Activation lymphocytaire T

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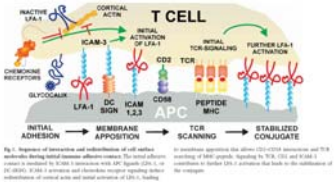
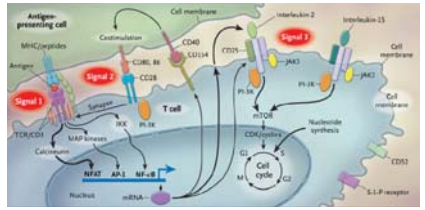
Activation lymphocytaire T

- **Le rapprochement cellulaire**
- **La synapse immunologique (cours SGD)**
- **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





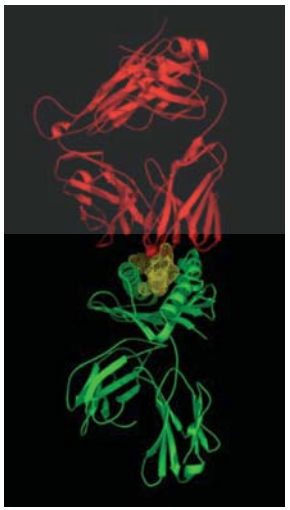
Plan

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

Bases moléculaires de la reconnaissance antigénique

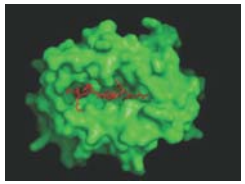
Influence des paramètres de l'interaction TCR/CMH-Ag

Reconnaissance antigénique



Interactions TCR/ MHC-Ag:

< Vert = MHC
Rouge = TCR
Jaune = peptide antigénique



Aff nité TCR /CMH-Ag: **KD ~1-50 ffM.** ff

Significativement plus faible que les autres interactions protéines-protéines aux conséquences biologiquesff

Paramètres de fixation au complexe CMH-Ag

Kon

TCR + CMHpep < > TCR/CMHpep

Koff

K_{on}: Taux d'association (M^{-1} s^{-1})
K_{off}: Taux de dissociation (s^{-1})

Demi-vie : $t_{1/2}$= ln 2 / k_{off}

K_A= K_{on}/K_{off} (M^{-1})

K_D= 1/K_A (M; 1///Affinité)

Importance de K_D ou de $t_{1/2}$ pour activation T ?

Paramètres

K_D 1-50 uM

Table 1. Binding measurements for wild-type T-cell receptors

TCR	Peptide/MHC	k_{on} (per M second)	k_{off} (per second)	$t_{1/2}$ (seconds)	K_D (μM)	Activity	References
CT26	AH1(A5)/L^d	58 000	0·11	6·3	1·9	Agonist	3
P14	gp33/D^b	400 000	0·975	0·7	2·4	Agonist	4
2C	p2Ca/L^d	8300	0·027	25·7	3·3	Agonist	5
2C	QL9/L^d	6350	0·025	26·8	3·9	Agonist	5
CT26	AH1/L^d	61 000	0·35	2·0	5·7	Agonist	3
OT-1	OVA/K^b	3720	0·022	31·5	5·9	Agonist	6
OT-1	OVA(G4)/K^b	900	0·009	77	10	Weak agonist	7
CT26	AH1(A7)/L^d	16 000	0·28	2·4	18	Weak agonist	3
2C	SIY/K^b	22 000	0·464	1·5	27·4	Agonist	8
AHIII 12.2	p1058/D^b	6610	0·538	1·2	81·4	Weak agonist	9
2C	dEV8/K^b	2200	0·185	3·7	84·1	Antagonist	5
B7	Tax/HLA-A2	96 000	0·13	5·2	1·2	Agonist	10
A6	Tax/HLA-A2	49 000	0·11	6·1	1·9	Agonist	10
G10	HIV_{gag}SLY/HLA-A2	330 000	0·06	11·2	2·2	Agonist	11
GRB	Flu/HLA-B27	39 000	0·09	7·4	3		12
JM22	Flu/HLA-A2	31 000	0·16	4·2	5·2		13
G10	HIV_{gag}SLF/HLA-A2	340 000	0·16	4·2	5·2	Agonist	11
CMV	pp65/HLA-A2	70 000	0·44	1·5	6·3		14
gp100	gp100/HLA-A2	31 000	0·23	2·9	7		12
AHIII 12.2	p1049/HLA-A2	26 200	0·295	2·3	11·3	Agonist	9
LC13	FLR(A)/HLA-B8	35 800	0·42	1·7	12·5	Agonist	15
AM3	EBV/HLA-A24	7300	0·21	3·2	28		12
1G4	NY-ESO-1/HLA-A2	40 000	0·128	6·4	32	Agonist	16
TEL	tel/HLA-A2	3500	0·14	4·8	40		12
LC13	FLR(F)/HLA-B8	2620	0·35	2·0	132	Antagonist	15
172.10	MBP1-11[4Y]/I-A^u	37 200	0·219	3·1	5·9	Agonist	17
3.L2	Hb/I-E^k	5557	0·06	10·8	12	Agonist	18
1934.4	MBP1-11[4Y]/I-A^u	5130	0·16	4·2	31	Weak agonist	17
2B4	MCC/I-E^k	633	0·057	11·7	90	Agonist	2
MAW 13	M-HSP/HLA-DR3	4000	0·12	5·6	30		12
AH1.23	C-HSP/HLA-DR4	4400	0·16	4·2	36		12
1A12	MBP/HLA-DR2	2100	0·17	3·9	81		12
2E11	MBP/HLA-DR2	5900	0·73	0·9	123		12

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°.

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*‡

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

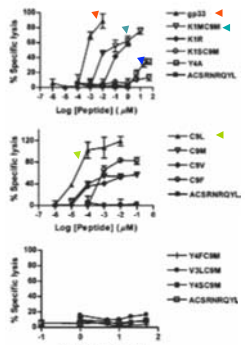
Table I. *Peptide variants of gp33*

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} (nM)[b]	K_D (μM)[c]	ΔG (kcal · mol^{-1})[d]	k_{on} ($\times 10^3$ M^{-1} · s^{-1})[e]	k_{off} (s^{-1})[f]	$t_{1/2}$ (s)[g]
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[i]	−4.94	ND[i]	ND[i]	ND[i]
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[j]	−4.38	ND[j]	ND[j]	ND[j]
Y4SC9M	>50,000	264.1 ± 157.5	530[j]	−4.53	ND[j]	ND[j]	ND[j]

[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 $k_{off} = K_D \times k_{on}$.



T-

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 (ACSRNEQYL) 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).

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

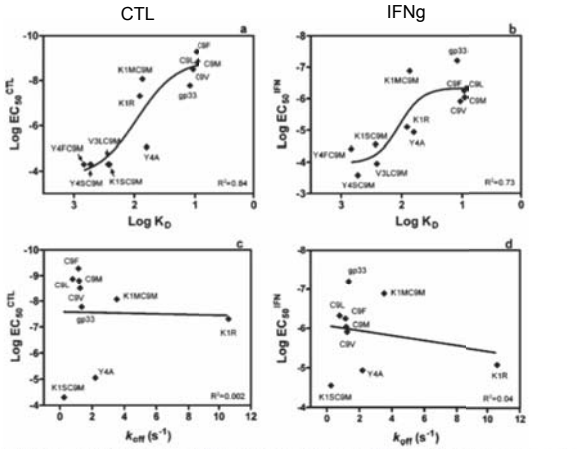


FIGURE 5. CTL response and IFN-γ production are correlated with binding affinity of peptide/D^b to P14 TCR. *a* and *b*, Correlations between log EC_{50}^{CTL} and log K_D (*a*), log EC_{50}^{IFN} and log K_D (*b*). All the data points are shown in Table II. The EC_{50}^{CTL} and EC_{50}^{IFN} values 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.

CTL ▲ ▸ Aff

CTL ▲ ▸ K diss

Importance de l'affinité

Kon

TCR + CMHpep < > TCR/CMHpep

Koff

K_{on}: Taux d'association
K_{off}: Taux de dissociation

Demi-vie : $t_{1/2}$= ln 2 (k_{off}) ◀

$K_A = K_{on}/K_{off}$

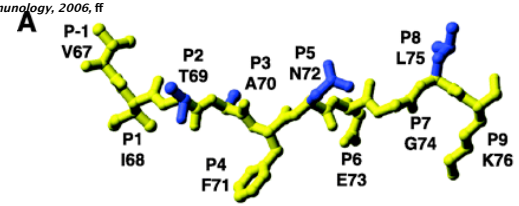
$K_D = 1/K_A$ (1///Affinité) ◀

Importance de K_D ou de $t_{1/2}$ pour activation T ?

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

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

Hb peptideff



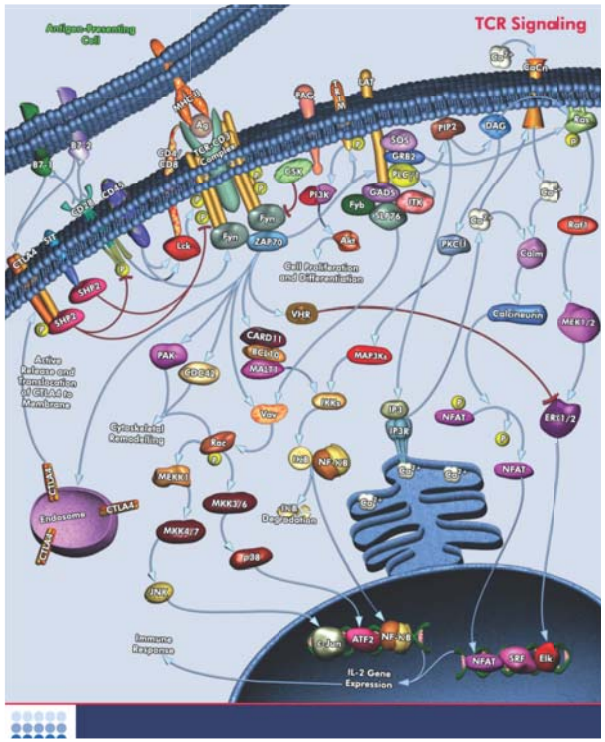
B

TCR mutantsff

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-aff nity 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 aff nity mutants** (M4, M14, and M15) with the location of their CDR mutations and respective KD, t1/2, kon, and koff values. 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).ff



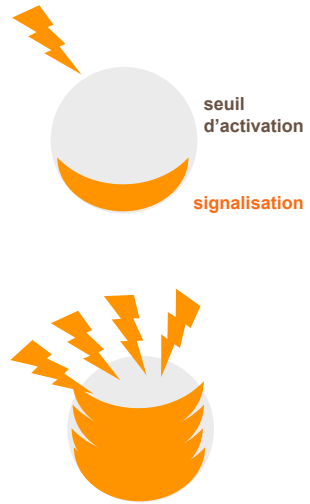
Activation T Transduction du signal

Lien entre Affinité et Transduction du signal ?

II. Activation T : un système dynamique

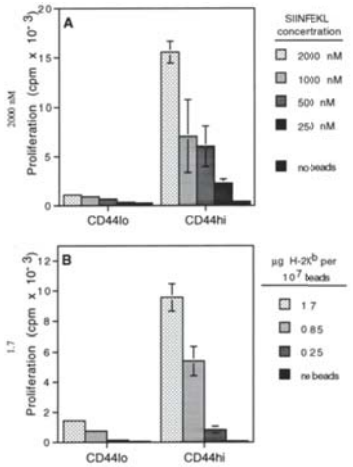
Stimulation réitérative du lymphocyte: « kinetic proofreading » model

- 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 (k_{off} faible, 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

Beads H-2Kb+OVA



- 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 µg protein/10^7 beads and pulsed with $OVA_{257-264}$ peptide at the indicated concentration.

Curtsinger, JI 1998

Seuil d'activation: T naïf vs T mémoire

T naïf

T mémoire

Affinité versus Avidité

force du signal

Affinité

++++

+

Avidité

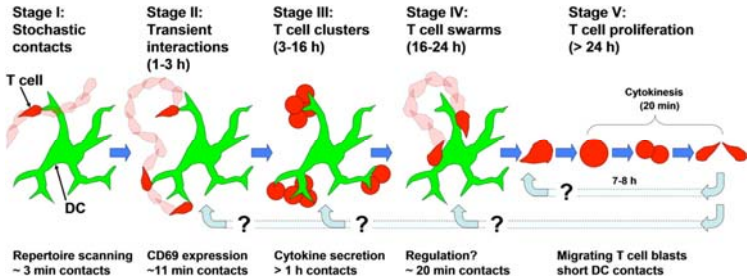
x n

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

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

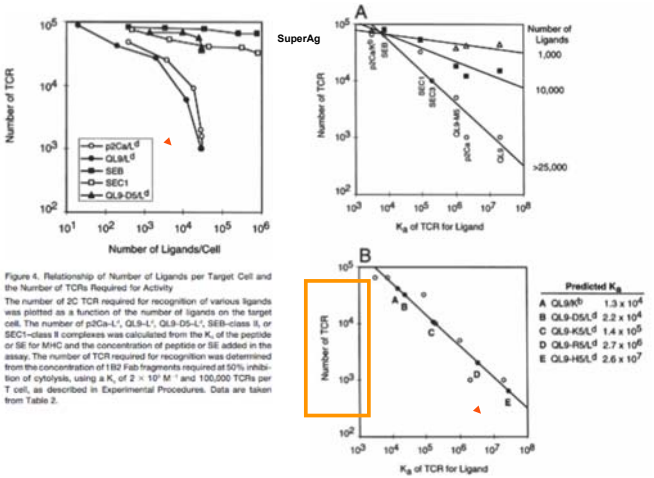


Figure 4. Relationship of Number of Ligands per Target Cell and the Number of TCRs Required for Activity

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.

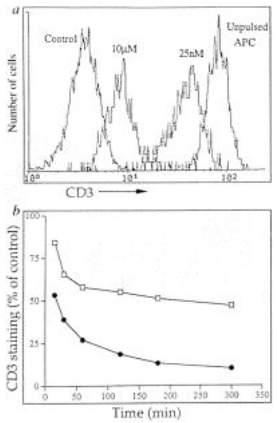
(B) K_a values for the TCR interaction with the indicated peptide–class I complexes were predicted from the number of TCRs required for activity. The curve determined from known K_a values for several TCR–ligand interactions was plotted versus number of TCRs at saturating ligand concentrations (open circles) to generate a standard curve. The inhibition analysis was used to determine the number of TCRs required at 3×10^{-9} M QL9-H5, QL9-R5, QL9-H5 and 10^{-8} M QL9-D5 with T2-L^d and 10^{-8} 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.

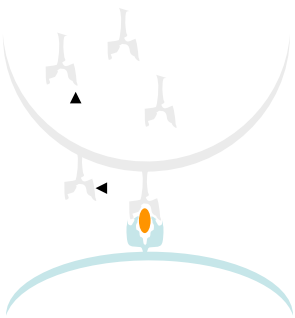
Modèle:
Clone 2C anti-AlloAg
Spé p2Ca/Ld; QL9/Ld
Nb ligands: β[peptide]
Nb TCR: [Fab 1B2], bloque TCR
Affinité: Mutants de QL9

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

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

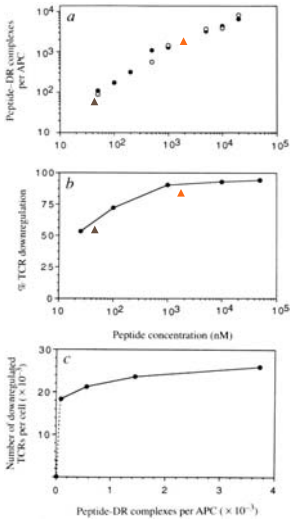




T cells conjugated with peptide-pulsed APCs undergo an antigen-dependent **downregulation of the TCR**/CD3 complex.ff

Nombre d'engagements

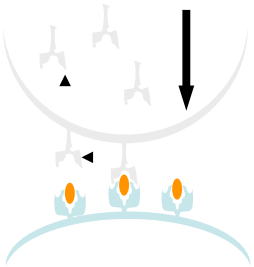
Serial triggering of many T-cell receptors by a few peptide-MHC complexes
Valitutti, Nature 1995



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 moéculaires

- **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

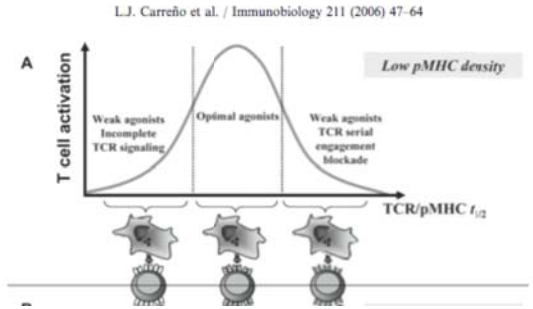


Fig. 2. T cell activation depends on TCR/pMHC interaction half-life and pMHC density. (A) When T cells interact with cognate pMHC at low density on the APC, efficient activation takes place within an optimal range of TCR/pMHC interaction half-life. Interactions with short half-lives cannot complete necessary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

Influence du temps de contact

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

Salvatore Valitutti and Antonio Lanzavecchia

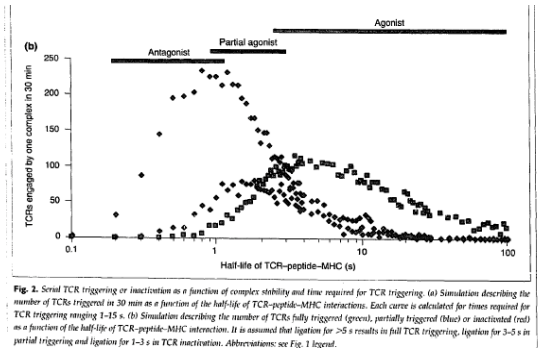
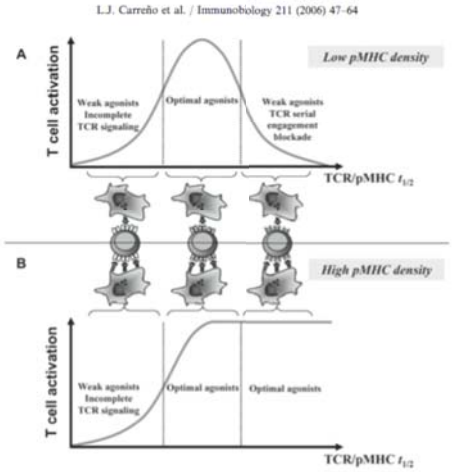


Fig. 2. Serial TCR triggering or inactivation as a function of complex stability and time required for TCR triggering. (a) Simulation describing the number of TCRs triggered in 30 min as a function of the half-life of TCR–peptide–MHC interactions. Each curve is calculated for times required for TCR triggering ranging 1–15 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 (par CMHp en 30min)	100	250

Influence de la densité de complexe CMH-Ag

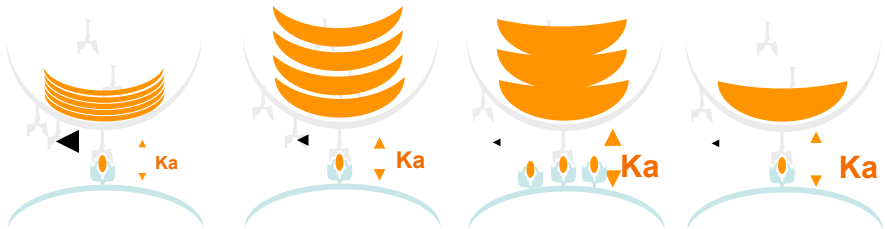


Conditions d'activation multiparamétriques

« demi-vie » de contact

Koff

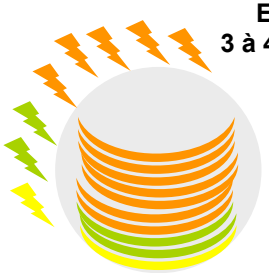
Ka



X n

Paramètres quantitatifs de l'activation

10^5 TCR / T



Engagement de 3 à 400 TCR différents

x 1- 200 / t = 2 - 30 s

Dépend de la présence de

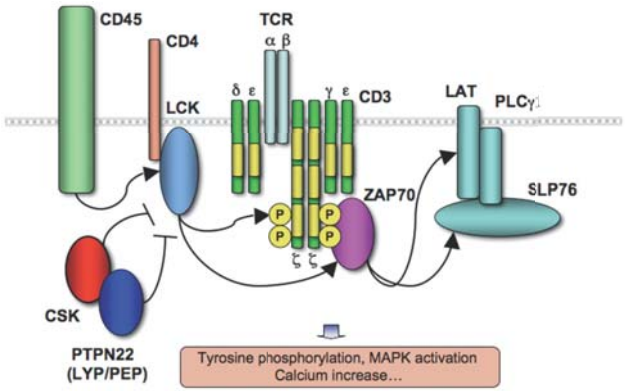
CD8

CD28
CD40L

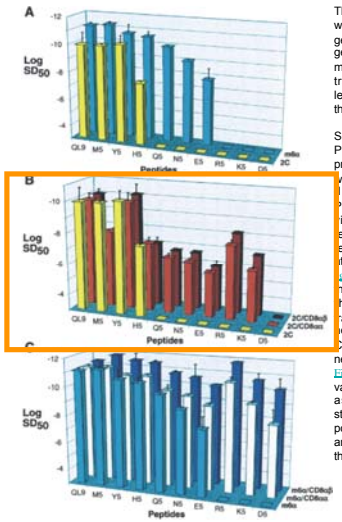
LFA-1

Rôle des molécules adaptatrices

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

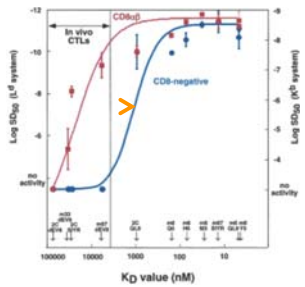


Rôle des molécules adaptatrices : CD8



The ββ-negative T cell hybridoma 58-/- was cotransfected with the 2C wt β chain gene and one of five different β chain genes from 2C and the mutants mβ m13β m33β and m67β . Stable transfectants that expressed similar levels of TCR were identified based on their staining with anti-Vβ 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.cate1.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 mβ CD8-negative; (B) 2C CD8-negative, 2C/CD8ββ and 2C/CD8ββ (C) mβCD8-negative, mβCD8ββ and mβCD8ββ 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).



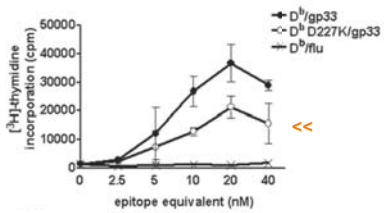
Relationship of Peptide Activity and TCR:pepMHC AffinitySD50 values of various peptides were plotted versus the equilibrium affinity constants (KD) of the corresponding TCR:pepMHC interaction (Table 1). In order to include values from the Ld and Kb systems, SD50 values from the two different antigen-presenting 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 CD8ββtransfectants (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).

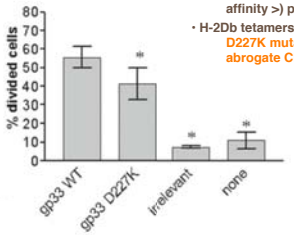
>> Aide à l'activation des clones T de faible affinité

Holler D , Imm 2003

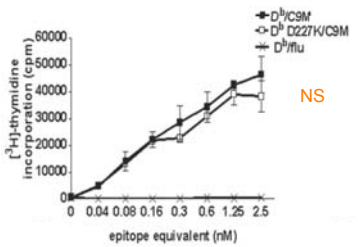
Molécule adaptatrices: indépendance pour les TCR de haute affinité

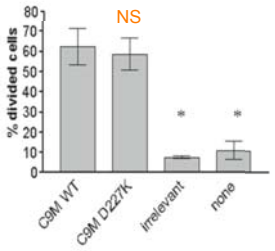
Kerry JI 2003





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

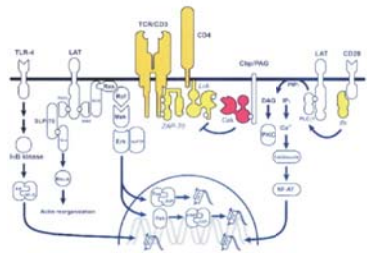




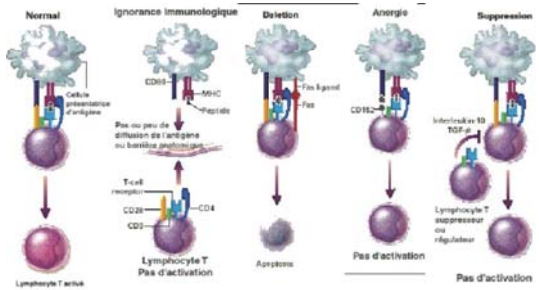
NB: Attention aux modèles de souris TCR transgéniques présentant un TCR de haute affinité

Rôle des molécules de costimulation:

- Mol de costimulation:
  - signal activateur complémentaire
  - CD28/ CD80
  - CD28/ CD86
  - CD40/ CD40L



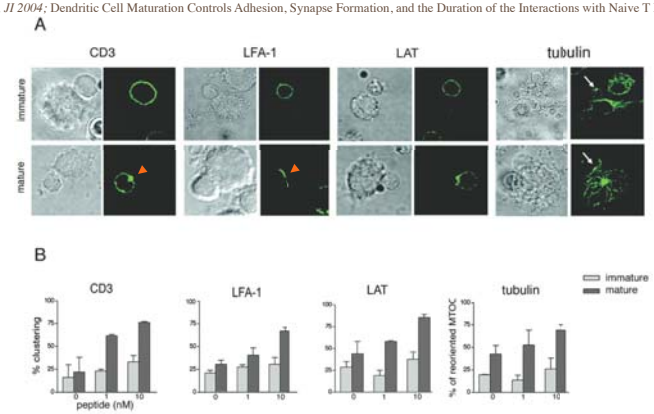
- Absence:
  - Induction d'anergie
  - mécanisme de tolérance périphérique



Mécanismes possibles de la tolérance périphérique

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



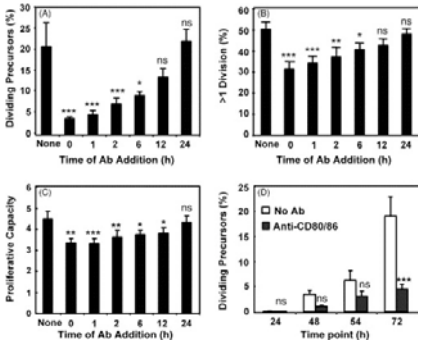
DC maturation is required for effective clustering and SMAC formation at the DC-T cell contact site

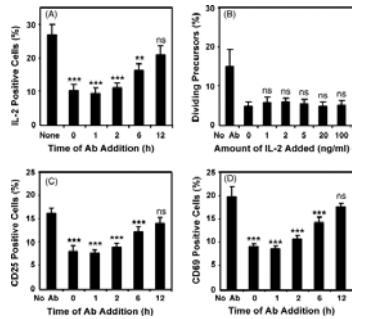
Efficient clustering in naive T cells requires DC maturation. Confocal images showing the distribution of CD3, LFA-1, LAT, and tubulin in T cells forming conjugates with immature or mature D1 pulsed with 10 nM H-Y peptide. Conjugates were formed for 30 min, washed five times, and fixed for immunostaining. *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, $n = 243$.

Nécessité d'un engagement prolongé de CD80/CD86

Liwski, Imm Letters 2006

CD4 anti-OVA ±anti-CD80/86



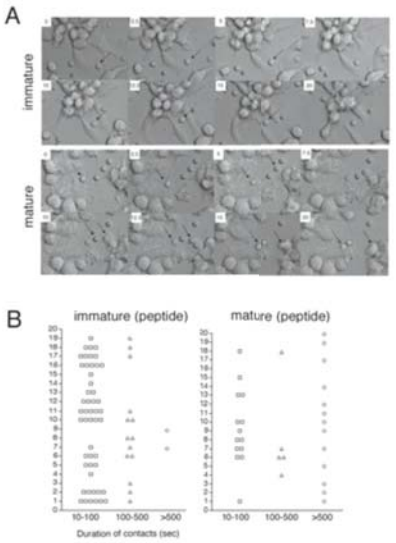


- **To investigate the functional effects of CD80/CD86 blockade on naive CD4+ T cell activation, freshly isolated CD4+ T cells from OVA-TCR transgenic DO11.10 mice [27] were labeled with CFSE and cultured with OVA-peptide pulsed, mature DC in the absence or presence of CD80 and CD86 specific mAbs.ff**

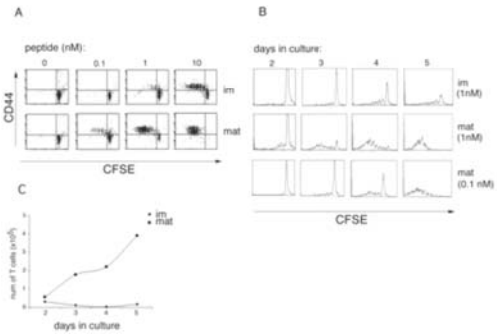
- **We demonstrated that DC mediated CD80/CD86 costimulation controls the magnitude of the naive T cell proliferative response by regulating both responder frequency as well as proliferative capacity.ff**

- **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.ff**

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

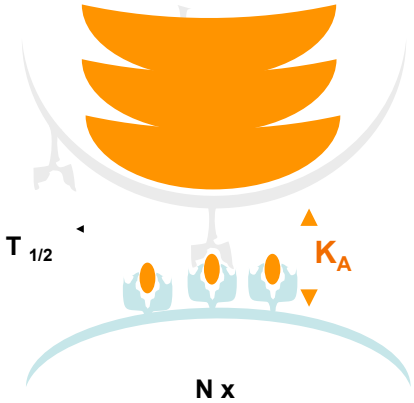


- Proliferation of naive T cells stimulated by **immature or mature DCs.** Immature or mature D1 pulsed with different doses of peptide were cocultured for 5 days with CFSE-loaded naive T lymphocytes (1:5 ratio). *A*, Representative dot blot profile showing the loss of CFSE and the up-regulation of CD44 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.

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



Stabilité du complexe CMH-peptide détermine la sélection clonale T

Peptide-MHC Class II Complex Stability Governs CD4 T Cell Clonal Selection

Christina K. Baumgartner, Andrea Ferrante, Mika Nagaoka, Jack Gorski, and Laurent P. Malherbe

C

peptide	amino acid sequence	$T_{1/2}$(h)	IC_{50}(μM)
MCC_{95A}	ANERADLAAYLKQATK	0.6 ± 0.1	42.3 ± 3.9
PCC_{88-104}	KAERADLIAYLKQATAK	4.7 ± 0.2	2.1 ± 0.5
MCC_{88-103}	ANERADLIAYLKQATK	73.3 ± 8.3	1.1 ± 0.1
PCC_{103K}	KAERADLIAYLKQATKK	229.5 ± 46.7	0.5 ± 0.04
I-E pocket		P1	P9

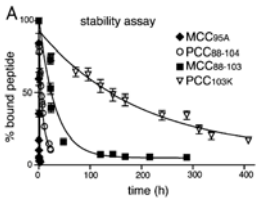


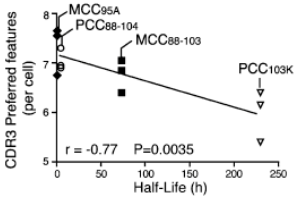
FIGURE 1. Generation of a peptide hierarchy to sample the importance of pMHCII stability for CD4 T cell clonal selection. Comparison of the (*A*) stability or (*B*) affinity of various I-E^k binding peptides at 37°C. The lines represent the fit of the data to (*A*) a single-exponential function or (*B*) a three-parameter sigmoid function from which the half-lives ($t_{1/2}$) or the IC_{50} could be determined. *C*, Peptide sequences are aligned according to their anchor positions (P1, P9). P1 and P9 pocket residues are in bold. Half-lives and IC_{50} of I-E^k/peptide complexes are indicated (mean ± SEM). Reactions were performed in triplicate, and data series represent one of three independent experiments.

Ag-specific TCR repertoire diversity correlates directly with pMHCII stability

To determine the clonal composition of the responding T cells induced by peptides with varying binding half-lives with MHCII molecules, we sorted single Ag-specific CD4 T cells ($V\alpha11^+V\beta3^+$ $CD44^{hi}CD62L^{lo}$ cells) from draining LNs 7 d after peptide immunization and sequenced their CDR3α and CDR3β regions using a single-cell RT-PCR approach. Previous studies have shown

A

Preferred Features	MCC_{95A}	PCC_{88-104}	MCC_{88-103}	PCC_{103K}
8				
7				
6				
≥6 (%)	95±5	90±3	83±4	72±6
5	•		••••	••••••
4		••	••	••••
3	•		•••	•••••
2	•	••		••
1		•		
n=	60	60	60	60



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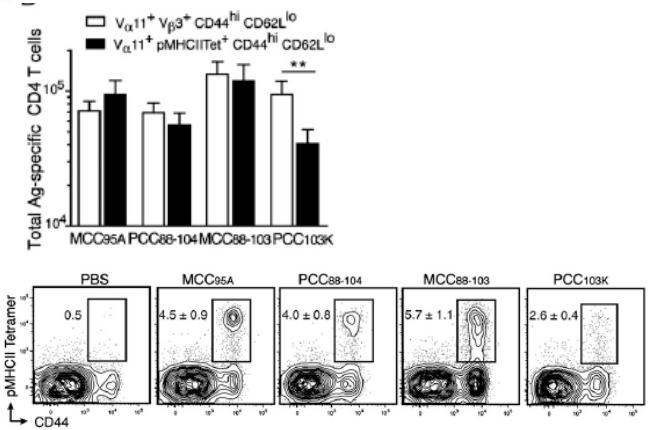
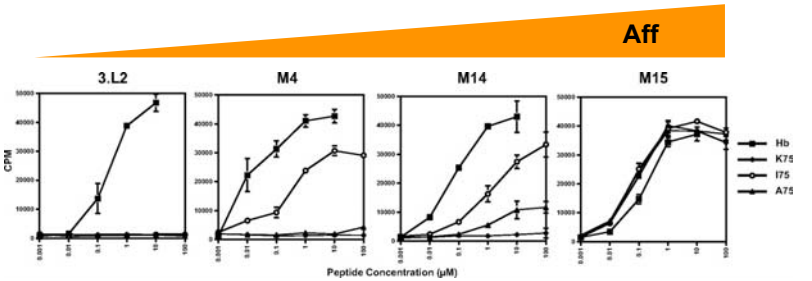


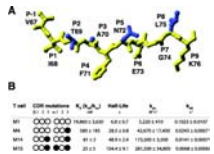
>>Preferential accumulation of high-affinity CD4 T cells with low-stability peptides

Affinité & Dégénérescence de la reconnaissance TCR

The Study of High-Affnity TCRs Reveals Duality in T Cell Recognition of Antigen: Specificity and Degeneracyff

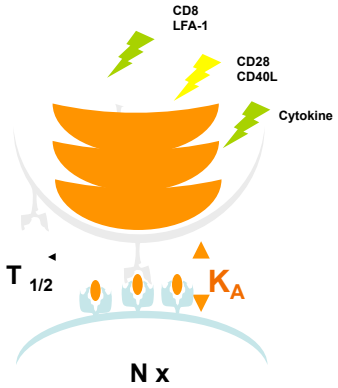
D Donermeyer et al; Journal of Immunology, 2006, ff

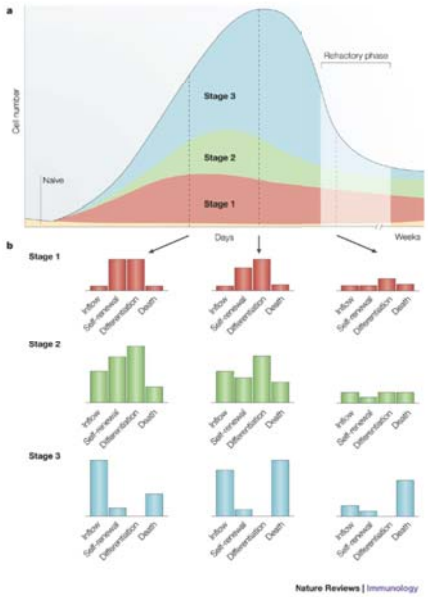




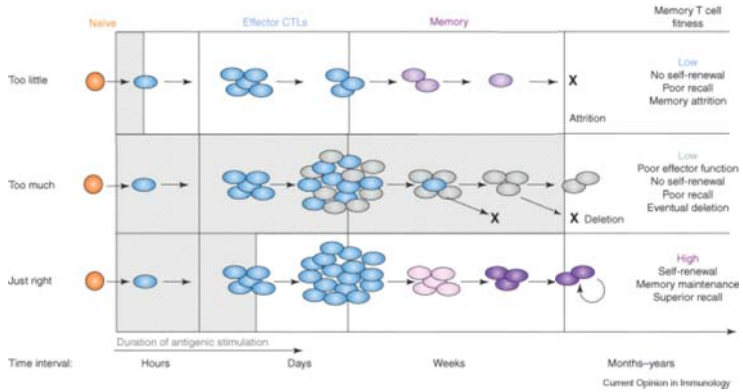
Degeneracy in the recognition of Hb correlates with increased aff nity. CD4+ T cell hybridomas expressing 3.L2, M4, M14, and M15 TCRs were stimulated with the indicated peptide concentrations of Hb or APLs of Hb at the P8 (75) position using CH27 as APC. The APLs are designated as described in Fig. 2. 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).ff

conclusion





Importance des paramètres d'activation : Génération des T mémoire



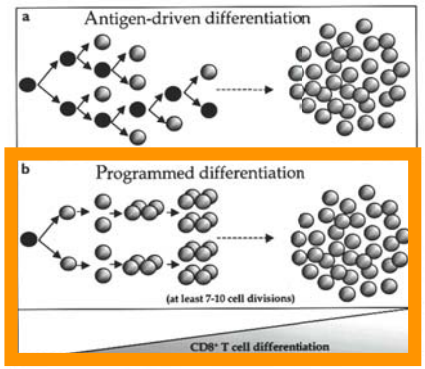
Masopust Curr Op Imm 2004

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.



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

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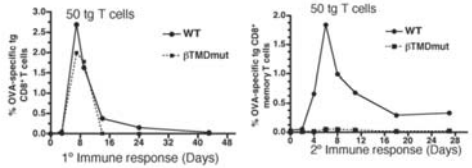
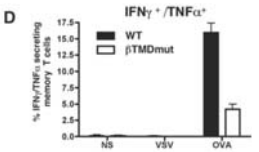
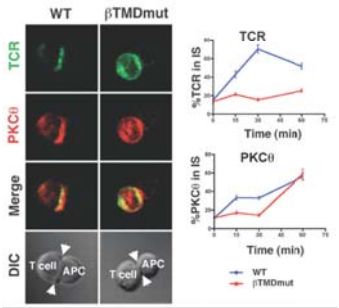
We generated OT-1 TCR transgenic mice expressing a point mutation in the βTMD, where the most carboxy-terminal tyrosine residue of the CART motif was replaced by a leucine (CART15 YL). TCR expression on mutant T cells was slightly decreased, but the mutant TCR-CD3 complex composition was unaltered .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

Using a mutant TCR transgenic model, we found that point mutations in the TCR β transmembrane domain (βTMD) 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 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-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.



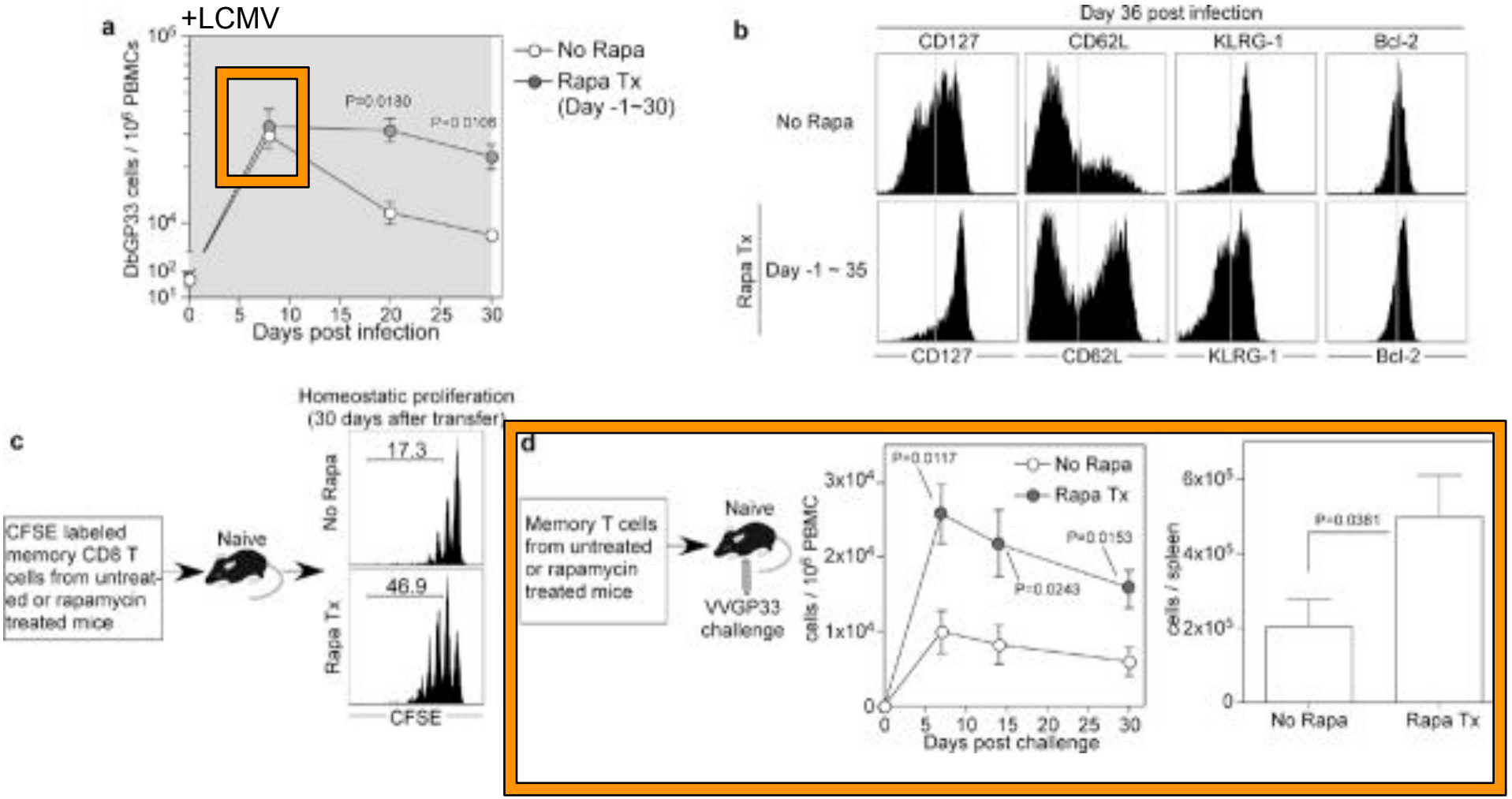




mTOR regulates memory CD8 T cell differentiation

B. Bellier

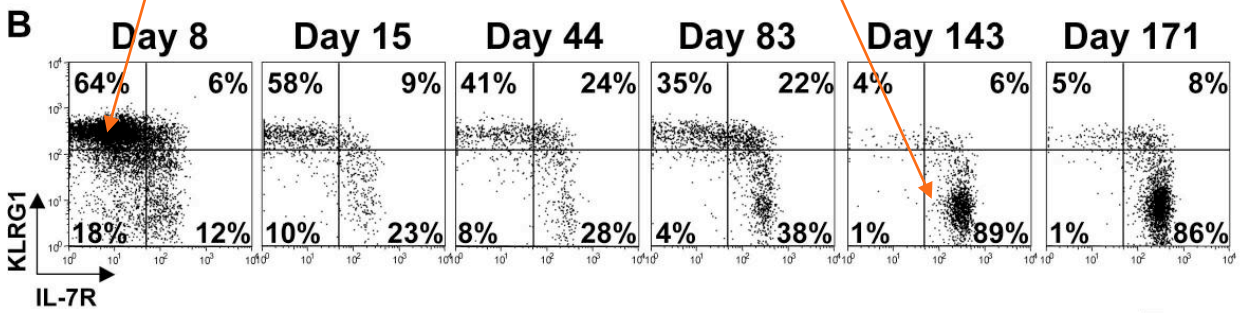
mTOR regulates memory CD8 T-cell differentiation.
Araki K & Ahmed R.
Nature. 2009 Jul



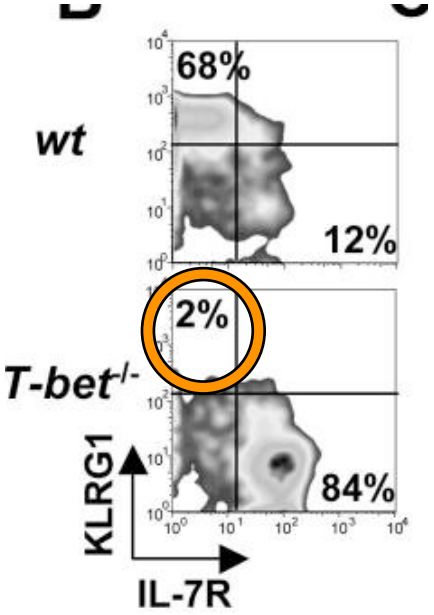
TCR >> mTOR >> Metabolism >> Memory differenciation

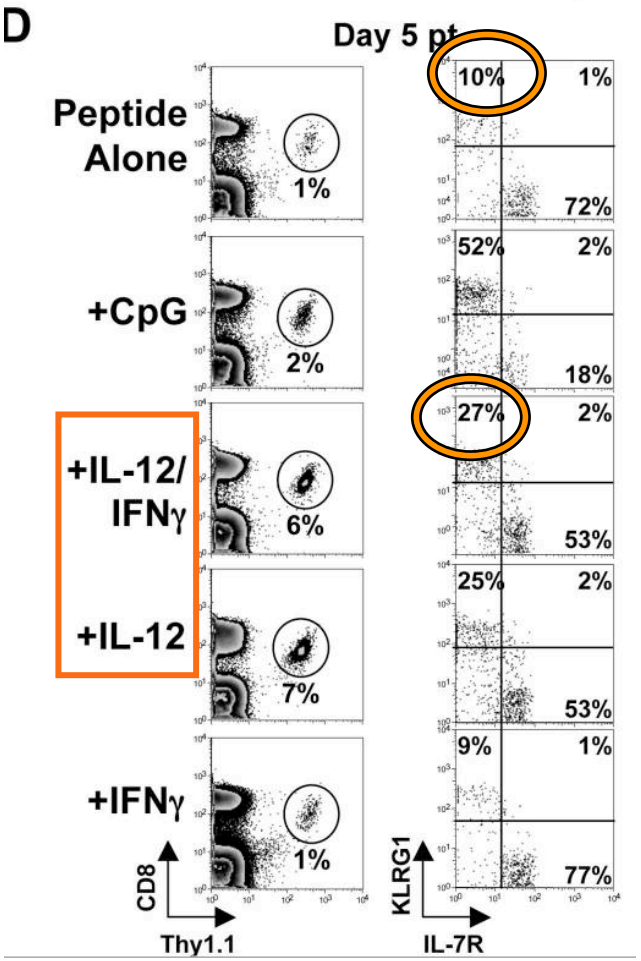
Short-live effector / Memory precursor modulation by T-bet

SLEC KLRG1^{hi} IL-7R^{lo} **MPEC** KLRG1^{lo} IL-7R^{hi}

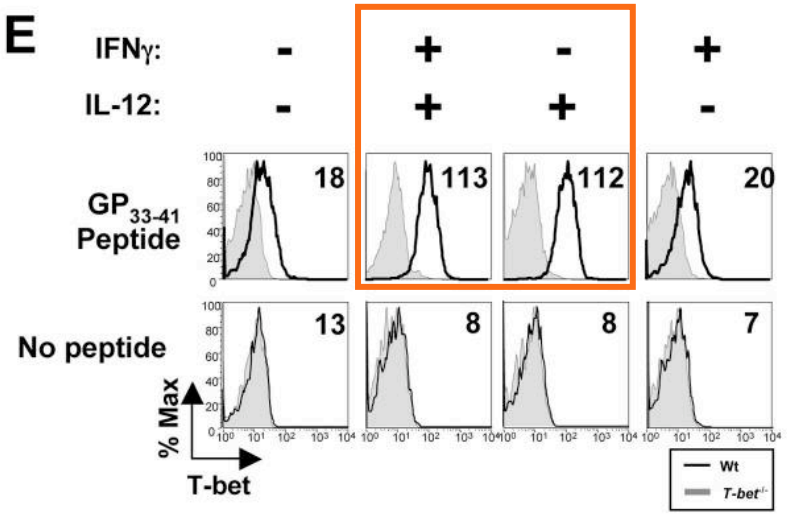


Inflammation Directs Memory Precursor and Short-Lived Effector CD8(+) T Cell Fates via the Graded Expression of T-bet Transcription Factor
Joshi et al, Immunity 2007





T-bet expression is necessary and sufficient for development of KLRG1^{hi} SLECs.



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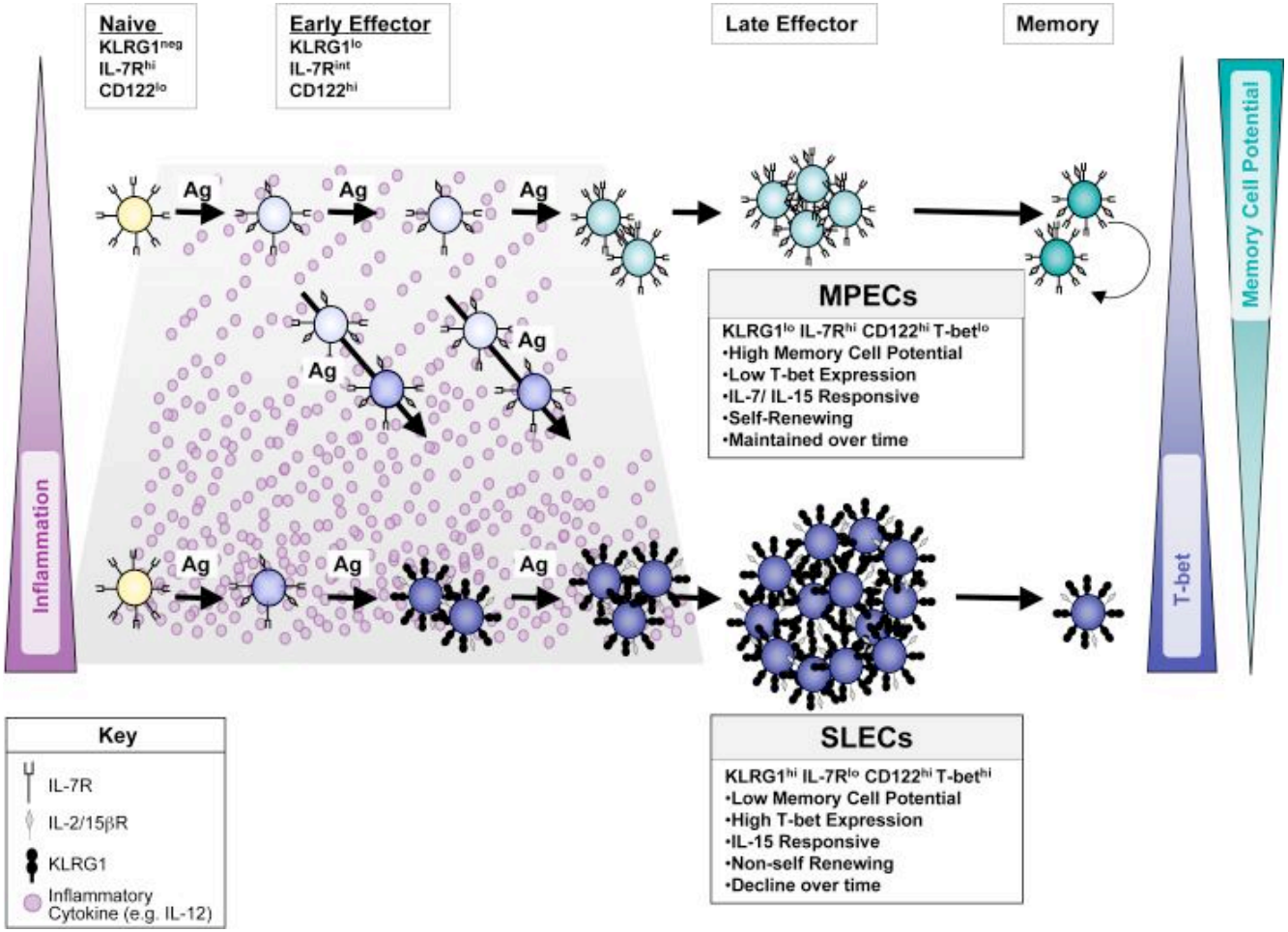
Model of SLEC and MPEC development during acute viral infection.



- Naive CD8 T cells are IL-7R^{hi}, CD122^{lo} (IL-2/15β), KLRG1^{neg} and T-bet^{neg} and are IL-7 dependent. Early during infection, most effector CD8 T cells become CD122^{hi} and downregulate IL-7R to an intermediate-to-low level, but expression of T-bet and KLRG1 is set depending on their exposure to inflammatory cytokines (e.g. IL-12). Effector CD8 T cells that are exposed to lower levels of inflammation express less T-bet (light blue cells) and begin to upregulate IL-7R to become KLRG1^{lo} IL-7R^{hi} MPECs (turquoise cells). Effector CD8 T cells that encounter higher levels of inflammatory cytokines express relatively more T-bet and KLRG1 (dark blue cells), stably repress IL-7R and consequentially become KLRG1^{hi} IL-7R^{lo} SLECs. SLECs become IL-15 dependent, however, IL-15 alone cannot support their long-term persistence or homeostatic turnover and they decline over time. In contrast, MPECs remain dually responsive to IL-7 and IL-15 and preferentially develop into long-lived memory CD8 T cells that can self-renew.

Conclusions

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

- Déterminent
  - **Activation lymphocytaire**
  - **Différenciation lymphocytaire**