

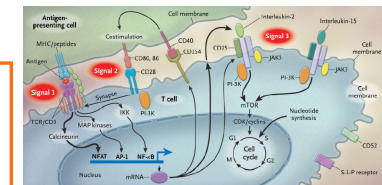
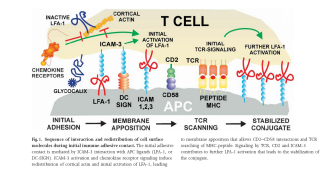
Dynamique de l'activation lymphocytaire T

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Activation lymphocytaire T

- Le rapprochement cellulaire
- La synapse immunologique (BMC403)
- Les molécules de l'activation
 - TCR: CMH-Ag + Co-récepteur CD4, CD8
 - Mol co-stimulation
 - Cytokines

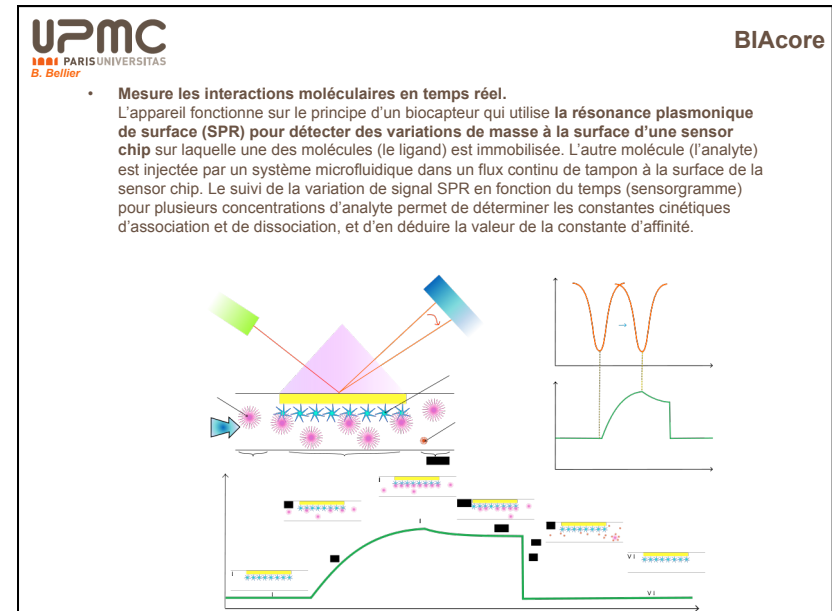
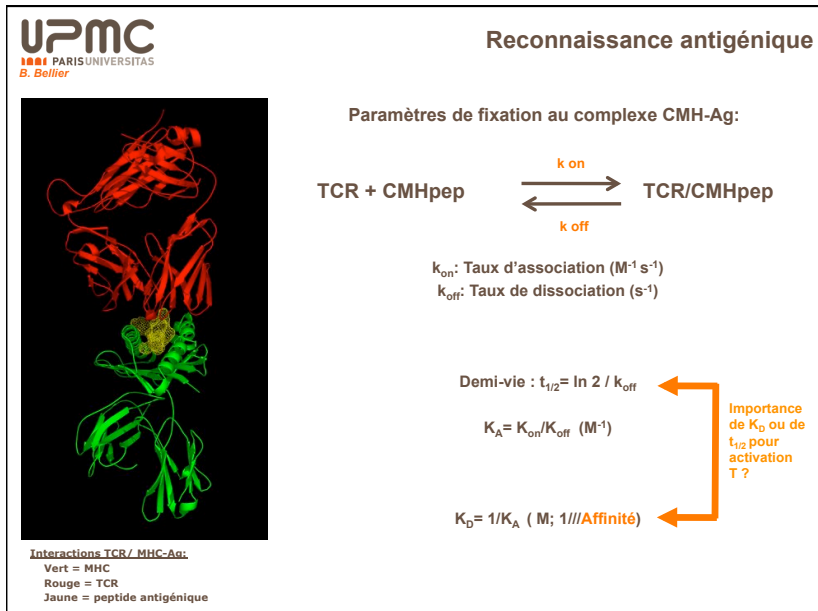
- Aspects quantitatifs et qualitatifs de l'activation
 - Affinité de l'interaction
 - Avidité de l'interaction
 - Dynamique des interactions moléculaires
- et ses conséquences sur le développement de la réponse immunitaire
 - Détermine le programme de différenciation T



- Bases moléculaires de la reconnaissance antigénique pour l'activation T
- Dynamique des interactions moléculaires
- Conséquences des interactions sur la différenciation T

**Bases moléculaires
de la reconnaissance antigénique**

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



UPMC PARIS UNIVERSITÉ B. Bellier	Table 1. Binding measurements for wild-type T-cell receptors							Paramètres
	TCR	Peptide/MHC	k_{on} (per M second)	k_{off} (per second)	$t_{1/2}$ (seconds)	K_D (μ M)	Activity	
Affinité TCR /CMH-Ag: KD ~1-50 μ M.	CT26	AH1(A3)/I ^L	58 000	0.11	6.3	1.9	Agonist	References
	P14	gp33/D ^b	400 000	0.975	0.7	2.4	Agonist	
Significativement plus faible que les autres interactions protéines-protéines aux conséquences biologiques	2C	p2Ca/L ^d	8300	0.027	25.7	3.3	Agonist	
	2C	QI9/L ^d	6350	0.025	26.8	3.9	Agonist	
	CT26	AH1/L ^d	61 000	0.35	2.0	5.7	Agonist	
	OT-1	OVA/K ^b	3720	0.022	31.5	5.9	Agonist	
	OT-1	OVA(G4)/K ^b	900	0.009	77	10	Weak agonist	
	CT26	AH1(A7)/L ^d	16 000	0.28	2.4	18	Weak agonist	
	2C	SIY/K ^b	22 000	0.464	1.5	27.4	Agonist	
	AHIII 12.2	p1058/D ^b	6610	0.538	1.2	81.4	Weak agonist	
	2C	dEV8/K ^b	2200	0.185	3.7	84.1	Antagonist	
	B7	Tax/HLA-A2	96 000	0.13	5.2	1.2	Agonist	
	A6	Tax/HLA-A2	49 000	0.11	6.1	1.9	Agonist	
	G10	HIV _{gp120} /SLY/HLA-A2	330 000	0.06	11.2	2.2	Agonist	
	GRB	Flu/HLA-B27	39 000	0.09	7.4	3	Agonist	
	JM22	Flu/HLA-A2	31 000	0.16	4.2	5.2	Agonist	
	G10	HIV _{gp120} /SLY/HLA-A2	340 000	0.16	4.2	5.2	Agonist	
	CMV	pp65/HLA-A2	70 000	0.44	1.5	6.3	Agonist	
	gp100	gp100/HLA-A2	31 000	0.23	2.9	7	Agonist	
	AHIII 12.2	p1049/HLA-A2	26 200	0.295	2.3	11.3	Agonist	
	LC13	FLR(A)/HLA-B8	35 800	0.42	1.7	12.5	Agonist	
	AM3	EBV/HLA-A24	7300	0.21	3.2	28	Agonist	
	IG4	NY-ESO-1/HLA-A2	40 000	0.128	6.4	32	Agonist	
	TEL	tel/HLA-A2	3500	0.14	4.8	40	Agonist	
	LC13	FLR(F)/HLA-B8	2620	0.35	2.0	132	Antagonist	
	172.10	MBP-11[4Y]/I-A ^a	37 200	0.219	3.1	5.9	Agonist	
	3L2	Hb/1-E ^b	5557	0.06	10.8	12	Agonist	
	1934.4	MBP-11[4Y]/I-A ^a	5130	0.16	4.2	31	Weak agonist	
	2B4	MCC/I-E ^b	633	0.057	11.7	90	Agonist	
	MAW 13	M-HSP/HLA-DR3	4000	0.12	5.6	30	Agonist	
	AH1.23	C-HSP/HLA-DR4	4400	0.16	4.2	36	Agonist	
	1A12	MBP/HLA-DR2	2100	0.17	3.9	81	Agonist	
	2E11	MBP/HLA-DR2	5900	0.73	0.9	123	Agonist	

K_D , equilibrium binding constant; k_{on} , association rate; k_{off} , dissociation rate; MHC, major histocompatibility complex; $t_{1/2}$, half-life of the interaction; TCR, T-cell receptor. Measurements performed at 25°.

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CD8⁺ T Cell Activation Is Governed by TCR-Peptide/MHC Affinity, Not Dissociation Rate¹

Shuomin Tian,* Robert Malle,*[†] Edward J. Collins,*[‡] and Jeffrey A. Frelinger*[§]

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

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

Table 1. Peptide variants of gp33

Mutation Site	Sequence	Peptide Mutant
None	KAQVNPATTC	gp33
P1	KAQVNPATTC	K1A, K1D, K1E, K1F, K1L, K1N, K1R, K1S, K1V, K1W, K1Y
P2	KAQVNPATTC	A2D, A2E, A2F, A2G, A2K, A2L, A2N, A2R, A2S, A2T, A2V, A2W, A2Y
P3	KAQVNPATTC	V1A, V1D, V1E, V1F, V1G, V1K, V1L, V1N, V1R, V1S, V1T, V1W, V1Y
P4	KAQVNPATTC	Y4A, Y4C
P6	KAQVNPATTC	P6L
P9	KAQVNPATTC	C9A, C9D, C9E, C9F, C9K, C9L, C9M, C9N, C9R, C9S, C9V, C9W
P1, P9	KAQVNPATTC	K1AC9M, K1MC9M, K1SC9M
P1, P9	KAQVNPATTC	Y4FCM, Y4SCM
P4, P9	KAQVNPATTC	Y4FC9M, Y4SC9M

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

Peptide	EC ₅₀ ^{1/2} (nM) ^a	EC ₅₀ ⁵⁰ (nM) ^a	K _D (nM) ^a	ΔG (kcal · mol ⁻¹) ^a	k _{on} (×10 ⁶ M ⁻¹ · s ⁻¹) ^a	k _{off} (s ⁻¹) ^a	t _{1/2} (s) ^a
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
C9P	0.53 ± 0.26	0.56 ± 0.44	1.9 ± 0.87	-6.05	1.19 ± 0.01	2.32 ± 0.07	0.60
C9L	1.36 ± 2.1	0.47 ± 0.43	8.9 ± 0.3	-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 ± 0.06	0.58
C9V	3.20 ± 5.60	1.23 ± 0.27	10.8 ± 0.3	-6.96	1.16 ± 0.03	1.25 ± 0.03	0.55
K1MC9M	8.58 ± 5.27	0.13 ± 0.09	14.5 ± 1.6	-5.70	0.33 ± 0.08	1.53 ± 0.1	0.20
K1R	49.0 ± 22.0	7.06 ± 1.06	11.6 ± 10.8	-5.65	1.31 ± 0.47	10.4 ± 3.5	0.065
K1SC9M	>50,000	28.0 ± 4.55	267	-4.94	0.01	0.26 ^b	2.67
Y4FCM	>50,000	112.8 ± 75.3	264 ± 103 ^c	-4.94	ND ^d	ND ^d	ND ^d
Y4A	8.870 ± 5.550	11.57 ± 7.47	63.0 ± 18.3	-5.8	0.38 ± 0.07	2.3 ± 0.57 ^e	0.32
Y4SC9M	>50,000	38.55 ± 22.03	847	-4.38	ND ^d	ND ^d	ND ^d
Y4SC9M	>50,000	264.1 ± 157.5	530 ^f	-4.53	ND ^d	ND ^d	ND ^d

^a EC₅₀^{1/2} 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 four experiments.

^b EC₅₀⁵⁰ 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 two to three experiments.

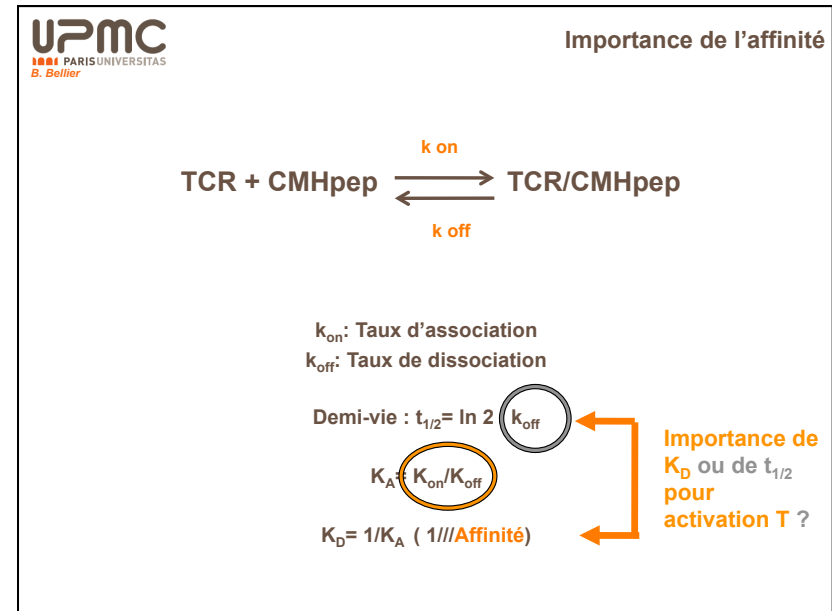
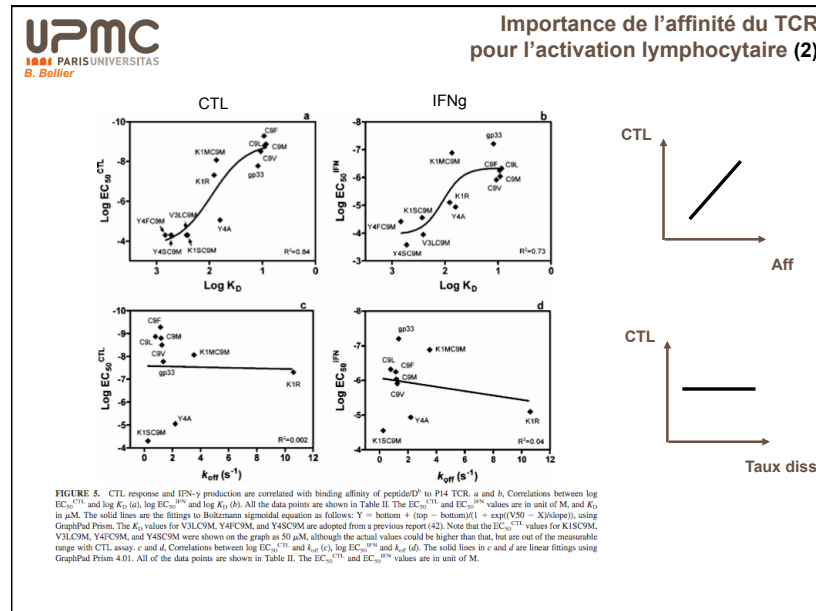
^c Determined by ΔG = -RT ln K_D, where RT = 25.7 cal · mol⁻¹ · °C⁻¹.

^d Determined from kinetic SPR data with software Scrubber. The values represent the mean ± SD of two to three experiments.

^e Half-life of dissociation of pMHC from P14 TCR. Determined by t_{1/2} = 0.693/k_{off}.

^f From Tian et al. (42). ND, not determined.

^g Derived from k_{on}, k_{off}, and K_D.

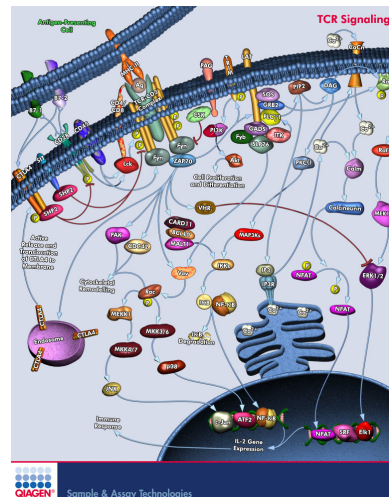


Le paradoxe de la reconnaissance par le TCR

- L'interaction TCR/CMH/peptide montre alors des paramètres cinétiques particuliers ; un taux d'association lent et un taux de dissociation rapide.

**Haute sensibilité,
faible affinité**

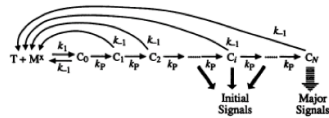
→ Comment une si faible interaction peut elle engendrer une transduction de signal ?



II. Activation T : un système dynamique

Modèle de « Kinetic proofreading » »

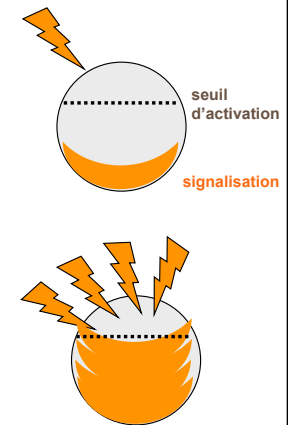
- Modèle de McKeithan (1995)
- S'inspire du modèle développé par Hopfield (1974) afin d'expliquer la remarquable exactitude de la réplication de l'ADN et de la synthèse protéique.
- L'hypothèse du modèle :
 - 1) Le complexe récepteur-ligand spécifique ou non spécifique C0 est converti via une série d'intermédiaires C_i en un complexe actif CN. Chacune de ces étapes requiert de l'énergie et implique la phosphorylation sur tyrosines.
 - 2) La dissociation du complexe emmène à la réversion des modifications, par le biais de phosphatases par exemple.
 - 3) Le taux de dissociation de complexes non spécifiques est suffisamment haut, afin que la dissociation intervienne avant que ce complexe génère des signaux.

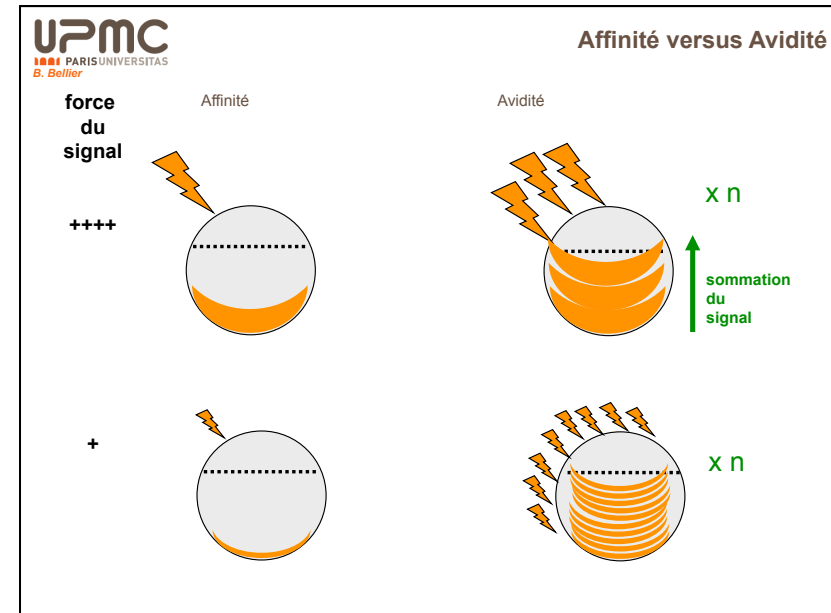
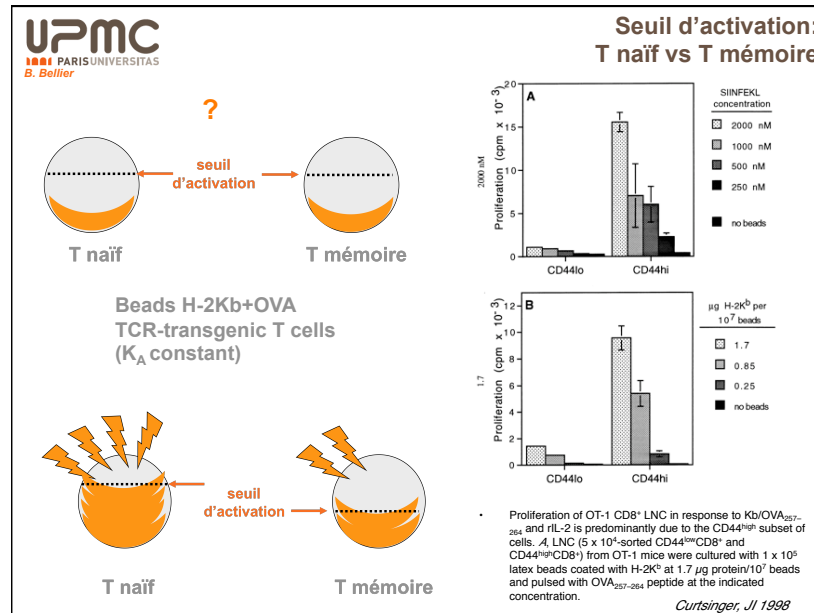


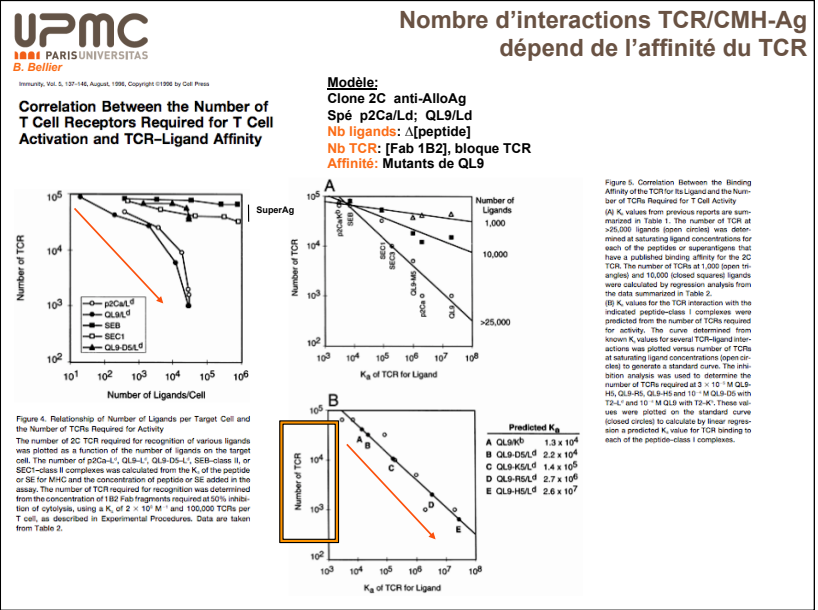
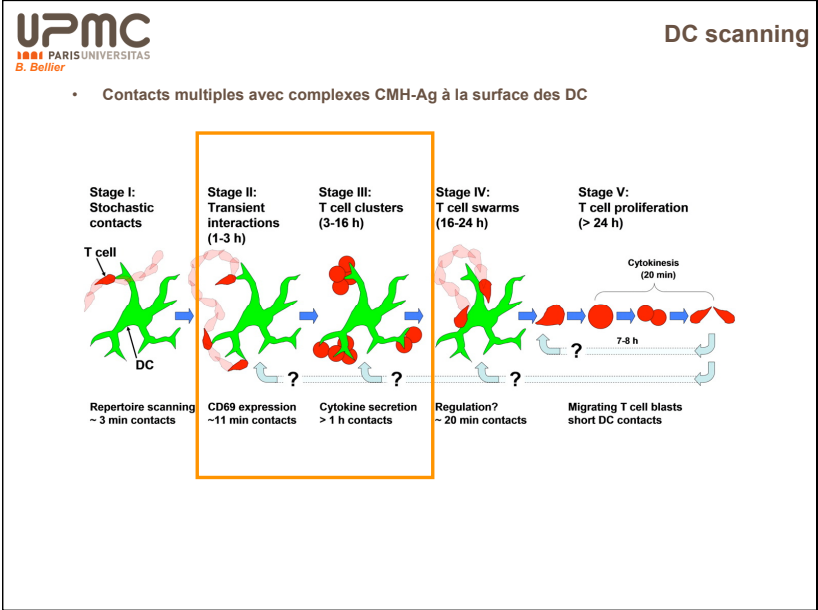
Des complexes initiaux C0 formés entre le TCR (T) et le complexe CMH/peptide (Mx) doivent subir N modifications avant de générer un complexe actif CN. A chaque étape, le complexe peut se dissocier emmenant à une complète réversion des sous unités à leur état basal. Pour une totale activation, les signaux doivent être générés à partir du complexe final C. D'après (McKeithan 1995).

Stimulation réitérative du lymphocyte:

- 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 (k_{off} fort, dissociation du complexe TCR/CMH-Ag) avant initiation de l'activation: les groupements phosphates seront éliminés par les phosphatases cellulaires







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

Engagement répété de la même molécule de TCR vs
différentes molécules de TCR ?

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

CD3 staining (% of control)

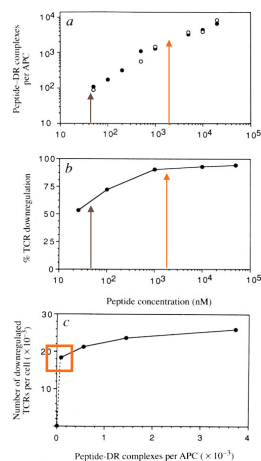
Time (min)

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

Le modèle du « Serial Triggering »

Nombre d'engagements

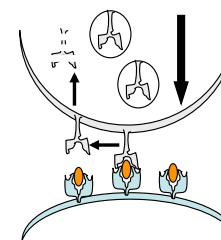
Serial triggering of many T-cell receptors by a few peptide-MHC complexes
 Valitutti, Nature 1995



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.

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

- Minimum 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

Influence de la « demi-vie » de contact

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

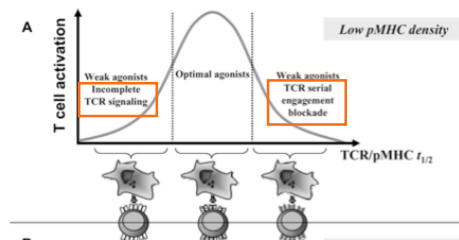


Fig. 2. T cell activation depends on TCR/pMHC interaction half-life and pMHC density. (A) When T cells interact with cognate pMHC at low density on the APC, efficient activation takes place within an optimal range of TCR/pMHC interaction half-life. Interactions with short half-lives cannot complete necessary 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 (Kalogris 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.

Influence du temps de contact

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

Salvatore Valitutti and Antonio Lanzavecchia

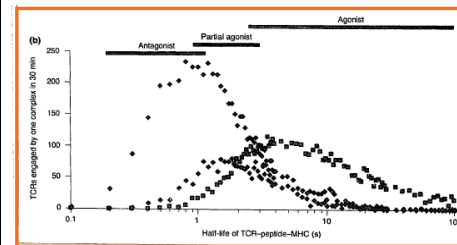
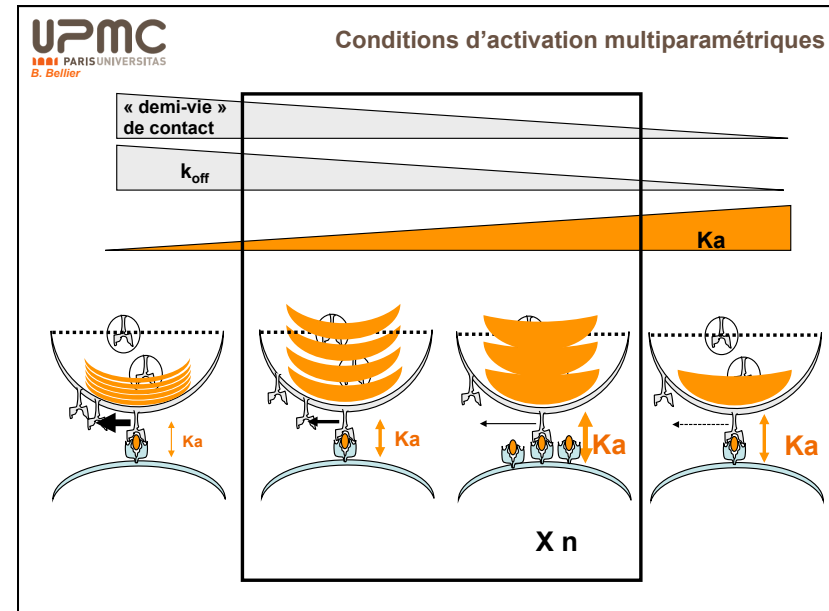
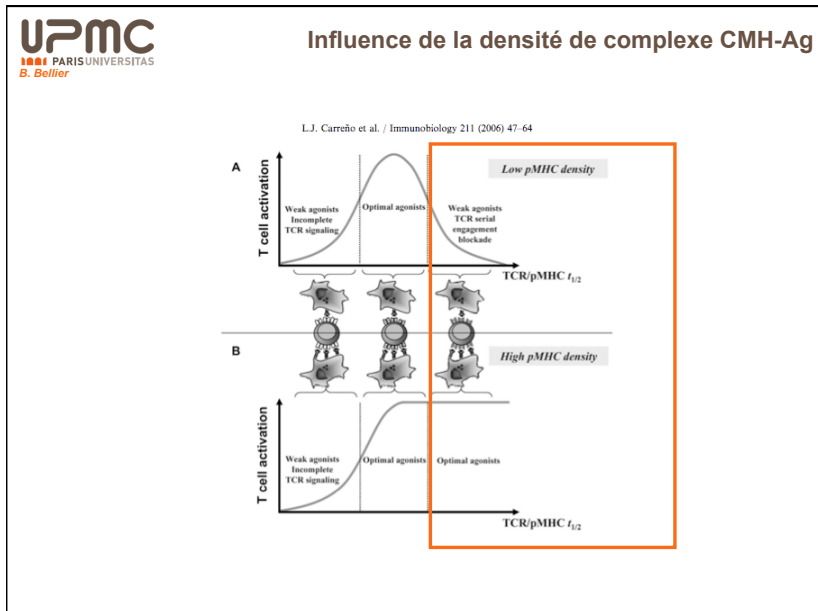
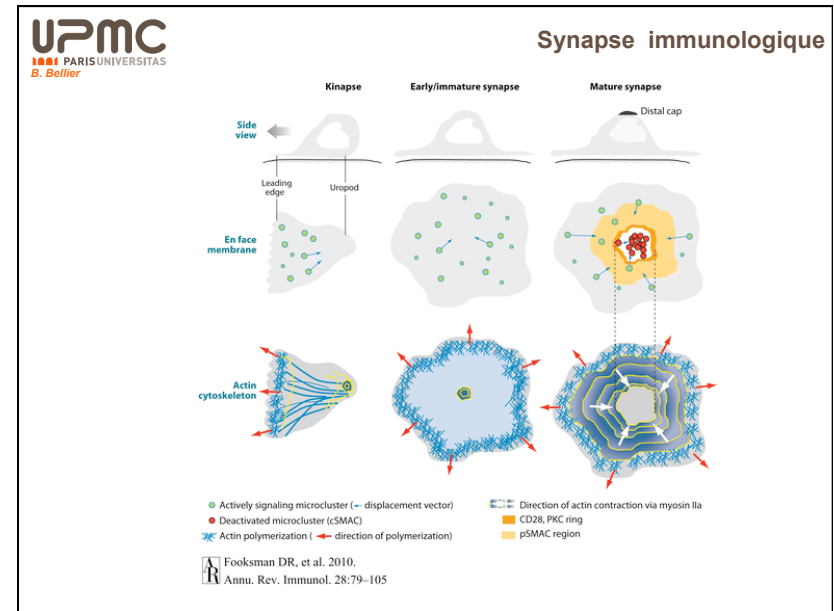
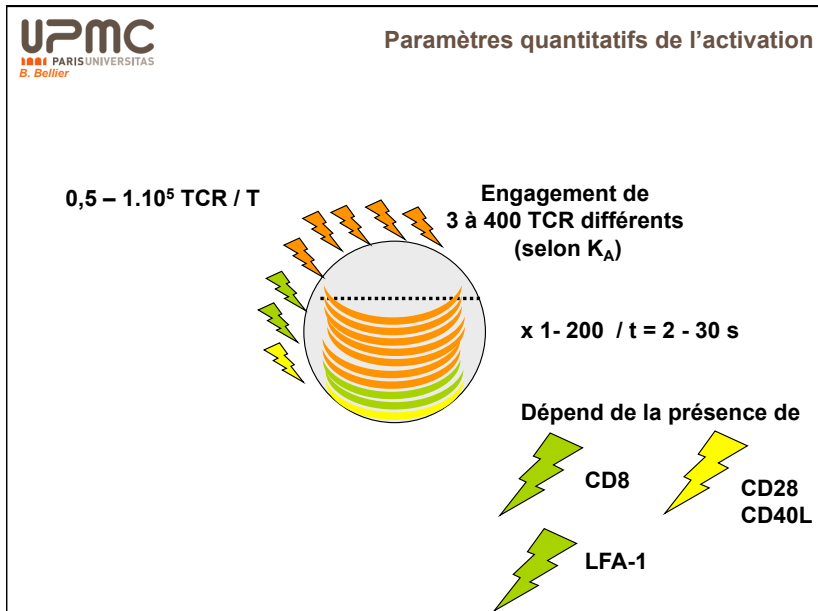


Fig. 2. Serial TCR triggering or inactivation as a function of complex stability and time required for TCR triggering. (a) Simulation describing the number of TCRs triggered in 30 min as a function of the half-life of TCR-peptide-MHC interactions. Each curve is calculated for times required for TCR triggering ranging 1–25 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 (par CMHp en 30min)	100	250





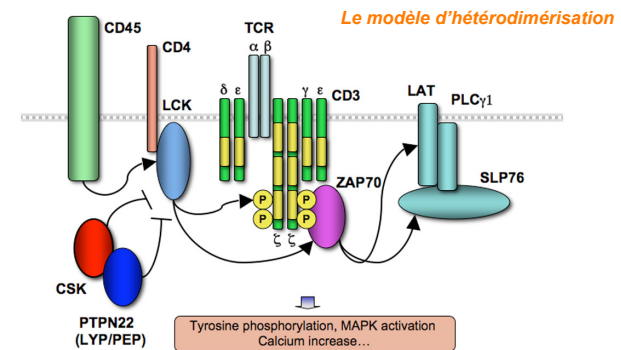
Conclusions

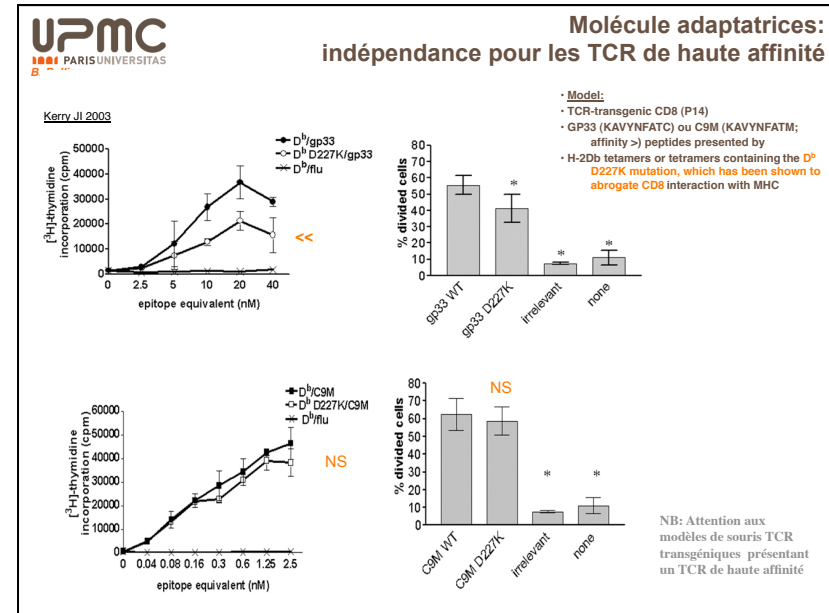
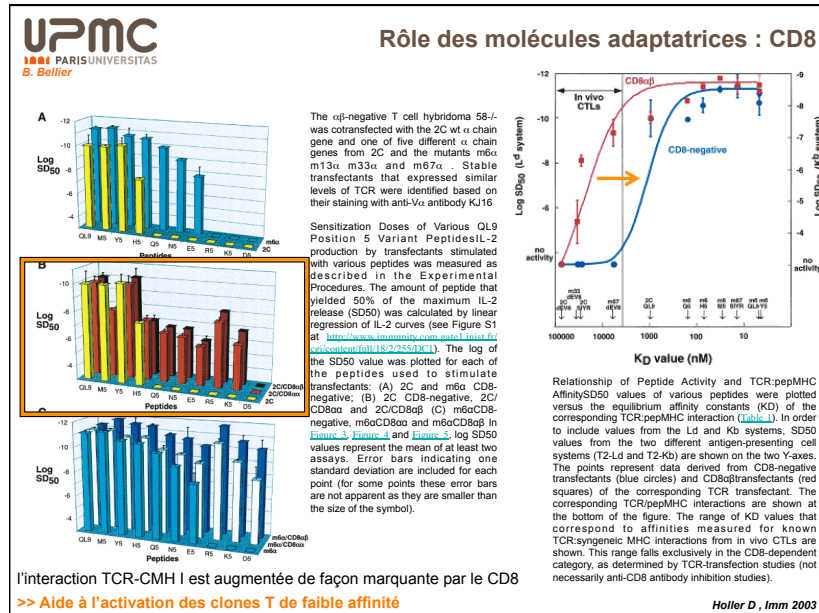
- En résumé,
- 2 théories de la dynamique moléculaire d'interaction:
 le « **kinetic proofreading** » se focalise **au niveau du récepteur individuel**. En effet, pour devenir activé, un TCR lié doit compléter une série de modifications biochimiques (phosphorylations des ITAMs, association puis activation de la ZAP-70 (Zeta chain-associated protein of 70 kDa), recrutement et phosphorylation d'autres molécules de signalisation additionnelles), avant de se dissocier avec le complexe CMH/peptide).

Par contre, le modèle du « **serial triggering** » porte à discuter au niveau cellulaire. Puisque, dans des conditions physiologiques où la densité de CMH/peptide spécifique sur la CPA est faible, la demi-vie de liaison du complexe TCR/CMH/peptide doit être assez courte pour permettre à un seul peptide d'engager en série de multiples TCR requis pour l'activation de la cellule T.

Rôle des molécules adaptatrices

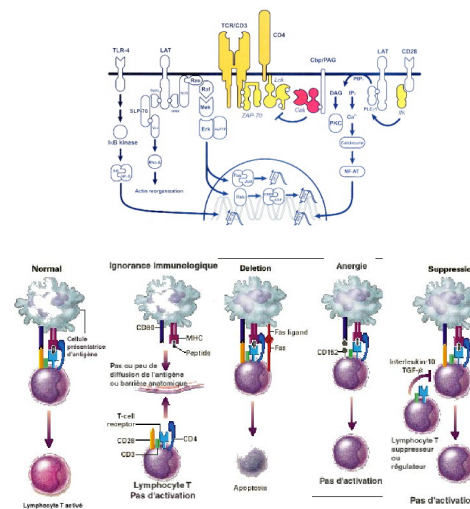
- CD4: CMH-II
- CD8: CMH-I
- Transduction du signal : renforcement des voies de signalisation





Rôle des molécules de costimulation:

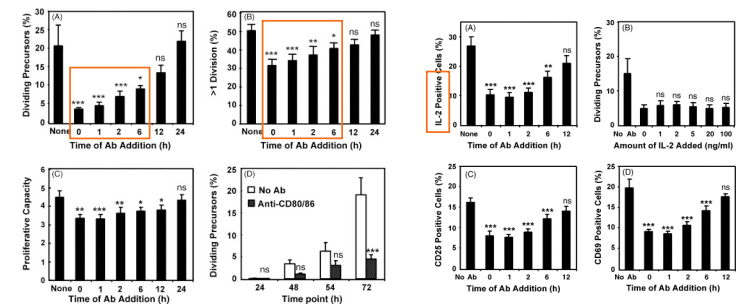
- Mol de costimulation:
 - signal activateur complémentaire
 - CD28/ CD80
 - CD28/ CD86
 - CD40/ CD40L
- Absence:
 - Induction d'anergie
 - mécanisme de tolérance périphérique



Nécessité d'un engagement prolongé de CD80/CD86

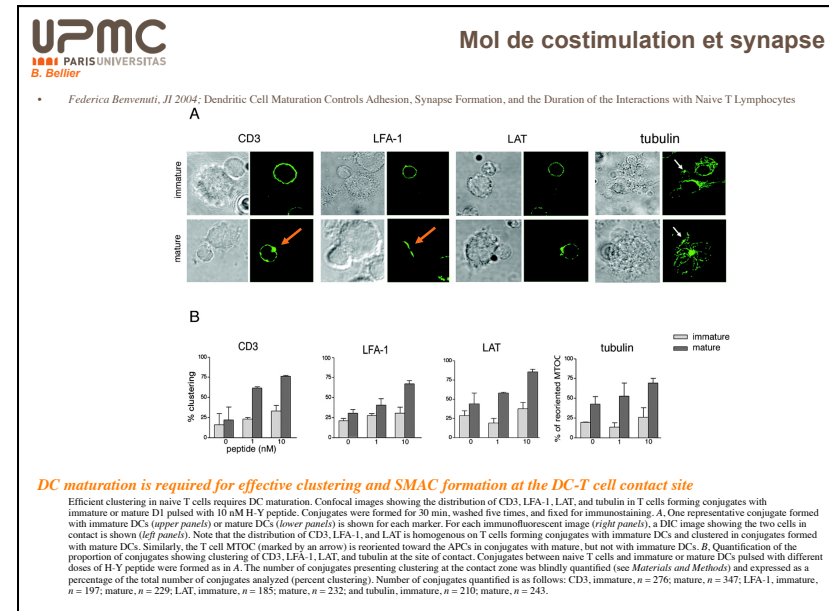
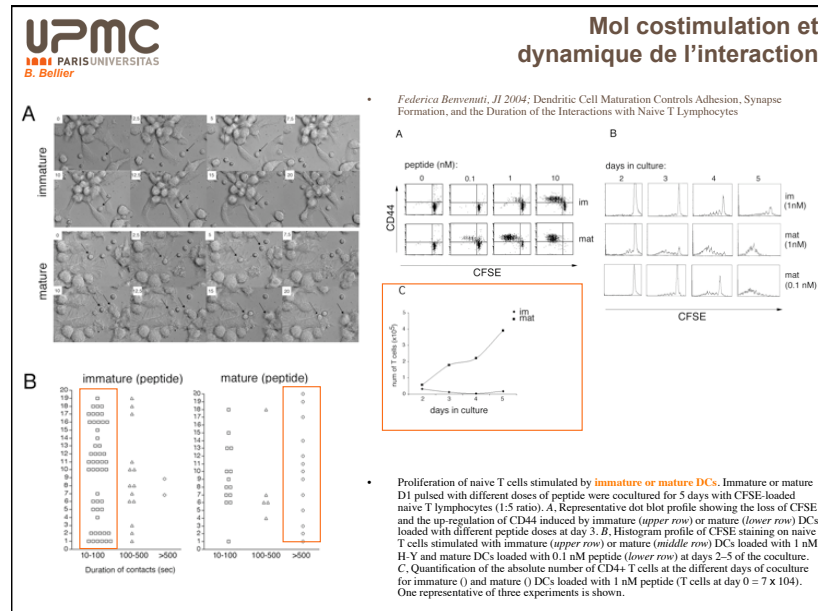
Liwski, Imm Letters 2006

CD4 anti-OVA ± anti-CD80/86



- 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.

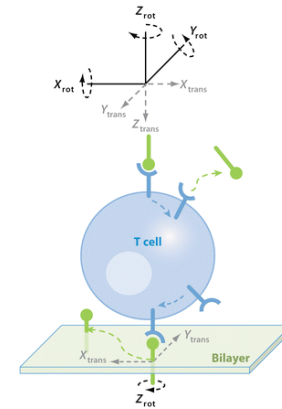
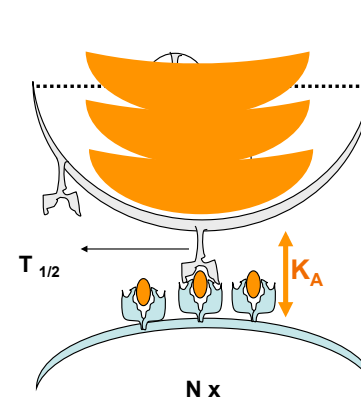
- We demonstrated that DC mediated CD80/CD86 costimulation controls the magnitude of the naive T cell proliferative response by regulating both responder frequency as well as proliferative capacity.
- Our data revealed that blocking CD80/CD86 signaling up to 6 h after conjugate formation resulted in 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.



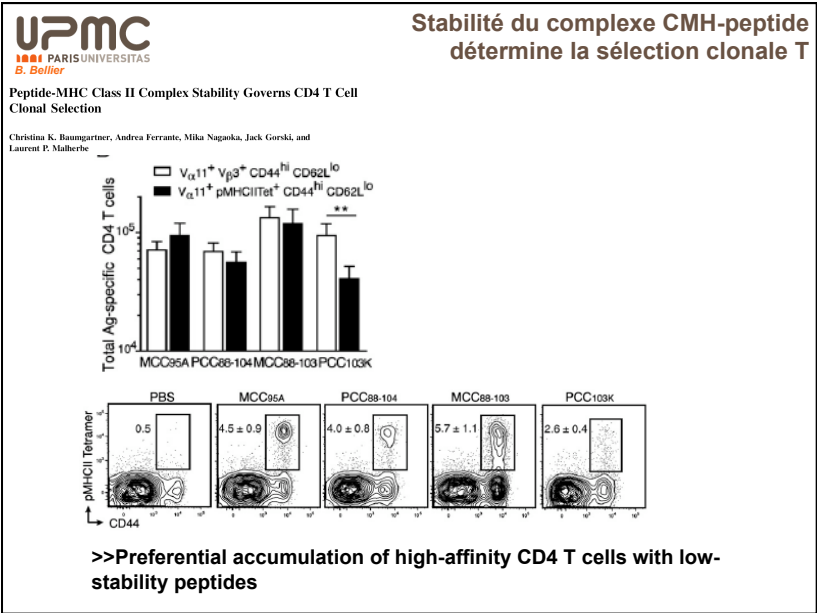
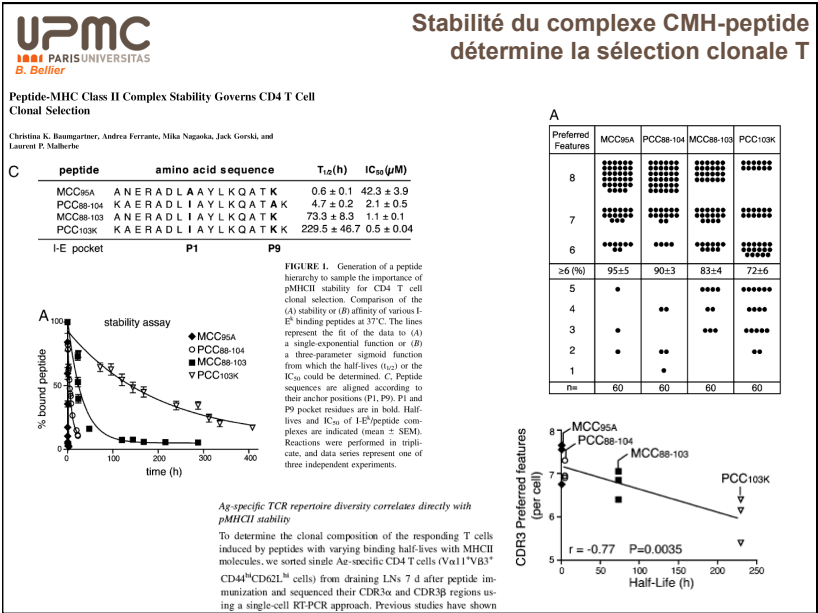
III. Conséquence des paramètres d'interactions T sur la réponse immunitaire

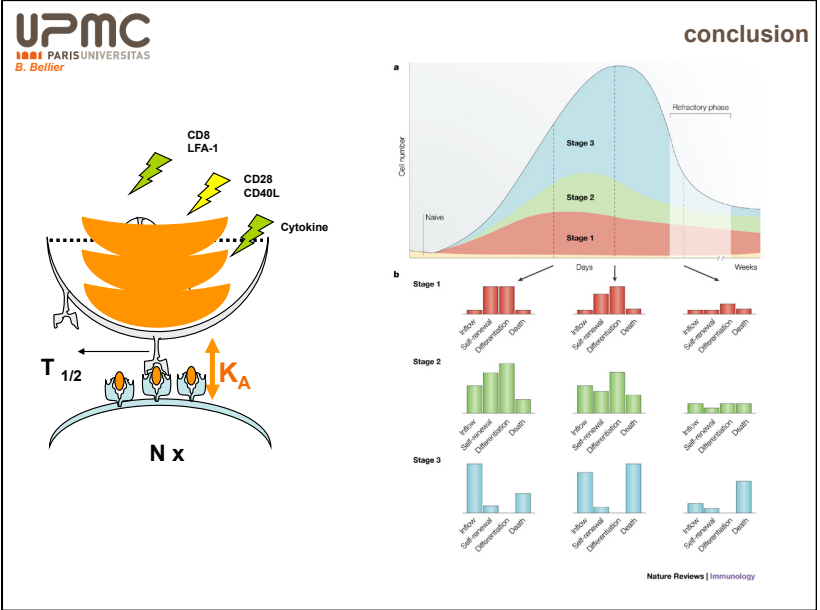
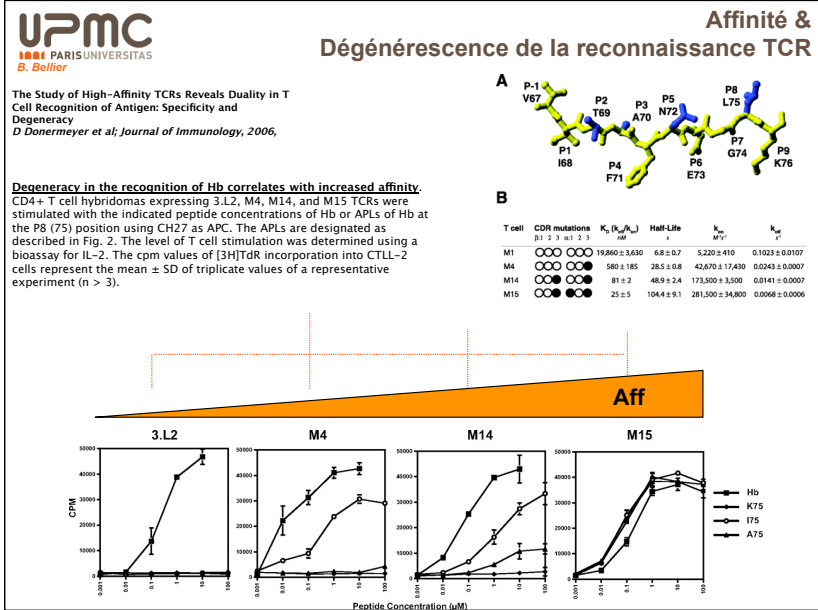
1. Mise en place de la réponse primaire
2. Différenciation T

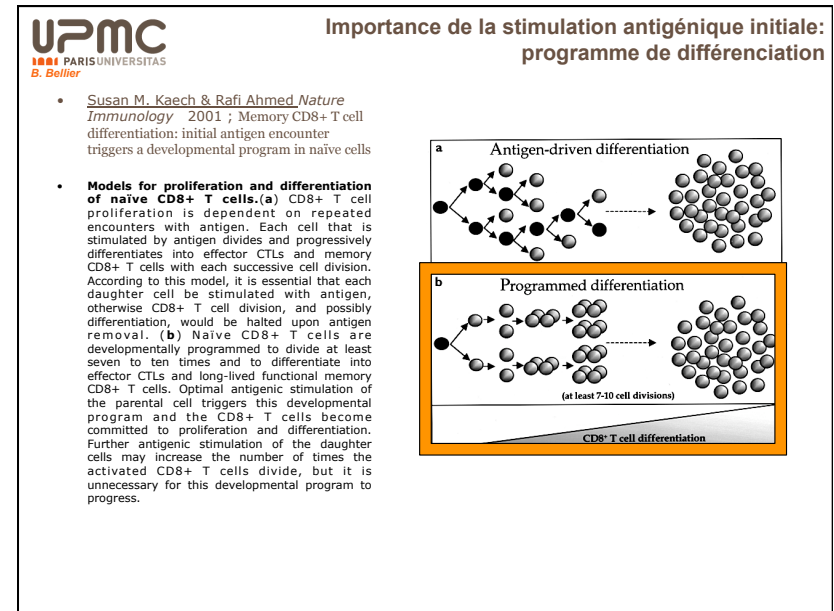
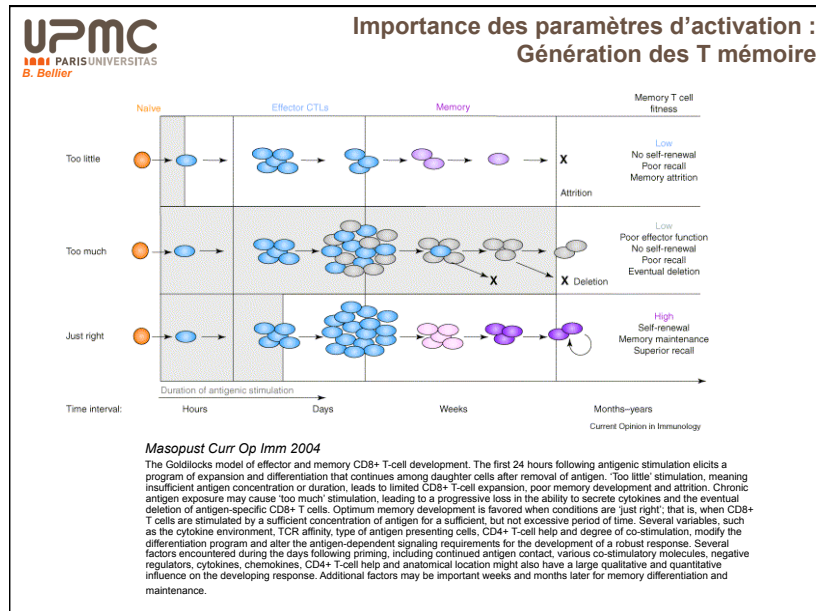
Paramètres de l'activation T



Fooksman DR, et al. 2010.
Annu. Rev. Immunol. 28:79–105







Different T Cell Receptor Signals Determine CD8⁺ Memory Versus Effector Development

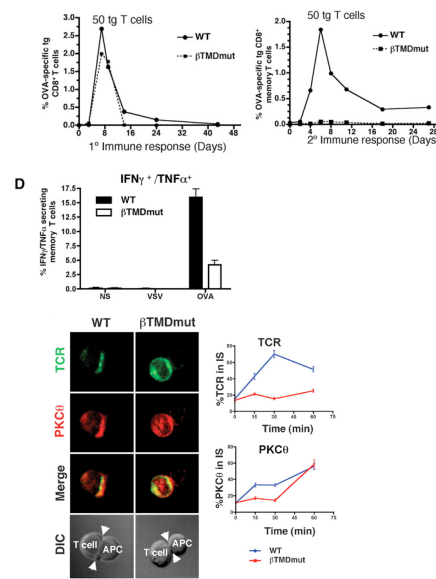
Emma Tehau,^{1,2} Mark A. Daniels,^{1,2} Sara E. Hamilton,² Adam G. Schrum,^{3,4} Rafael Bragado,⁵ Stephen C. Jameson,² Ed Palmer¹

23 JANUARY 2009 VOL 323 SCIENCE

We generated OT-1 TCR transgenic mice expressing a point mutation in the β TMD, where the most carboxy-terminal tyrosine residue of the CART motif was replaced by a leucine (CART15 YL). TCR expression on mutant T cells was slightly decreased, but the mutant TCR-CD3 complex composition was unaltered. T cell maturation and homeostasis in β TMD mutant mice (β TMDmut) were normal. The OT-1 and β TMDmut OT-1 TCRs recognize the ovalbumin peptide 257 to 264 (OVA_p) bound to H-2Kb.

Using a mutant TCR transgenic model, we found that point mutations in the TCR β transmembrane domain (β TMD) impair the development and function of CD8⁺ memory T cells without affecting primary effector T cell responses. Mutant T cells are deficient in polarizing the TCR and in organizing the nuclear factor κ B signal at the immunological synapse. Thus, effector and memory states of CD8⁺ T cells are separable fates, determined by differential TCR signaling.

>> Importance of the TCR in regulating the NF- κ B signal required for memory development. We show here that effector and memory programming can be dissociated by the induction of a different arrangement of TCR signals in CD8⁺ T cells.



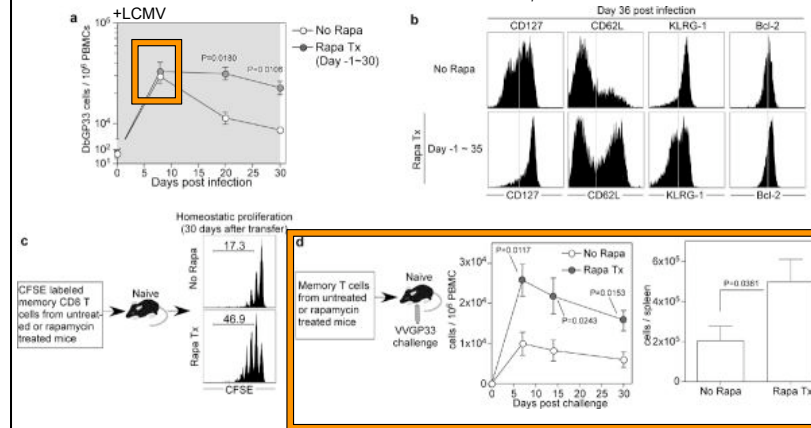
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mTOR regulates memory CD8 T cell differentiation

mTOR regulates memory CD8 T-cell differentiation.
Araki K & Ahmed R.

Nature. 2009 Jul

mammalian Target of Rapamycin
enzyme de la famille des sérine/thréonine kinase qui régule la prolifération cellulaire, la croissance cellulaire, la mobilité cellulaire, la survie cellulaire, la synthèse protéique et la transcription



TCR >> mTOR >> Metabolism >> Memory differentiation

