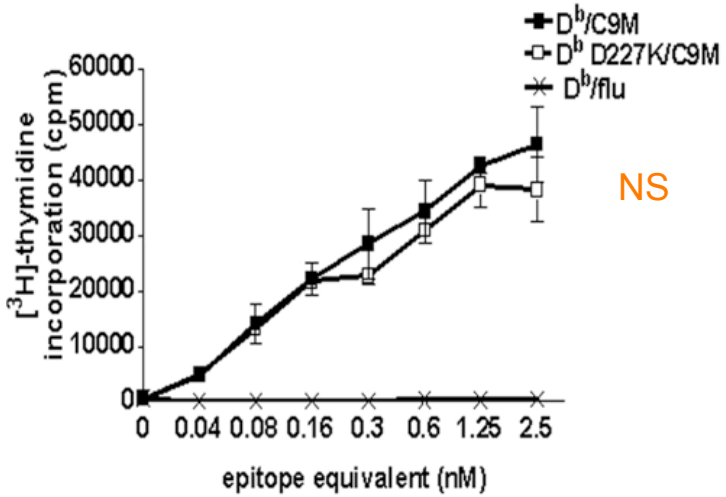


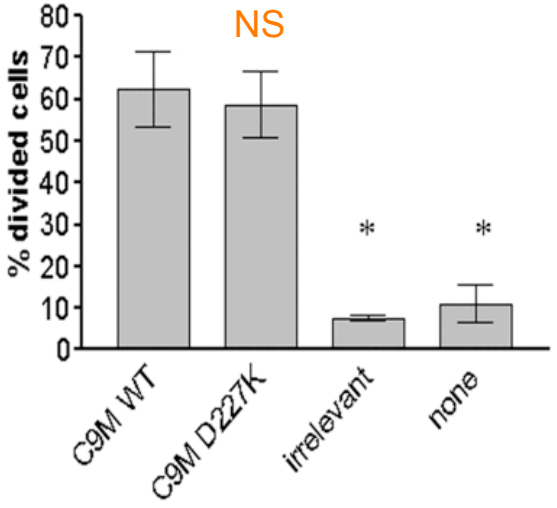
Molécule adaptatrices: indépendance pour les TCR de haute affinité

Kerry JI 2003





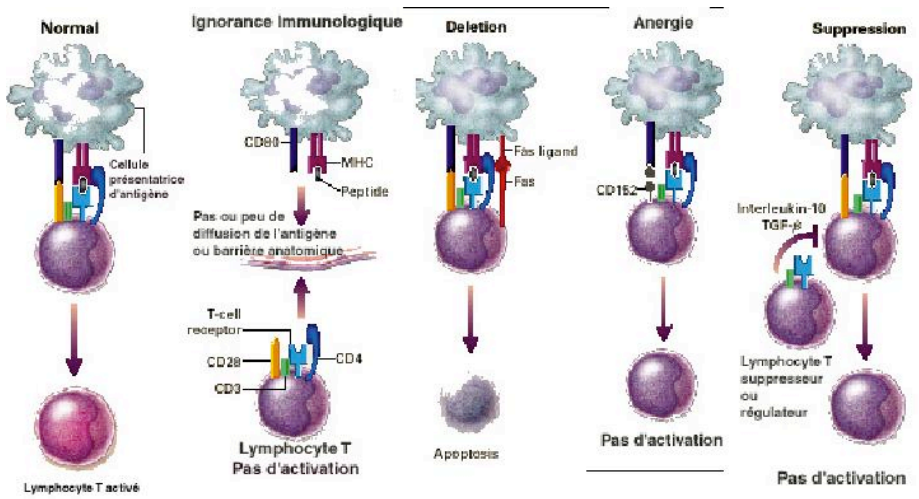




NB: Attention aux modèles de souris TCR transgéniques présentant un TCR de haute affinité

Costimulation / anergie

- **Absence:**
  - mécanisme de tolérance périphérique:
    ignorance immunologique
  - Induction d'anergie

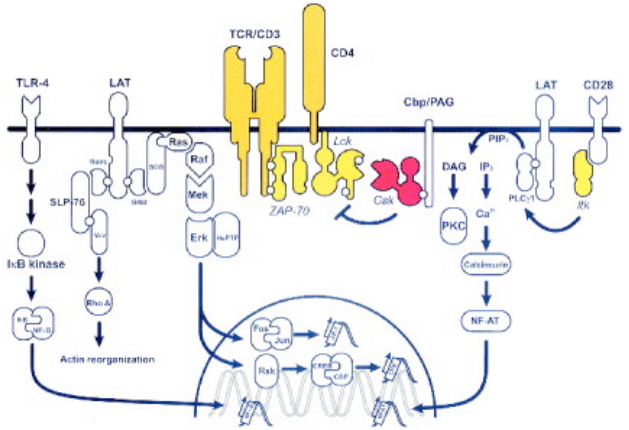


Mécanismes possibles de la tolérance périphérique

Figure N°22

New England J Med 2001, 344, 65

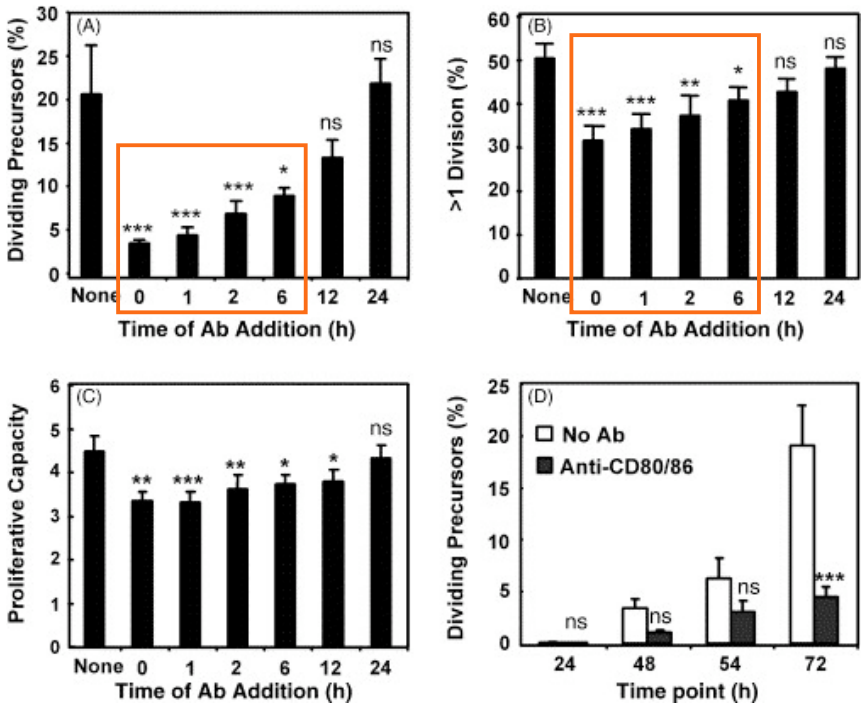
- **Mol de costimulation: signal complémentaire**

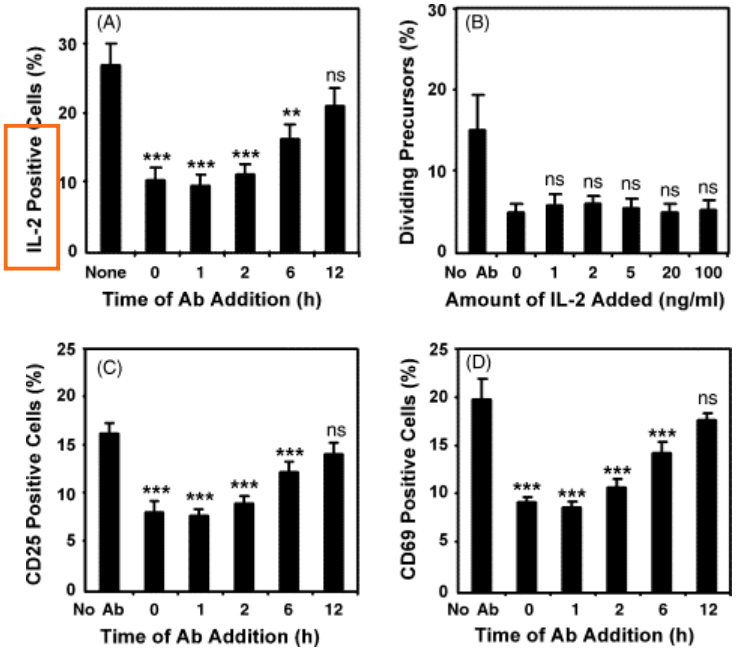


B. Bellier

Nécessité d'un engagement prolongé de CD80/CD86

Liwski, Imm Letters 2006





- To investigate the functional effects of CD80/CD86 blockade on naive CD4+ T cell activation, freshly isolated CD4+ T cells from OVA-TCR transgenic DO11.10 mice [27] were labeled with CFSE and cultured with OVA-peptide pulsed, mature DC in the absence or presence of CD80 and CD86 specific mAbs.

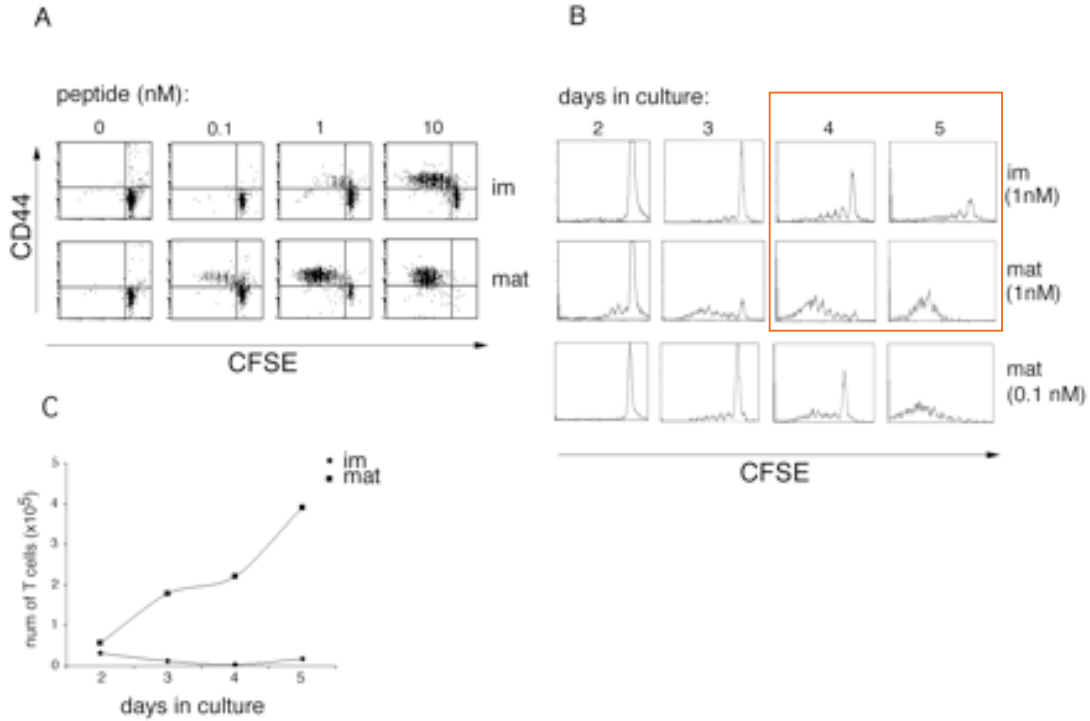
- We demonstrated that DC mediated CD80/CD86 costimulation controls the magnitude of the naïve T cell proliferative response by regulating both responder frequency as well as proliferative capacity.

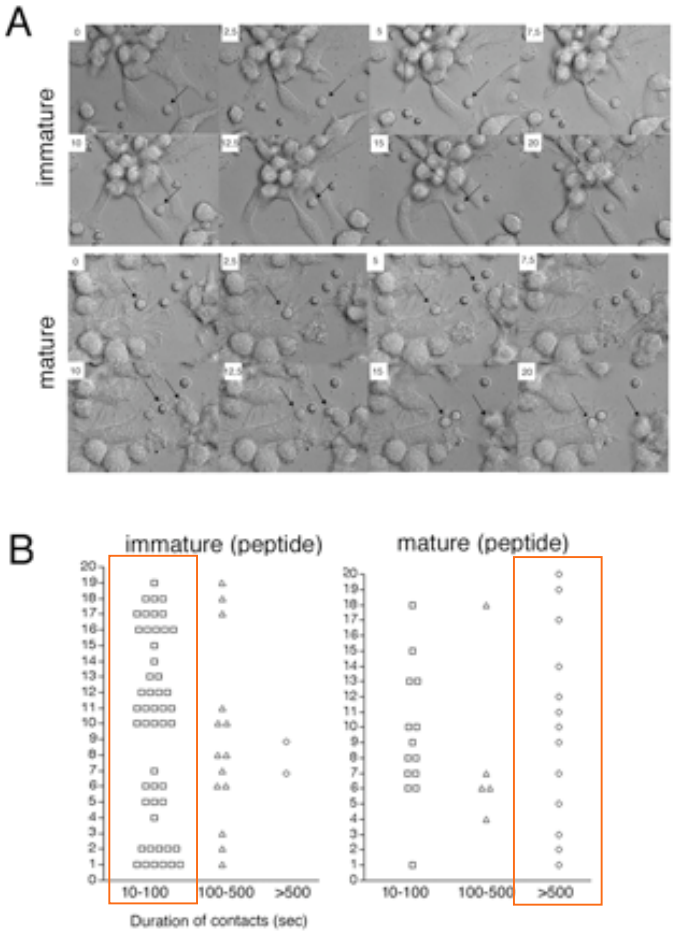
- Our data revealed that blocking CD80/CD86 signaling up to 6 h after conjugate formation resulted in a significant decrease in both the number of naive T cells entering the proliferative cycle and the number of daughter cells generated by each cell.

B. Bellier

Mol costimulation et dynamique de l'interaction

- *Federica Benvenuti, JI 2004;* Dendritic Cell Maturation Controls Adhesion, Synapse Formation, and the Duration of the Interactions with Naive T Lymphocytes



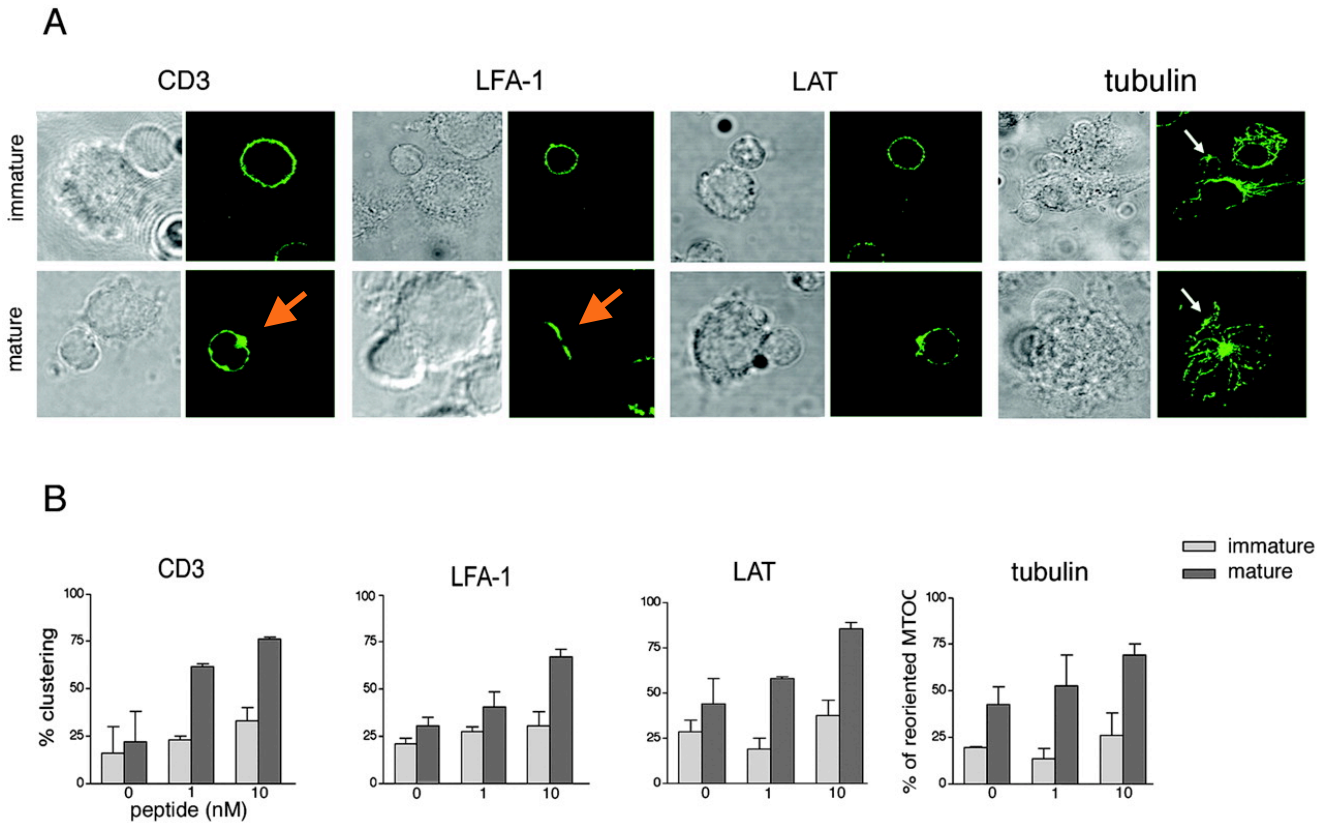


- Proliferation of naive T cells stimulated by **immature or mature DCs**. Immature or mature D1 pulsed with different doses of peptide were cocultured for 5 days with CFSE-loaded naive T lymphocytes (1:5 ratio). *A*, Representative dot blot profile showing the loss of CFSE and the up-regulation of CD44 induced by immature (*upper row*) or mature (*lower row*) DCs loaded with different peptide doses at day 3. *B*, Histogram profile of CFSE staining on naive T cells stimulated with immature (*upper row*) or mature (*middle row*) DCs loaded with 1 nM H-Y and mature DCs loaded with 0.1 nM peptide (*lower row*) at days 2–5 of the coculture. *C*, Quantification of the absolute number of CD4+ T cells at the different days of coculture for immature () and mature () DCs loaded with 1 nM peptide (T cells at day 0 = 7 x 104). One representative of three experiments is shown.

B. Bellier

Mol de costimulation et synapse

- *Federica Benvenuti, JI 2004;* Dendritic Cell Maturation Controls Adhesion, Synapse Formation, and the Duration of the Interactions with Naive T Lymphocytes

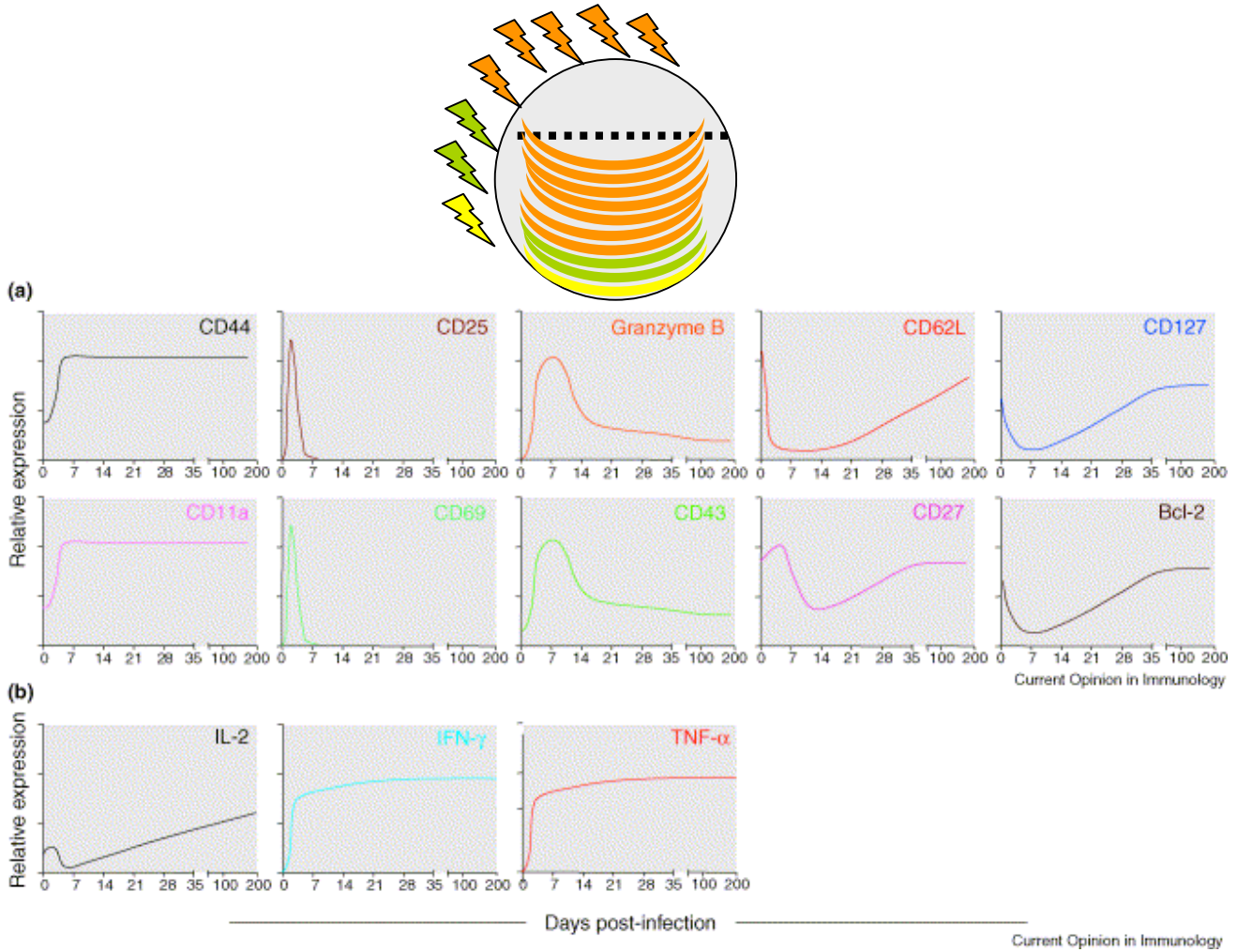


DC maturation is required for effective clustering and SMAC formation at the DC-T cell contact site

Efficient clustering in naive T cells requires DC maturation. Confocal images showing the distribution of CD3, LFA-1, LAT, and tubulin in T cells forming conjugates with immature or mature D1 pulsed with 10 nM H-Y peptide. Conjugates were formed for 30 min, washed five times, and fixed for immunostaining. *A*, One representative conjugate formed with immature DCs (*upper panels*) or mature DCs (*lower panels*) is shown for each marker. For each immunofluorescent image (*right panels*), a DIC image showing the two cells in contact is shown (*left panels*). Note that the distribution of CD3, LFA-1, and LAT is homogenous on T cells forming conjugates with immature DCs and clustered in conjugates formed with mature DCs. Similarly, the T cell MTOC (marked by an arrow) is reoriented toward the APCs in conjugates with mature, but not with immature DCs. *B*, Quantification of the proportion of conjugates showing clustering of CD3, LFA-1, LAT, and tubulin at the site of contact. Conjugates between naive T cells and immature or mature DCs pulsed with different doses of H-Y peptide were formed as in *A*. The number of conjugates presenting clustering at the contact zone was blindly quantified (see *Materials and Methods*) and expressed as a percentage of the total number of conjugates analyzed (percent clustering). Number of conjugates quantified is as follows: CD3, immature, $n = 276$; mature, $n = 347$; LFA-1, immature, $n = 197$; mature, $n = 229$; LAT, immature, $n = 185$; mature, $n = 232$; and tubulin, immature, $n = 210$; mature, $n = 243$.

B. Bellier

Importance des paramètres d'activation
Induction du programme de différenciation



Masopust Curr Op Imm 2004

Dynamics of phenotypic changes as mouse CD8+ splenocytes respond to viral infection. The CD8+ T-cell differentiation program is coupled to progressive phenotypic changes that persist through memory development. Temporal expression pattern of various proteins (a) **directly *ex vivo*, or** (b) **following five hours of *in vitro* re-stimulation, among antigen-specific CD8+ T cells after viral infection. Expression of these markers has largely been defined by analysis of blood- or lymphoid-derived CD8+ T cells, and a much more comprehensive characterization of non-lymphoid memory cells is warranted.**

UPMC PARIS UNIVERSITAS

B. Bellier

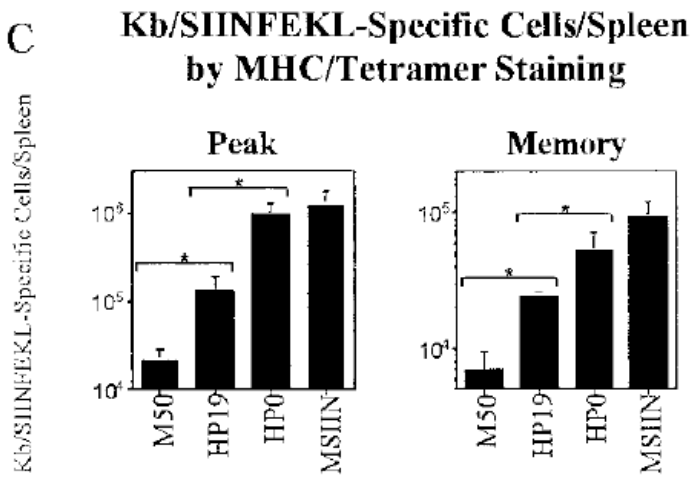
Génération des CD8 mémoire: Influence de la quantité d'Ag

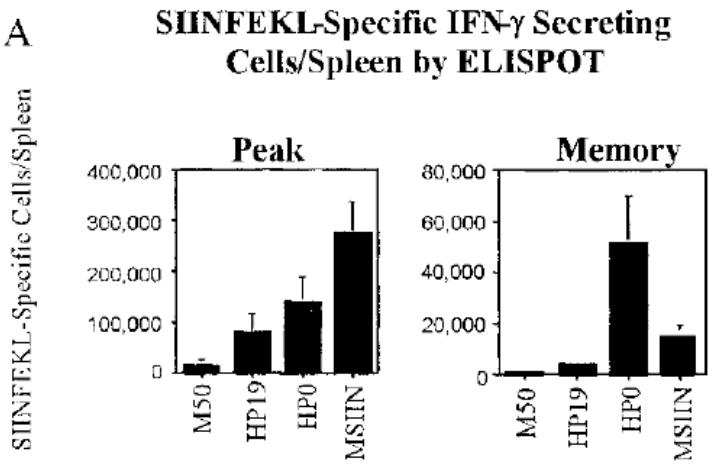
- *E. John Wherry, JI* 2002, Generation of CD8+ T Cell Memory in Response to Low, High, and Excessive Levels of Epitope

Table I. *VV recombinants*

Recombinant	Description	Epitopes/Cell[a] (copies/cell)
M50	Control virus-expressing an irrelevant minigene (NP_{50-57}; H-$2K^b$)	0
HP19	Full-length NP/S (influenza NP + SIINFEKL) behind a 19bp HP	~800
HP0	Full-length NP/S (influenza NP + SIINFEKL) without a HP (HP size = 0)	~30,000
(M)SIINFEKL	Minigene version of $OVA_{257-264}$ (M designates the initiating methionine)	~60,000

Espaceur:
< expresion
de l'Ag





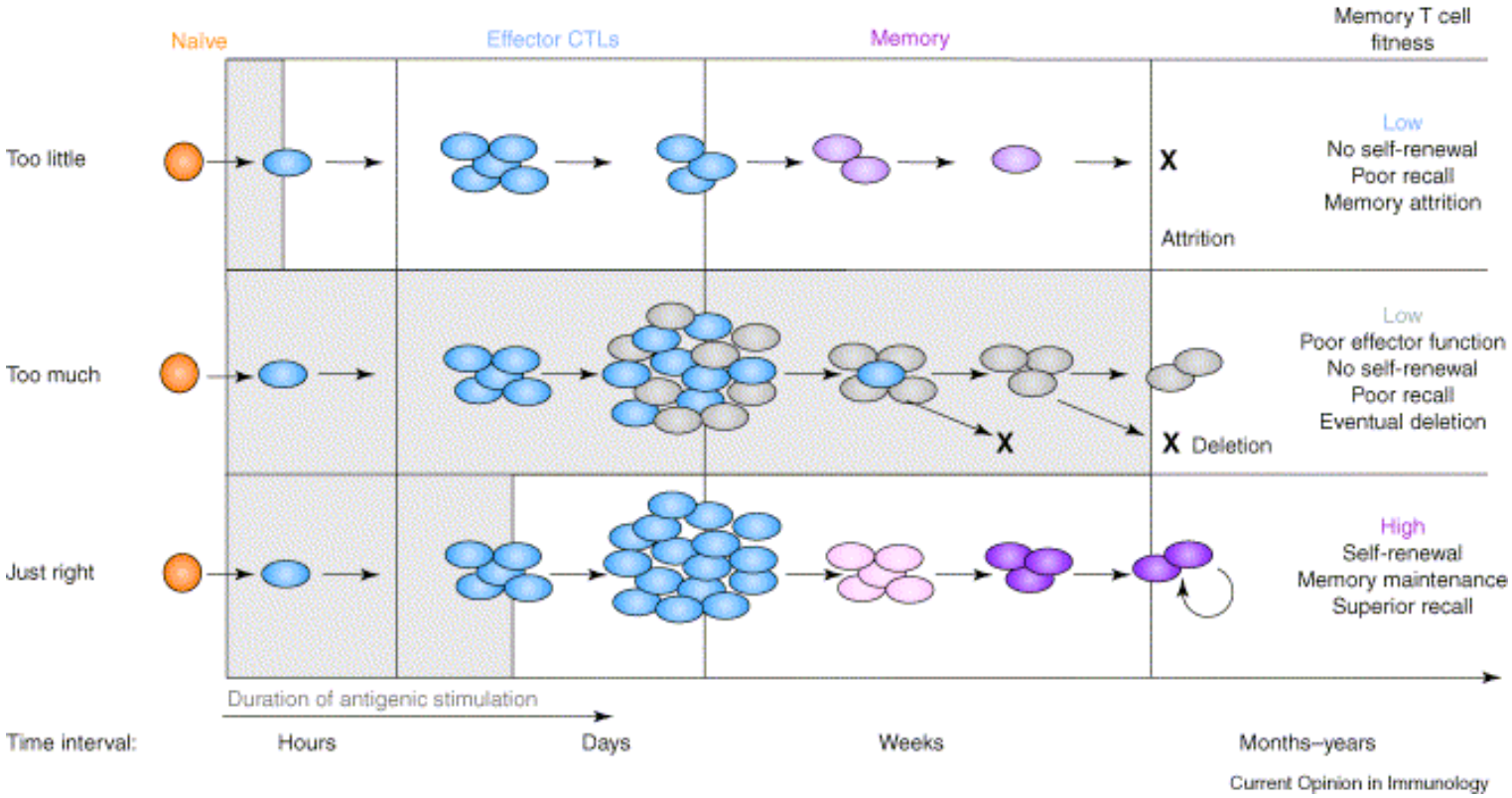
➢**recrutement d'un nombre de clones plus ou moins important**

➢**et d'affinité variable**

selon quantité d'Ag lors de la stimulation

B. Bellier

Importance des paramètres d'activation : Génération des CD8 mémoire



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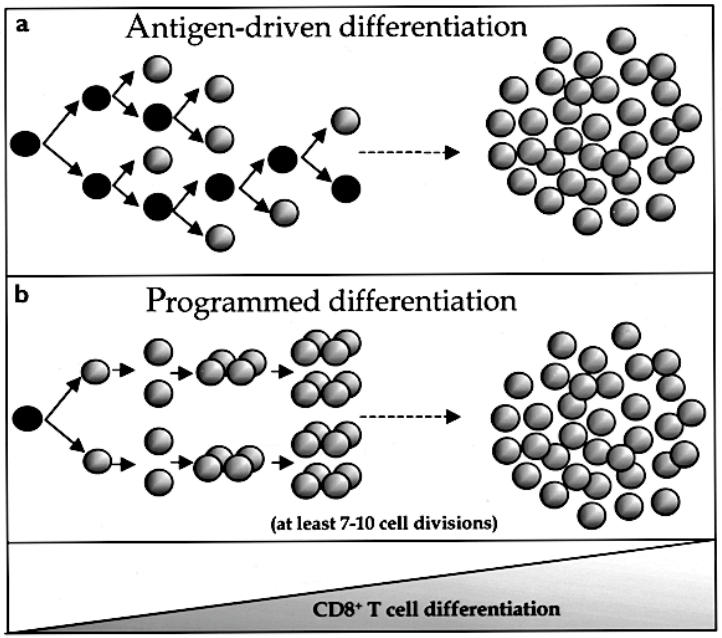
The Goldilocks model of effector and memory CD8+ T-cell development. The first 24 hours following antigenic stimulation elicits a program of expansion and differentiation that continues among daughter cells after removal of antigen. 'Too little' stimulation, meaning insufficient antigen concentration or duration, leads to limited CD8+ T-cell expansion, poor memory development and attrition. Chronic antigen exposure may cause 'too much' stimulation, leading to a progressive loss in the ability to secrete cytokines and the eventual deletion of antigen-specific CD8+ T cells. Optimum memory development is favored when conditions are 'just right'; that is, when CD8+ T cells are stimulated by a sufficient concentration of antigen for a sufficient, but not excessive period of time. Several variables, such as the cytokine environment, TCR affinity, type of antigen presenting cells, CD4+ T-cell help and degree of co-stimulation, modify the differentiation program and alter the antigen-dependent signaling requirements for the development of a robust response. Several factors encountered during the days following priming, including continued antigen contact, various co-stimulatory molecules, negative regulators, cytokines, chemokines, CD4+ T-cell help and anatomical location might also have a large qualitative and quantitative influence on the developing response. Additional factors may be important weeks and months later for memory differentiation and maintenance.

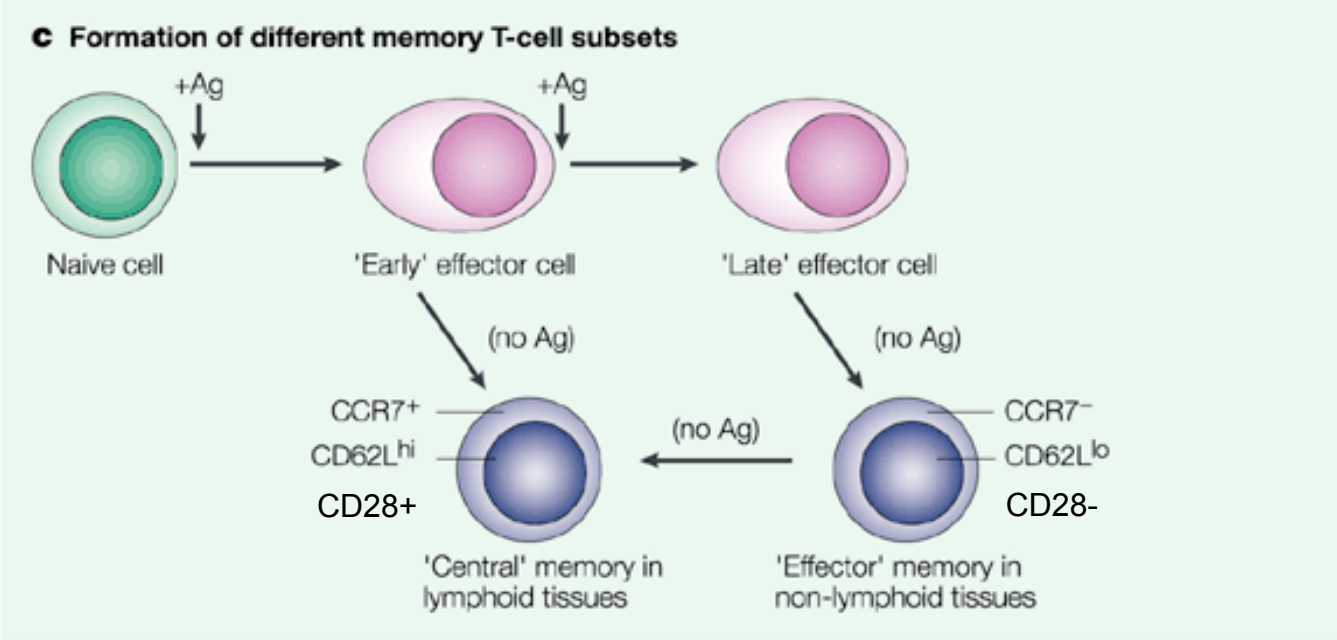
Importance de la stimulation antigénique initiale: programme de différenciation

- Susan M. Kaech & Rafi Ahmed *Nature Immunology* 2001 ; Memory CD8+ T cell differentiation: initial antigen encounter triggers a developmental program in naïve cells

- **Models for proliferation and differentiation of naïve CD8+ T cells.**(**a**) CD8+ T cell proliferation is dependent on repeated encounters with antigen. Each cell that is stimulated by antigen divides and progressively differentiates into effector CTLs and memory CD8+ T cells with each successive cell division. According to this model, it is essential that each daughter cell be stimulated with antigen, otherwise CD8+ T cell division, and possibly differentiation, would be halted upon antigen removal. (**b**) Naïve CD8+ T cells are developmentally programmed to divide at least seven to ten times and to differentiate into effector CTLs and long-lived functional memory CD8+ T cells. Optimal antigenic stimulation of the parental cell triggers this developmental program and the CD8+ T cells become committed to proliferation and differentiation. Further antigenic stimulation of the daughter cells may increase the number of times the activated CD8+ T cells divide, but it is unnecessary for this developmental program to progress.

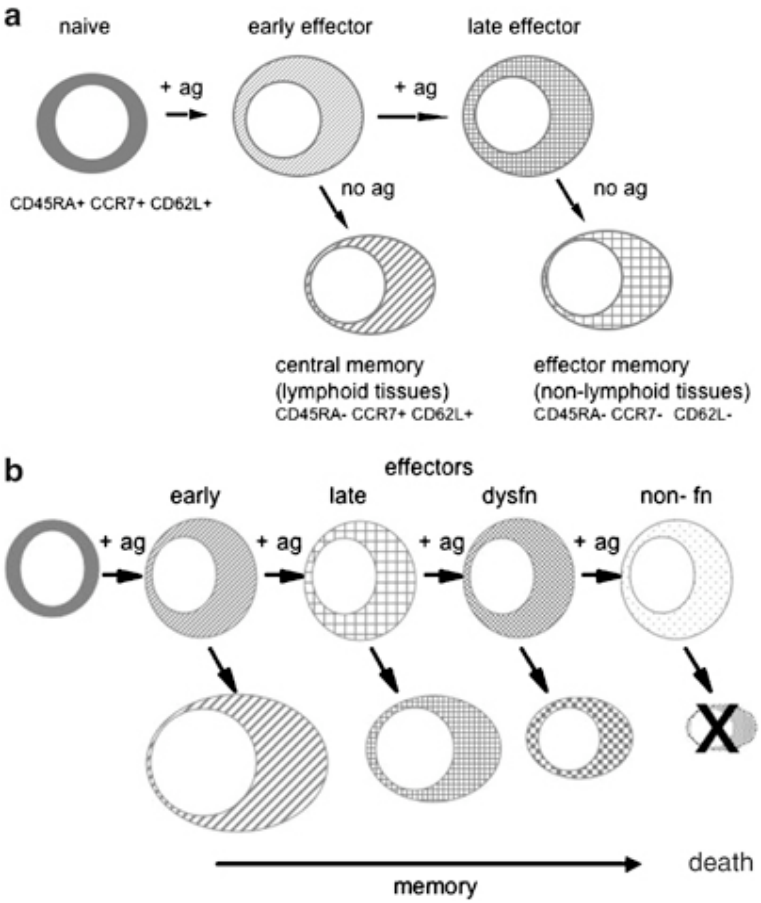


Prolongation de la stimulation antigénique: conséquence sur la différenciation en cellules mémoire



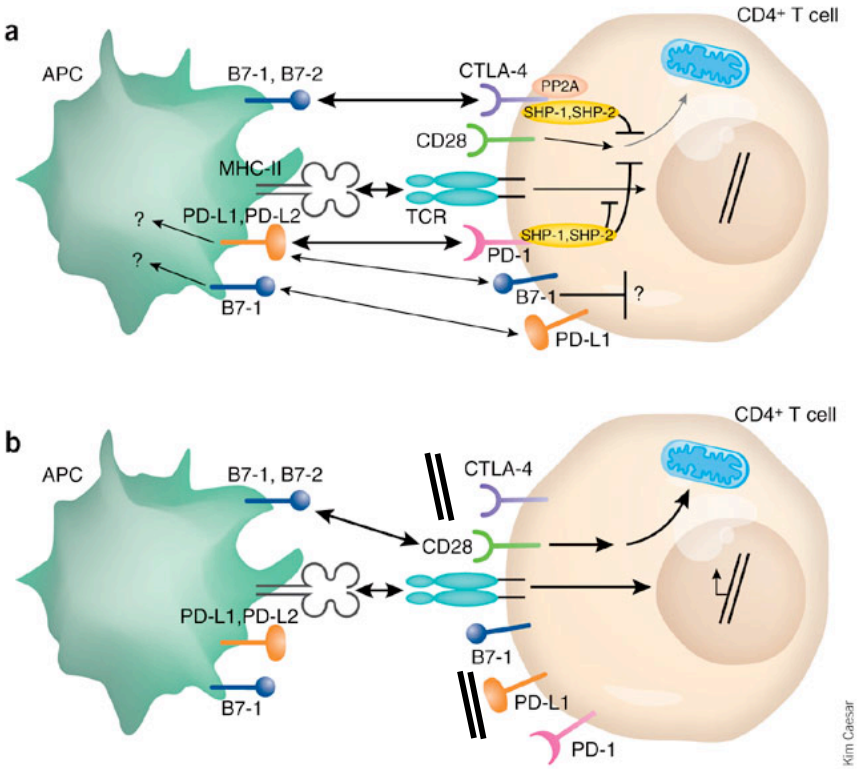
Kaech, Nature Reviews Immunology, 2002

Stimulation antigénique chronique



M L Munier and A D Kelleher

Program death 1 (**PD-1**) receptor



Inhibitory receptors: whose side are they on?
Alison Crawford & E John WherryNature
Immunology 8, 1201 - 1203 (2007)

Conclusions

- Paramètres de l'interaction TCR/CMH-Ag
  - **Affinité**
  - **Avidité**
  - **Temps contact**
  - **Flexibilité du CMH-Ag**
  - **Molécules adaptatrices / costimulation**

- Déterminent
  - **Activation lymphocytaire**
  - **Sélection des thymocytes**