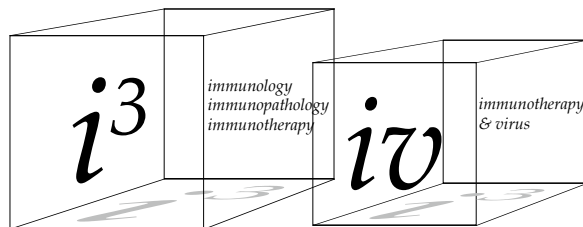


UPMC BMC423 IF

Tolérance Immunitaire



Bertrand Bellier

UPMC CNRS

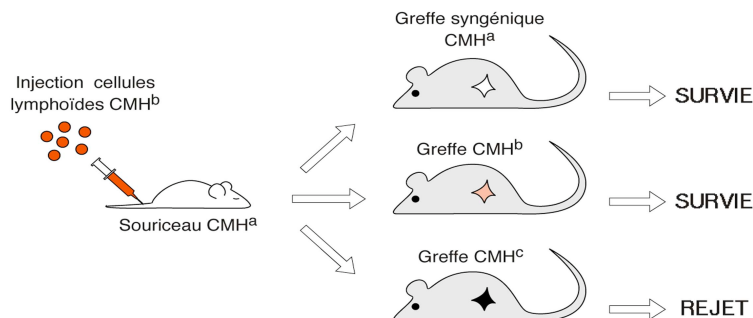
Immunology Immunopathology Immunotherapy

Pitié-Salpêtrière - Paris - France

bertrand.bellier@upmc.fr

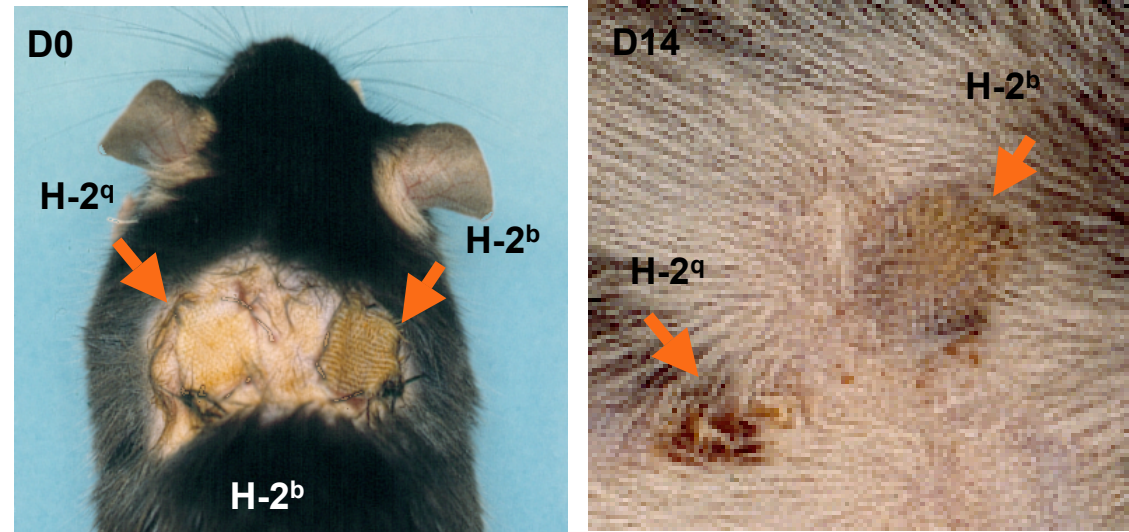
- **Propriété essentielle du système immunitaire**
- **Assure la discrimination du soi / non-soi**
- **Inné (Éléments étrangers ou cellules anormales)**
- **Adaptatif (Ag soi et non soi)**
- **Tolérance naturelle**
 - La tolérance naturelle au soi a été acquise après des centaines de millions d'années d'évolution qui ont sélectionné des récepteurs permettant de différencier les produits microbiens ou les cellules infectées des produits du soi ...
 - Mise en place de mécanismes régulateurs
- **Tolérance induite**
 - Induction d'une non-réactivité immunitaire (soi / non-soi)
 - Infections chroniques
 - Immunothérapies actives (MAI)
- **Conséquences:**
 - Tolérance au soi
 - Réponse immunitaire et élimination des pathogènes
 - Rejet des greffes allogéniques
 - En cas de rupture de la tolérance : Maladies Auto Immunes

- **Tolérance vis-à-vis d'antigènes du soi:**
 - Debut XXeme siècle: **Ehrlich and Morgenroth** (1901)
Absence de réponse immunitaire de la chèvre vis à vis de ses propres hématies; par contre production d'Ac hémolytiques contre des hématies d'un animal tiers
- **Les périodes foétale et néonatale sont rapidement apparues comme des stades privilégiés d'acquisition d'une tolérance par exposition à des antigènes étrangers:**
 - **1938- Traub** induit un état de tolérance en injectant le virus LCMV in utero : Nouveau-nés deviennent porteurs chroniques sains
 - **1949- Macfarlane Burnett:** Nature de la réponse induite lors du premier contact antigénique (réponse versus tolérance) dépend de l'âge de l'individu.
 - **1953: Medawar** induction d'une tolérance néonatale par injection de cellules allogéniques chez le nouveau-né :

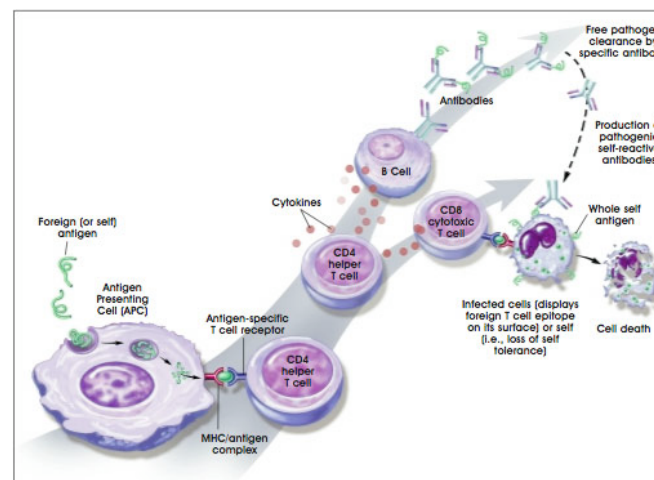


Conséquences physiologiques: Tolérance au soi / Rejet du non-soi

- Greffes:**

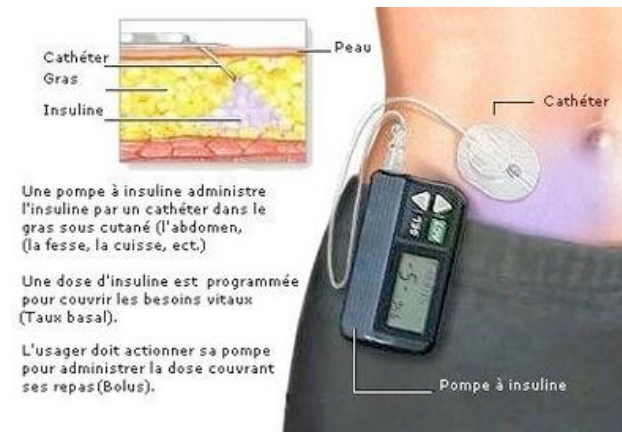
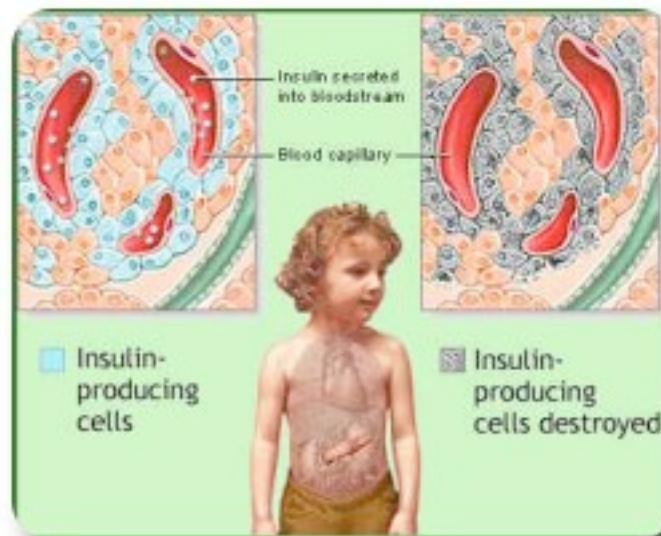


- Infections:**

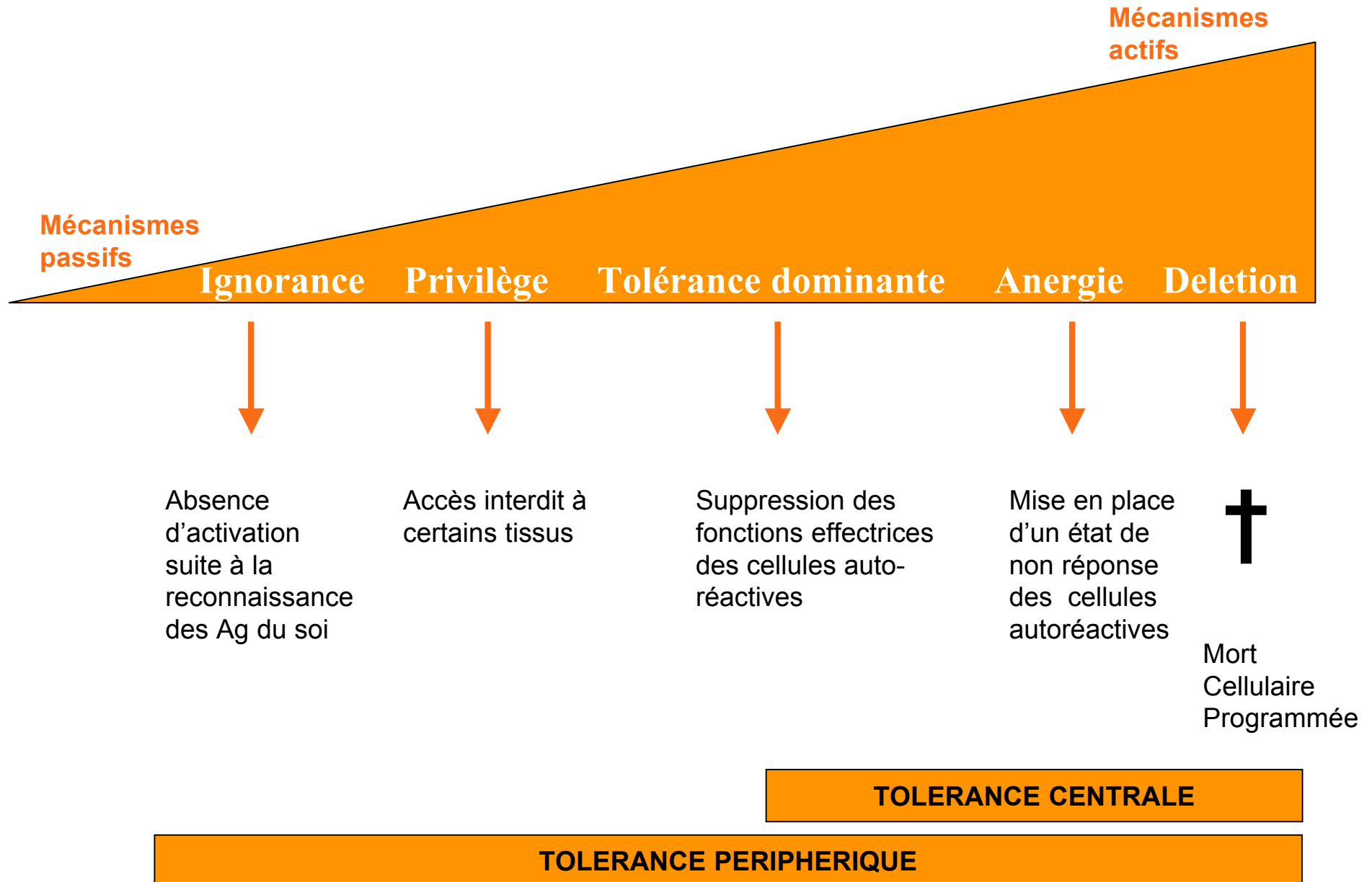


Conséquences physiologiques: Rupture de Tolérance / Rejet du soi

- **Maladies auto-immunes:**
 - Ex: les thyroïdites auto-immunes, la polyarthrite rhumatoïde, la spondylarthrite ankylosante, le diabète type 1, la maladie de Crohn, etc...

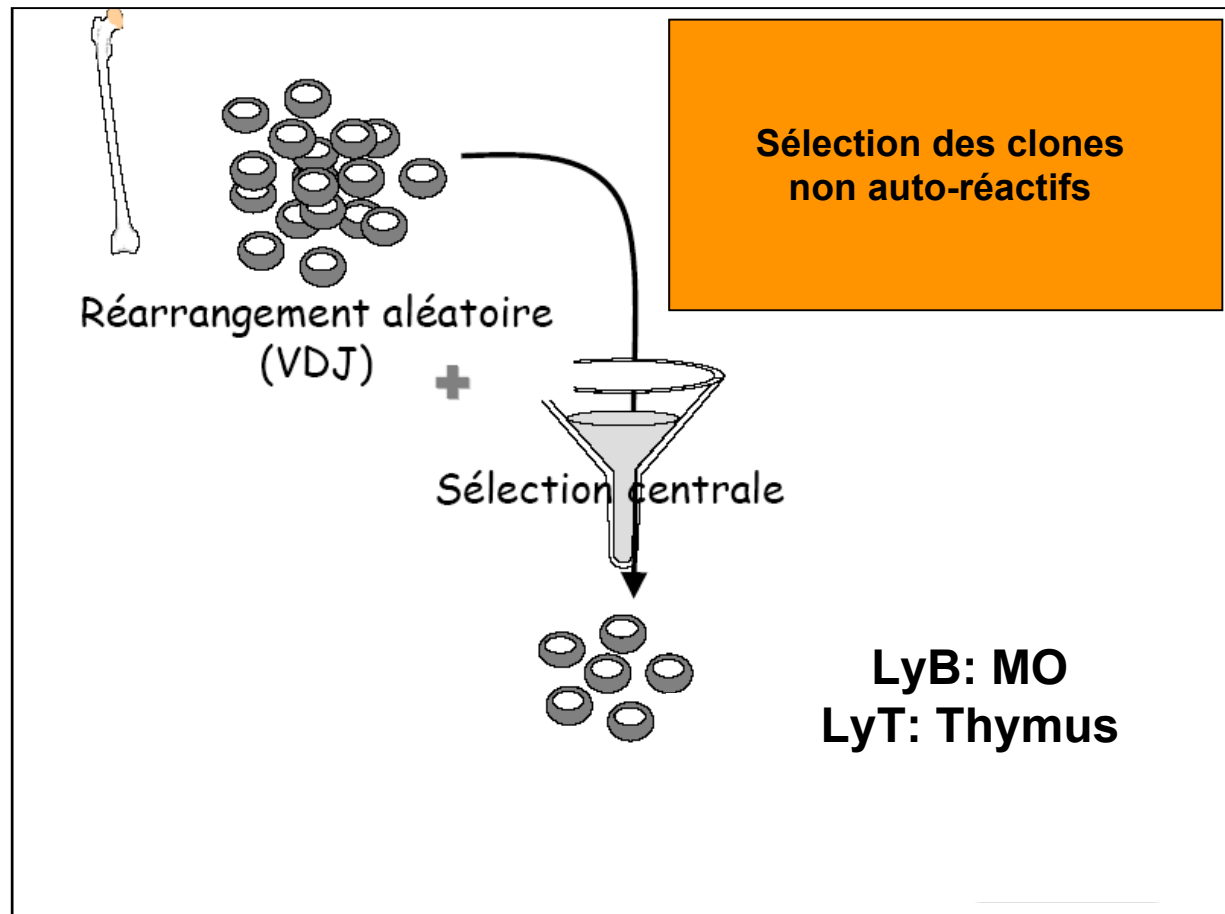


Cette absence de réponse aux Ag du soi est assurée par différents mécanismes



Tolérance centrale

Tolérance centrale: Sélection lymphocytaire dans les organes lymphoïdes primaires



Sélection lymphocytaire : Une nécessité en raison du caractère stochastique de la génération des récepteurs T/B

- Ontogénie T et B:**

Récepteur TCR et BCR sont générés par recombinaison génétique des locus

>> Diversité combinatoire

>> Diversité jonctionnelle

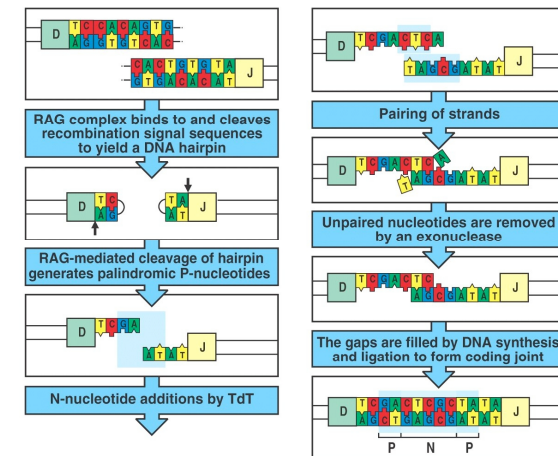
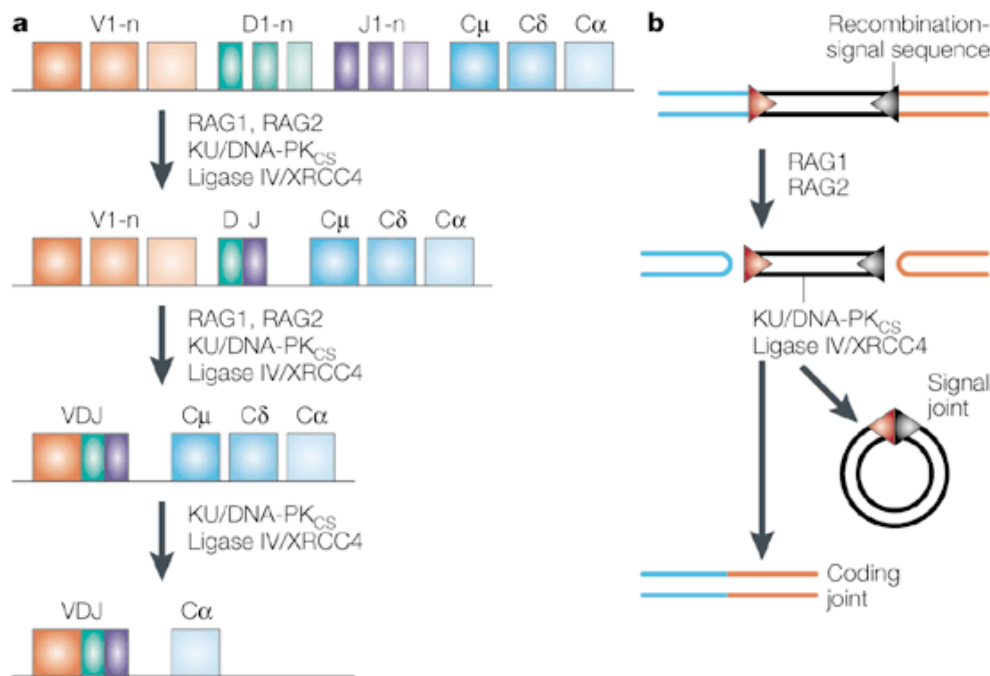
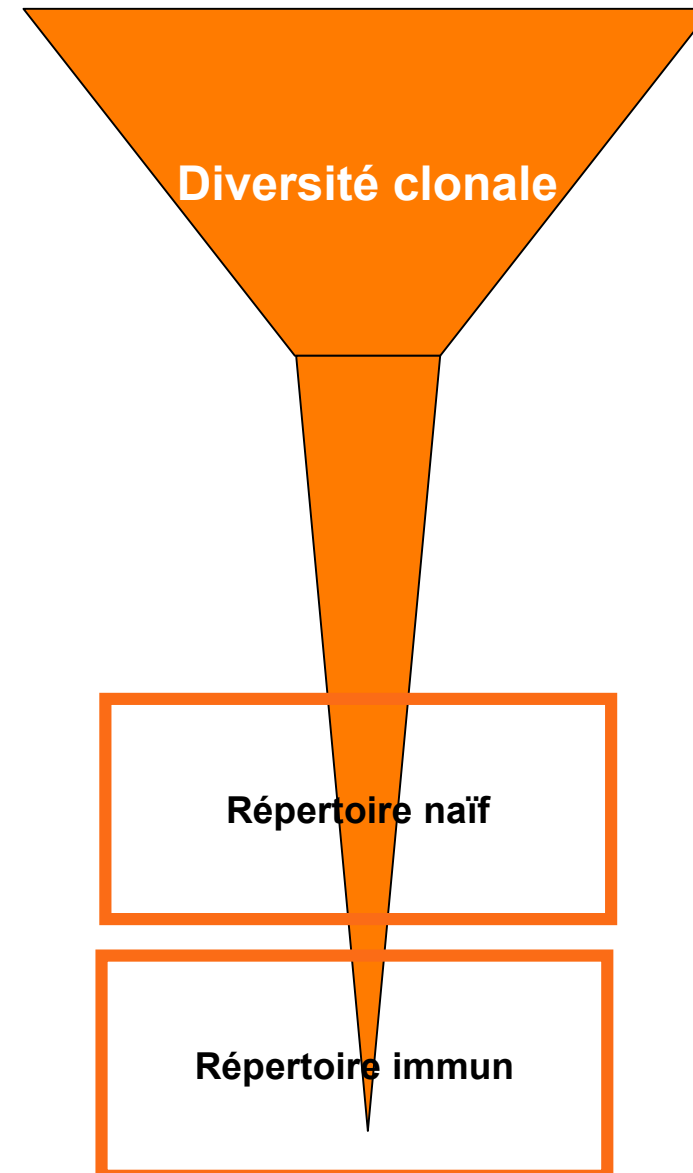
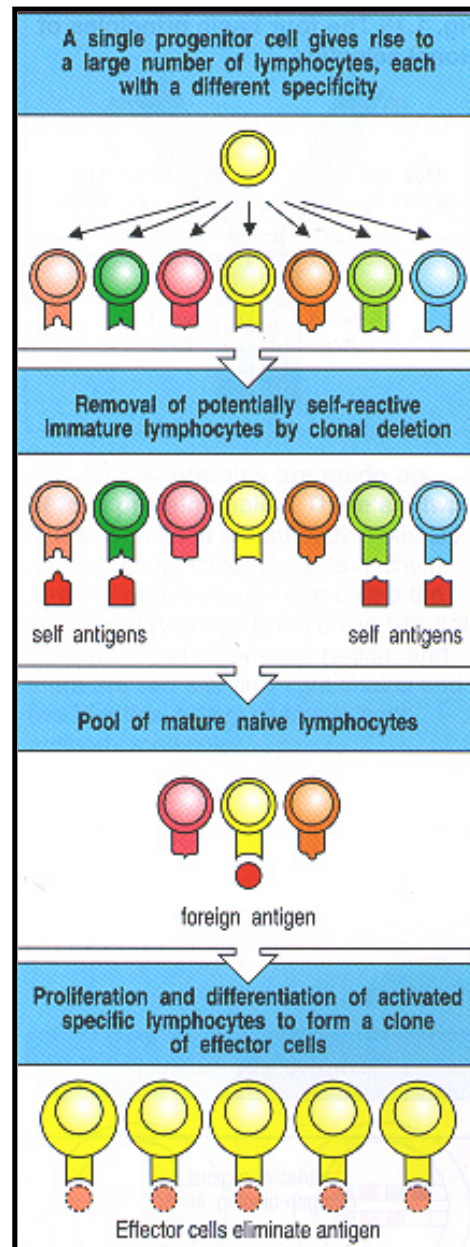
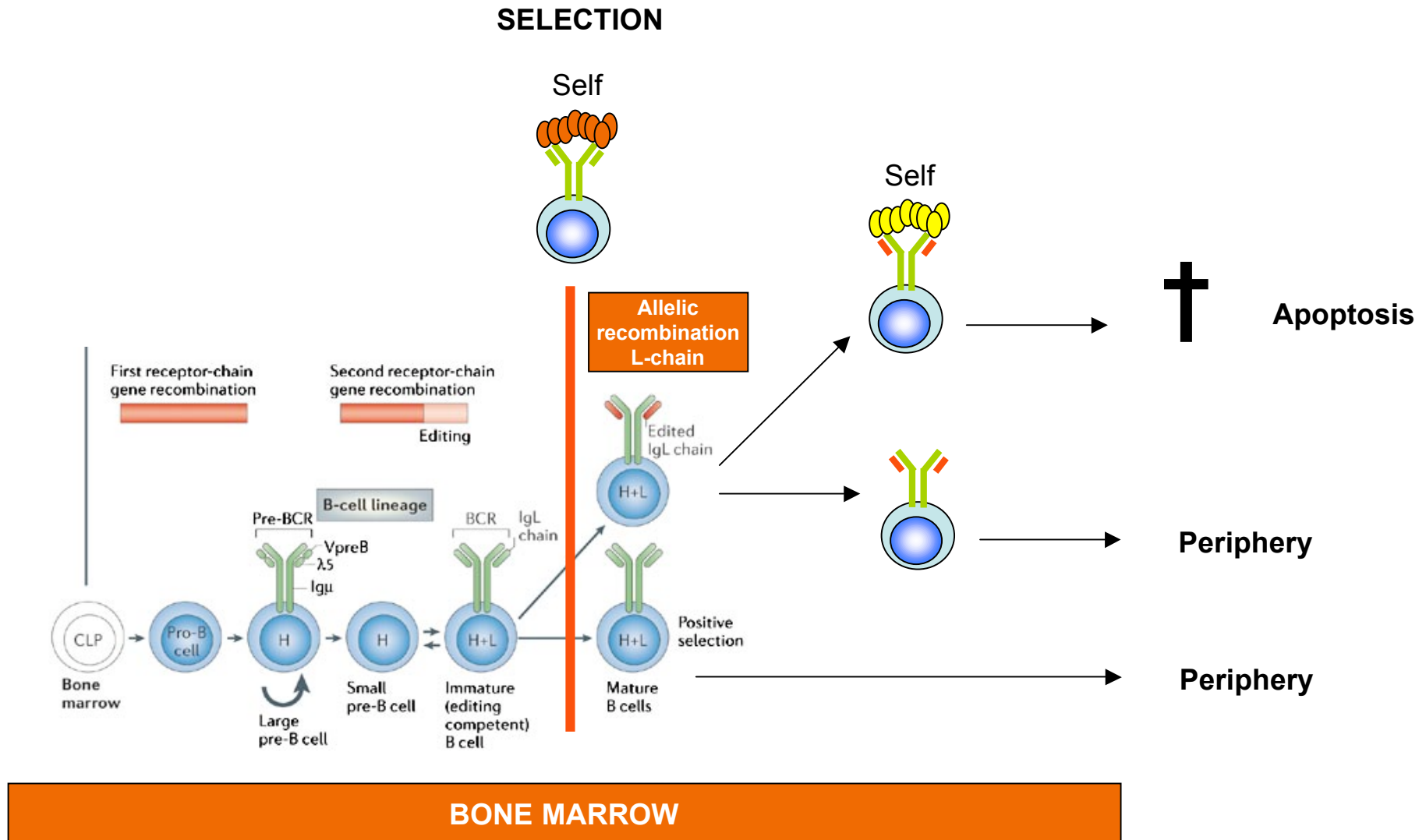


Figure 4-8 Immunobiology, 6/e. (© Garland Science 2005)

Sélection lymphocytaire : Une nécessité élimination des clones auto-réactifs



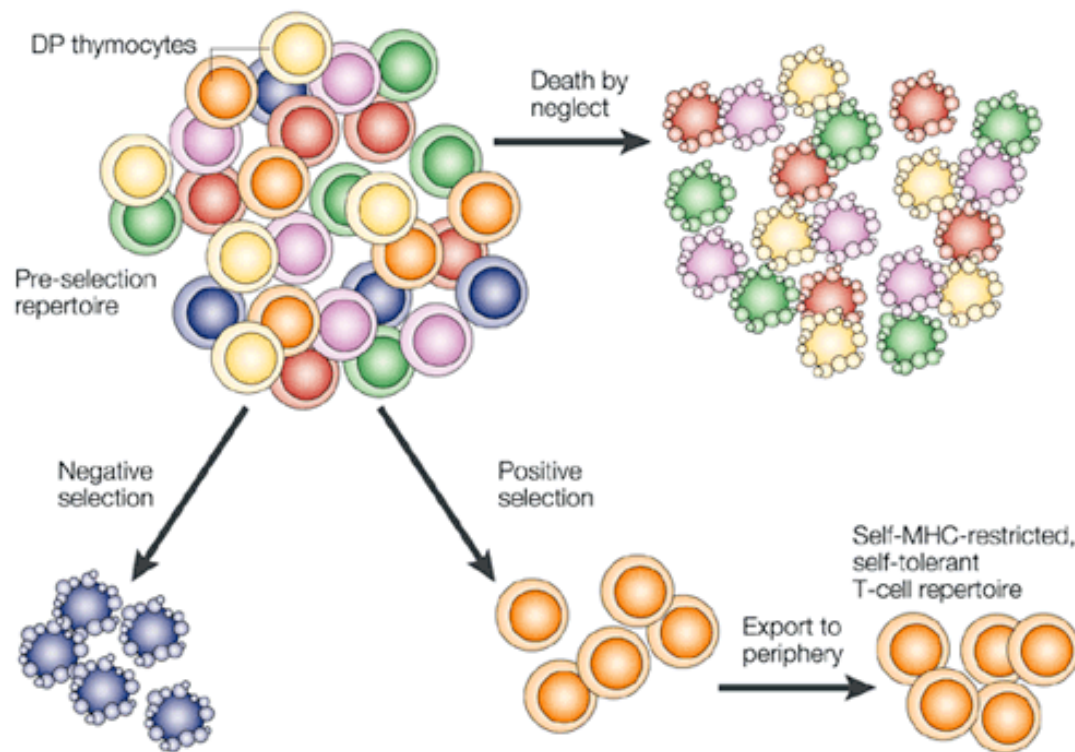
Sélection lymphocytaire B: Survie après « Editing » ou Mort programmée



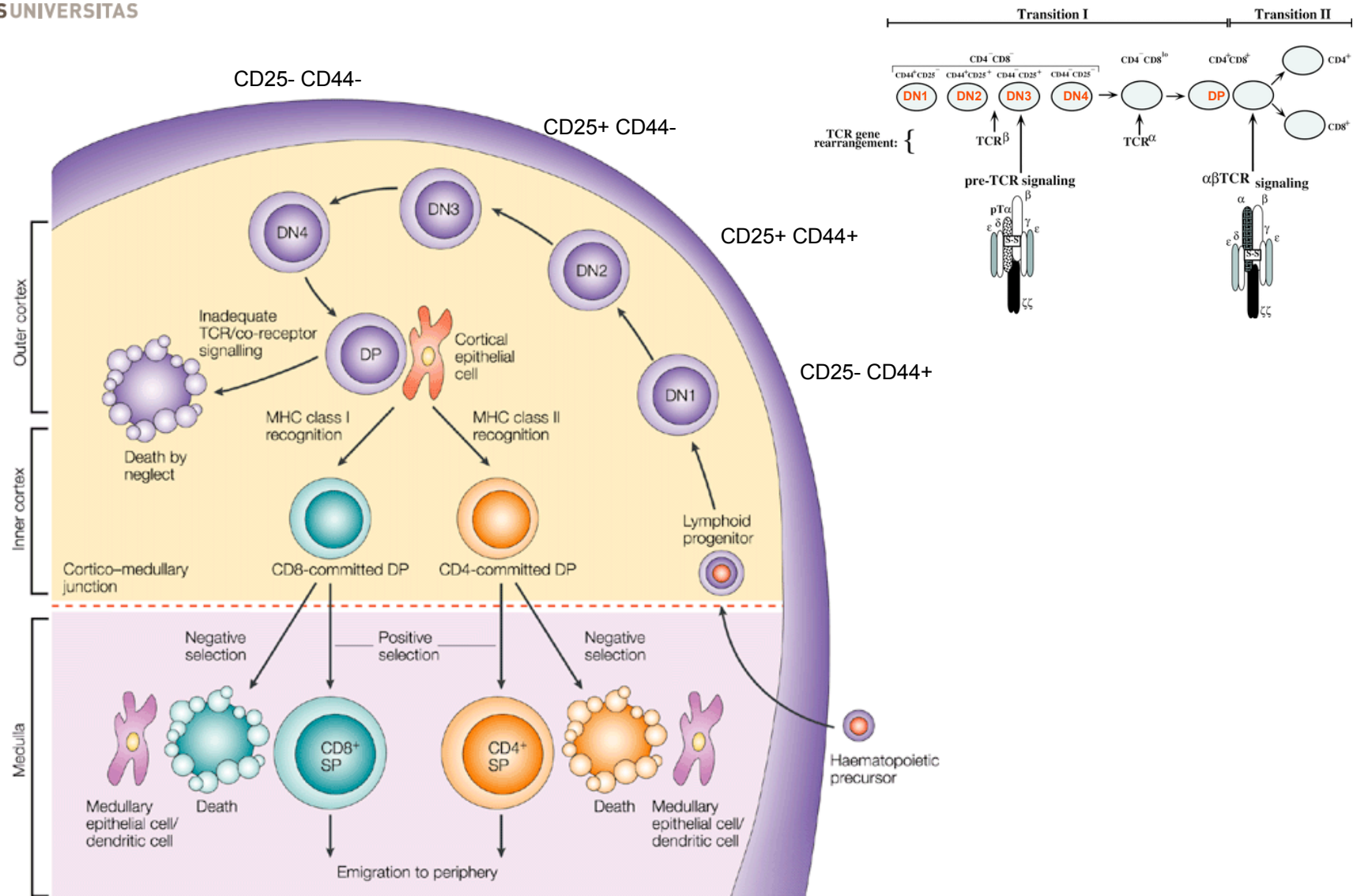
Sélection lymphocytaire T

- **Double sélection :**

- **Sélection positive** des clones T dont le TCR peut interagir avec les molécules de CMH
 >> apporte un **signal de survie**
- **Sélection négative** des clones T reconnaissant les Ag du soi
 >> délétion par **apoptose**

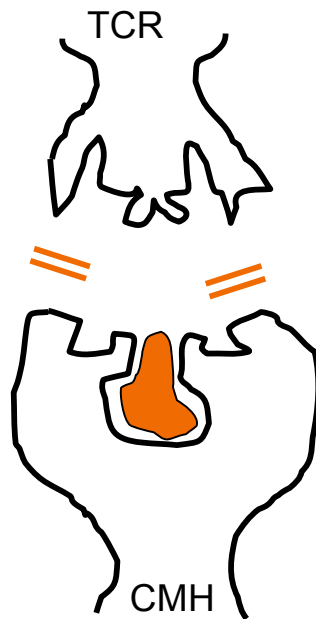


Mécanisme de sélection thymique



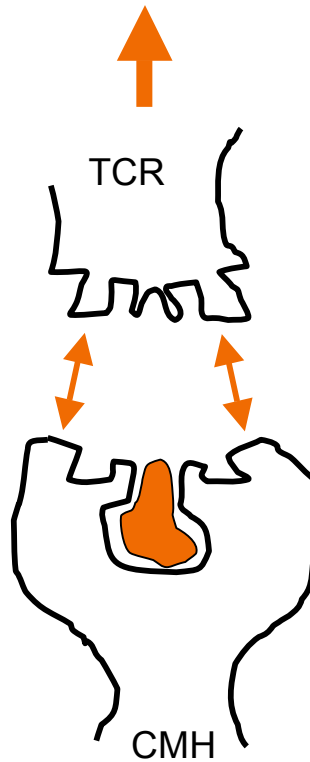
Sélection positive Signal de survie

Absence
de signal

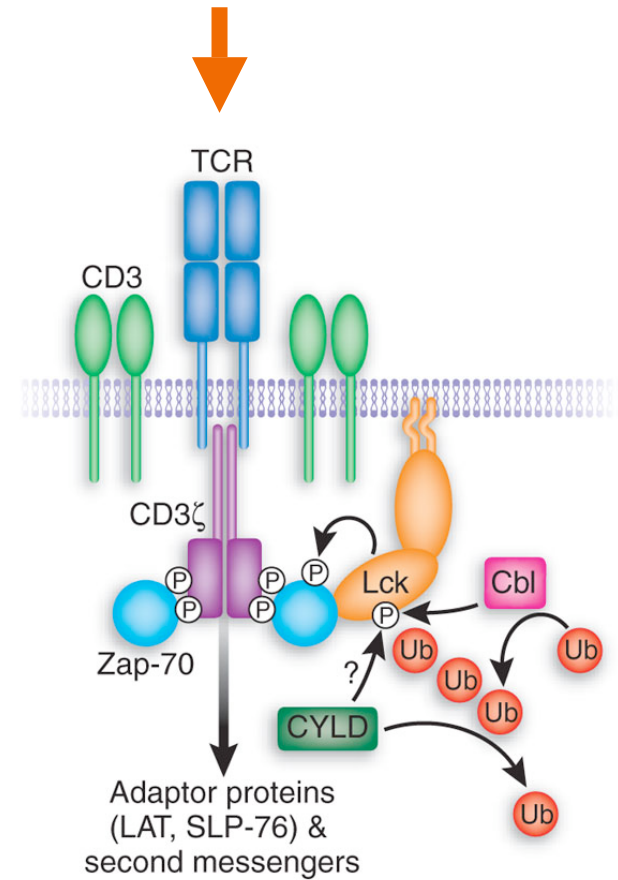


Mort par négligence

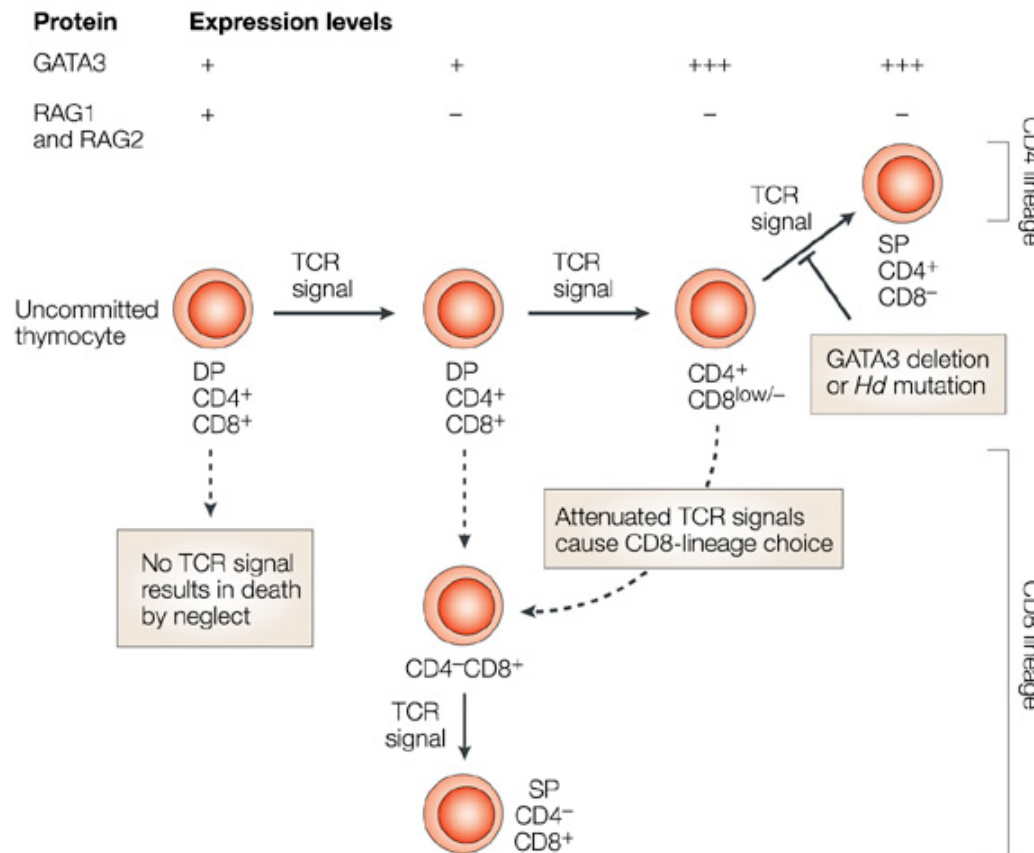
Signal



Survie

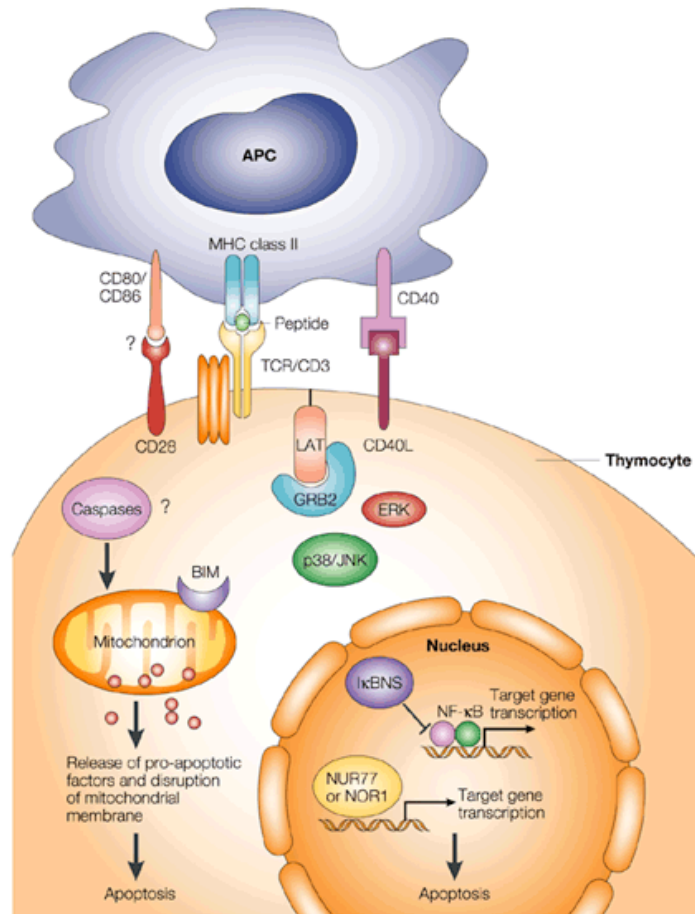


Pendant la sélection positive, les interactions lors de la sélection positive détermine la différenciation CD4/CD8



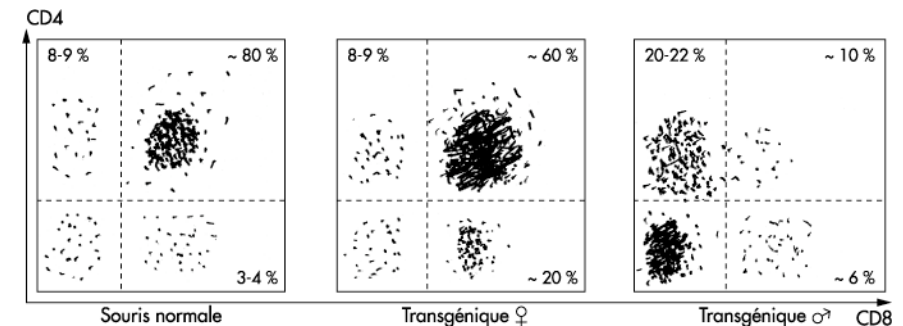
- T-cell receptor (TCR)-signal-induced and TCR-independent (dotted arrows) developmental stages are shown, based on a hypothetical model of thymocyte development. In the absence of TCR signalling, double-positive (DP) thymocytes die by neglect prior to lineage differentiation. Initial TCR signalling in DP cells causes the termination of recombina-activating gene 1 (RAG1) and RAG2 gene expression¹⁵¹ (thereby fixing TCR- specificity), the upregulation of CD5 and CD69 expression¹⁵², and the downregulation of CD8 gene expression^{31, 32}. The kinetic signalling hypothesis proposes that such initial TCR signalling makes thymocytes competent for lineage choice. Persistent TCR signalling, despite downregulation of CD8 expression, causes CD4-lineage differentiation, through the upregulation of GATA3 expression. Downregulation of TCR signalling before or during CD8 downregulation causes CD8-lineage differentiation^{31, 80}. A terminal TCR signalling step 'proofreads' CD8-lineage differentiation^{80, 127} and contributes to the terminal differentiation of CD8-lineage thymocytes, notably by allowing interleukin-7 signalling¹⁵³. This model predicts that MHC class-II-restricted CD8 single-positive (SP) populations develop when TCR and CD4 signalling, and thereby thymocyte survival, is maintained by enforcing CD4 expression after CD8-lineage differentiation. Indeed, such populations are detected when CD4 expression is maintained in CD8-lineage cells using either CD4 transgenes^{24, 27} or germline deletion of the CD4 silencer¹⁵⁴. Hd, helper cell deficient.

Sélection négative Signal de mort cellulaire

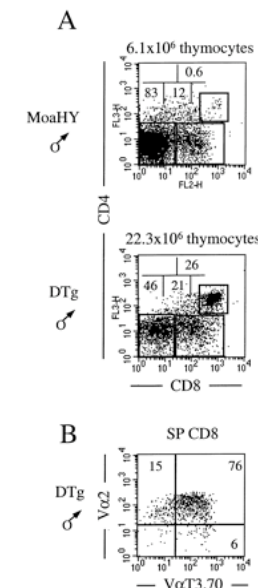


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- Although the signalling pathway that mediates negative selection is not fully understood, many of the important signalling molecules have been identified and studied. Future work should identify the other signalling components and establish the pathway, which begins with engagement of the T-cell receptor (TCR) with a high-affinity peptide–MHC ligand and ends with the irreversible death of the self-reactive thymocyte. APC, antigen-presenting cell; BIM, BCL-2-interacting mediator of cell death; ERK, extracellular-signal-regulated kinase; **GRB2, growth-factor receptor-bound protein 2**; IBNS, inhibitor of NF- κ B; JNK, JUN N-terminal kinase; LAT, linker for activation of T cells; NF- κ B, nuclear factor- κ B; NOR1, neuron-derived orphan receptor 1; NUR77, nuclear receptor 77.



Profil de sous-populations thymiques (cytométrie de flux) chez des souris normales et transgéniques mâles ou femelles exprimant le récepteur T pour l'antigène H-Y (uniquement exprimé chez les souris mâles).



Souris male
TCR-transgénique

Anti-HY

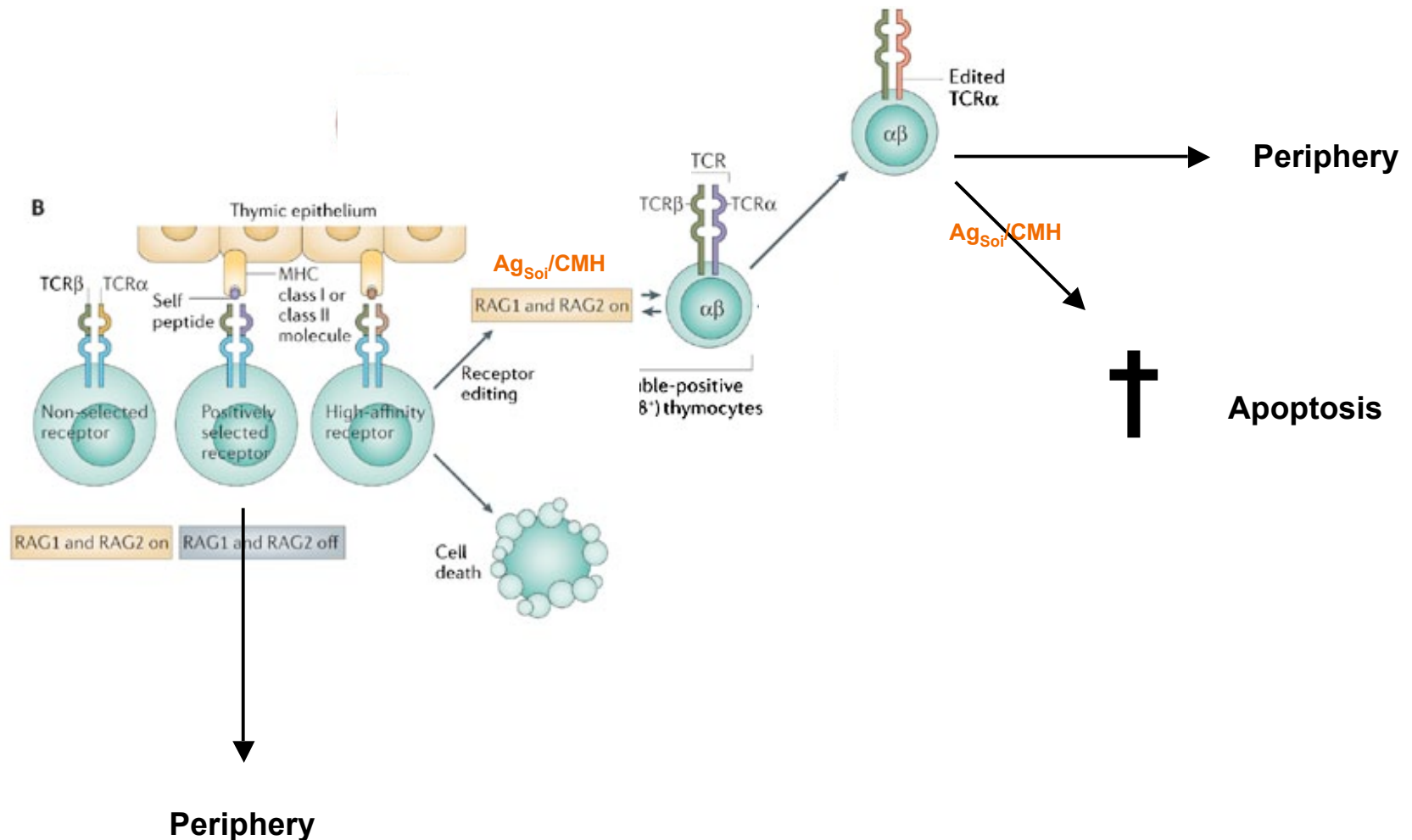
Anti-HY & Anti-GP33

Legrand et al., JI 2001

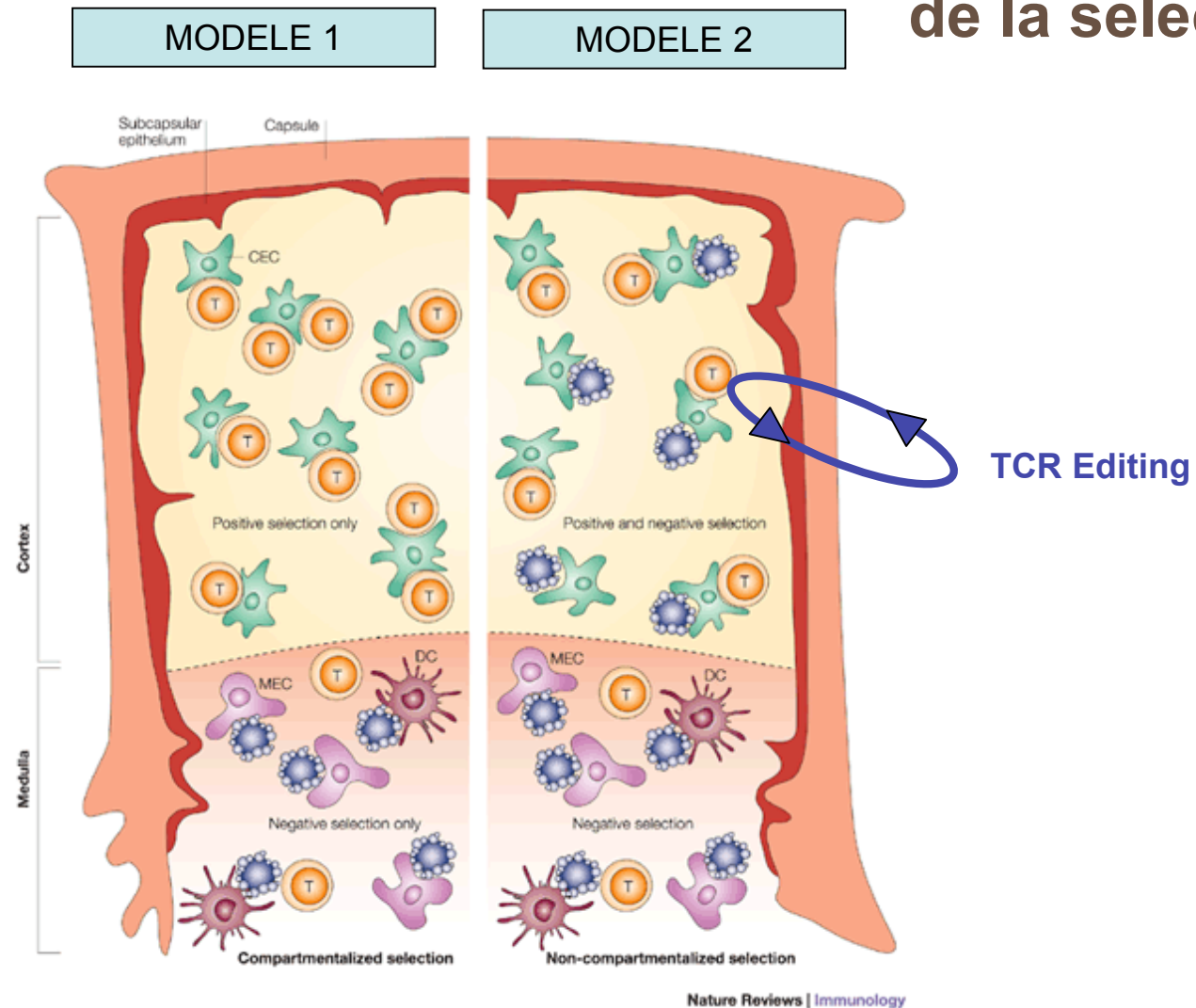
Détournement de la Sélection négative

Editing, une alternative à l'apoptose

- « Editing » du TCR en cas de reconnaissance des complexes: Ag_{Soi}/CMH

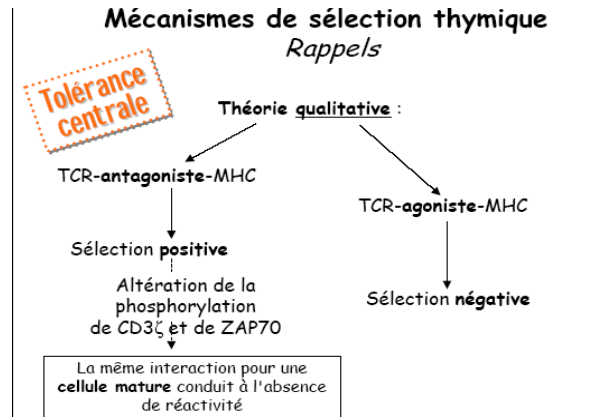


Absence de « compartimentalisation » de la sélection

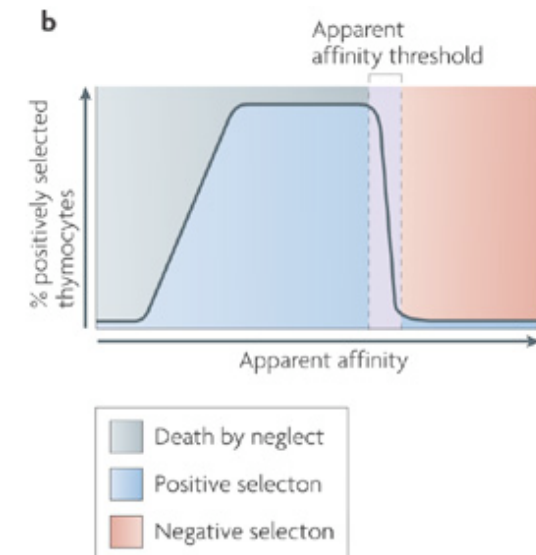
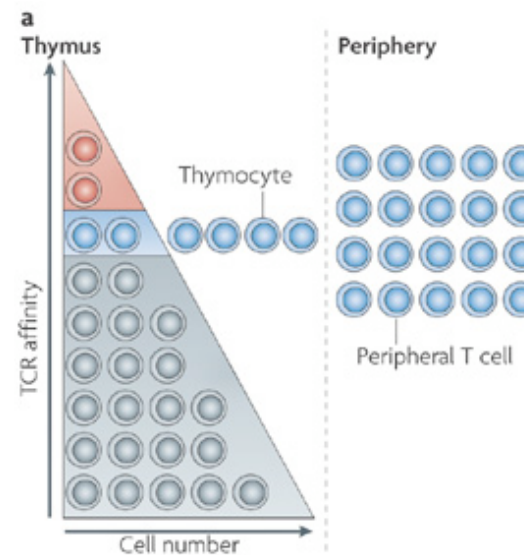
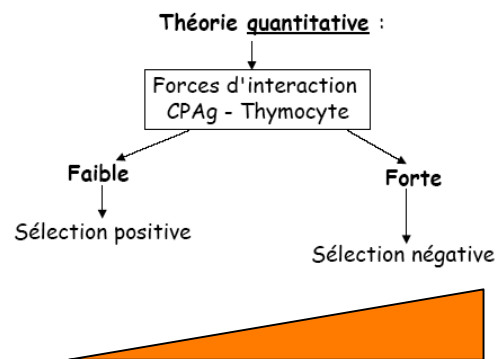


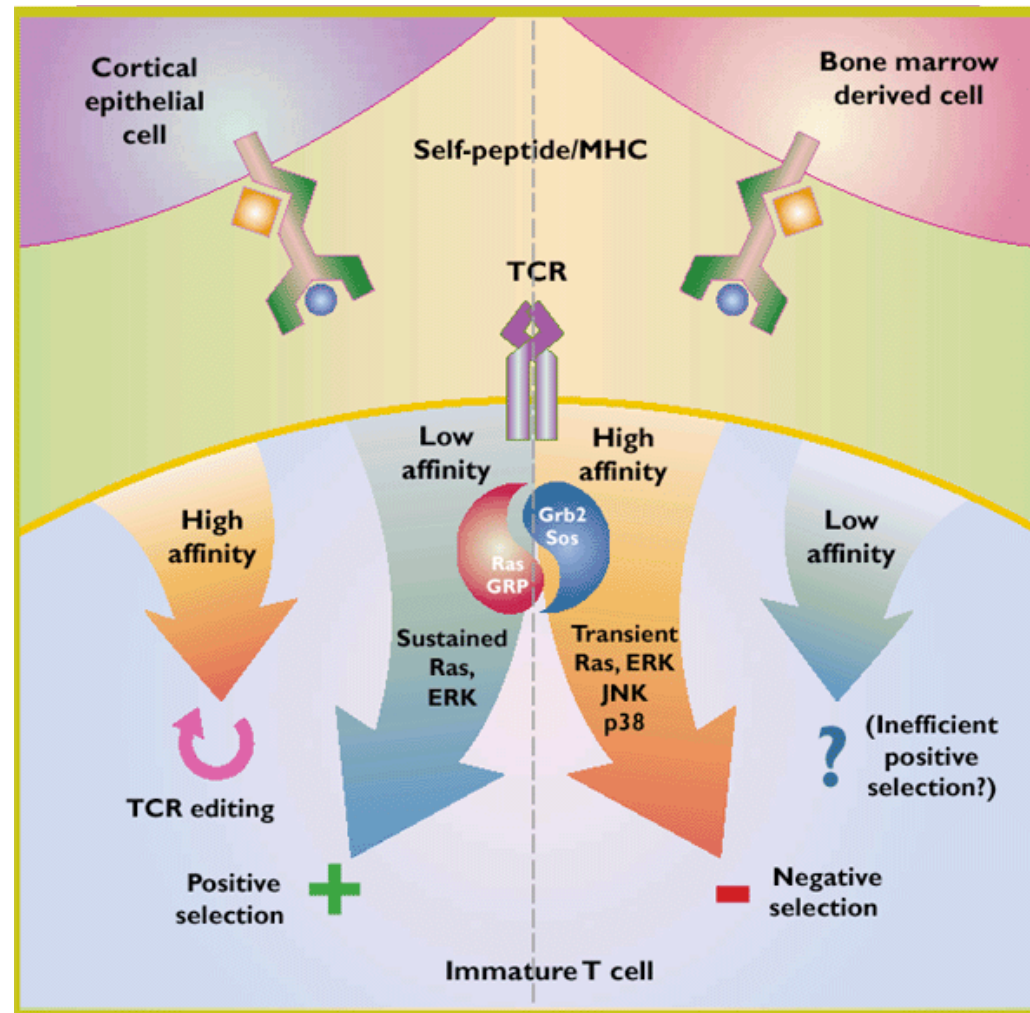
- Epithelial cells in the thymic cortex are the antigen-presenting cells (APCs) that mediate positive selection, whereas the medulla contains epithelial and dendritic cells (DCs) that are efficient APCs for negative selection. For these reasons, positive selection occurs in the cortex, whereas deletion of self-reactive thymocytes occurs predominantly in the medulla (left panel). An alternative view is that some negative selection occurs in the cortex, such that **negative selection is not strictly compartmentalized in the thymus**. For a full description of thymic anatomy and development see Ref. 1. CEC, cortical epithelial cell; MEC, medullary epithelial cell.

Modèle alternatif de la sélection: « une question d'affinité... »



Mécanismes de sélection thymique (2)

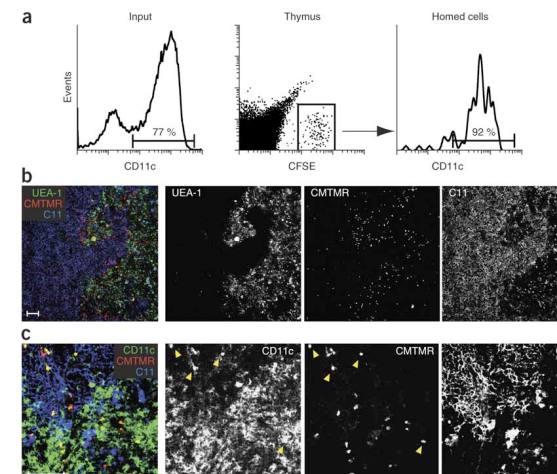
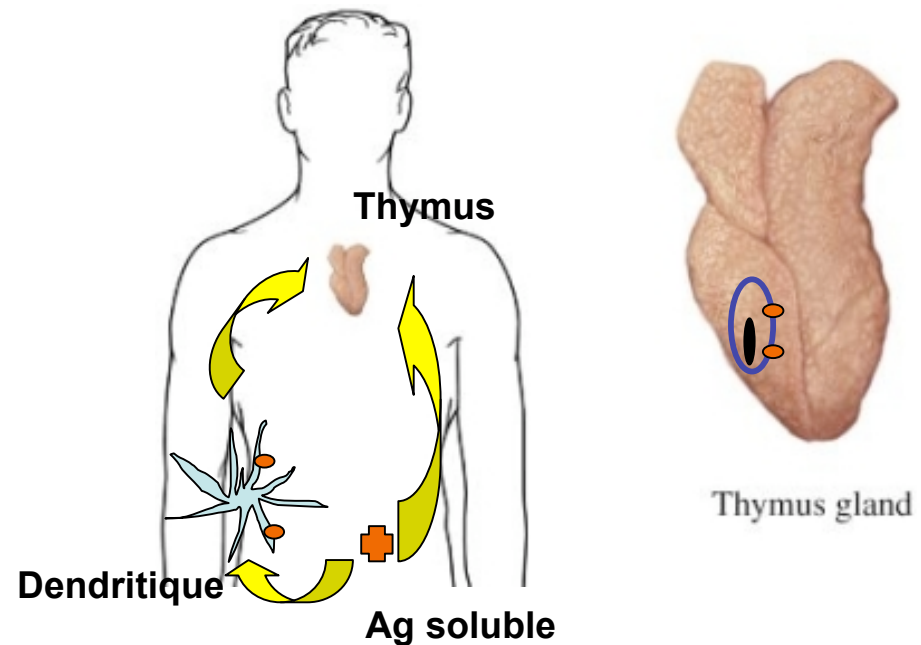




Présentation intrathymique des Ag du soi

- **Antigènes d'histocompatibilité:**
 - Epitopes dérivés des molécules du CMH
- **Antigènes ubiquitaires**
 - Protéines cellulaires
- **Antigènes solubles**
 - Accessibilité au thymus
- **Antigènes tissulaires**
 - Accès limité au tissu
 - Apportés par les DC (+/-)

Clonal deletion of thymocytes by circulating dendritic cells homing to the thymus. Bonasio R, Nat Immunol. 2006



Ag soluble versus membranaire

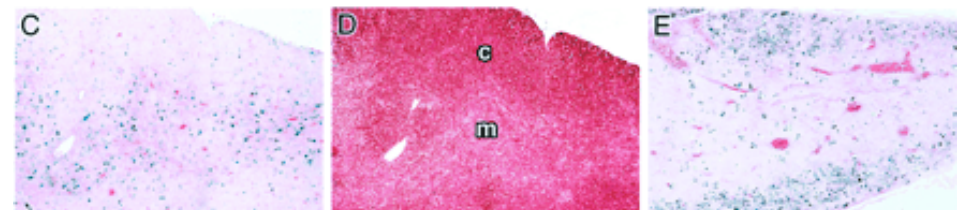
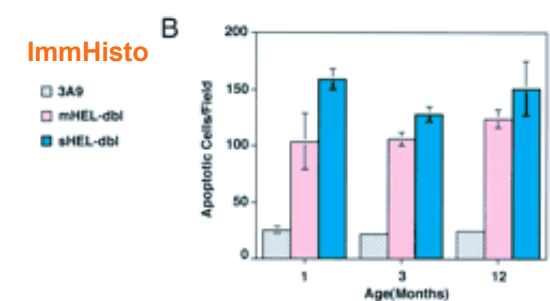
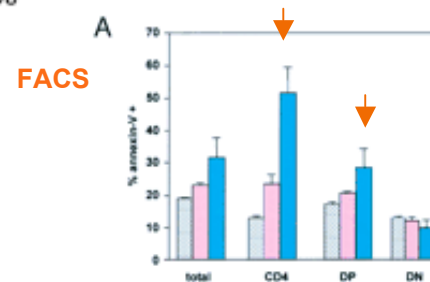
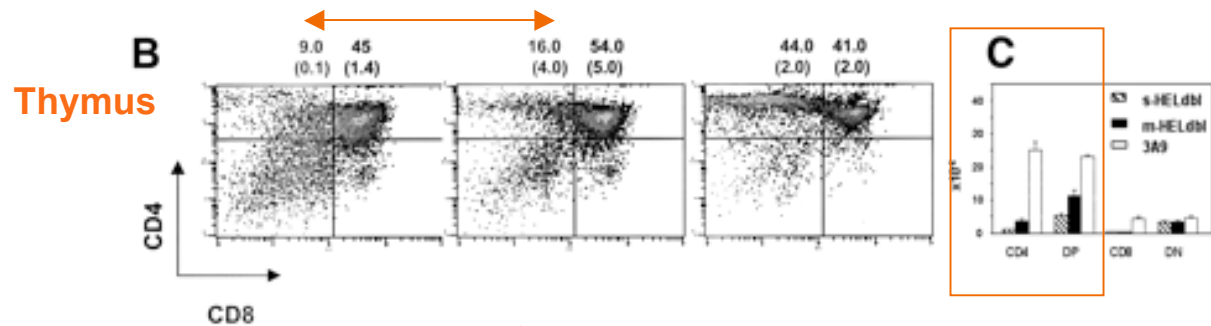
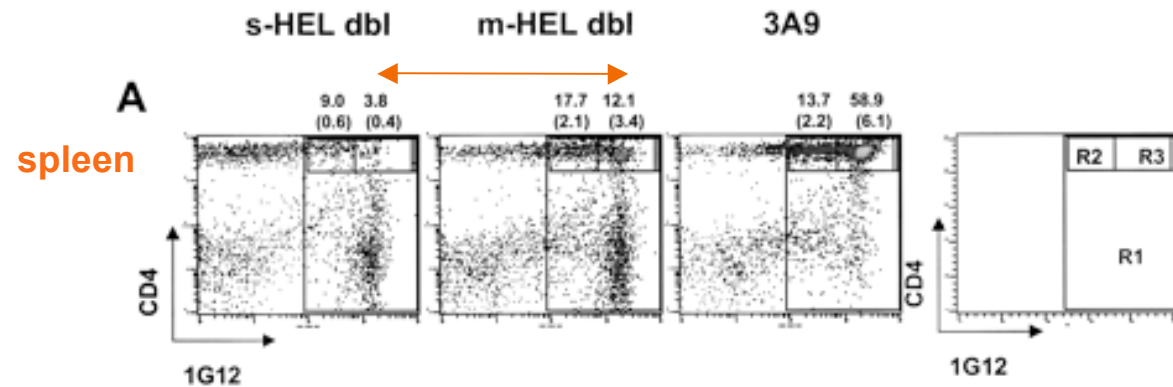
T Cell Tolerance to a Neo-Self Antigen
Expressed by Thymic Epithelial Cells: The
Soluble Form Is More Effective Than the
Membrane-Bound Form

Zhang M, et al, J Immunol 2003

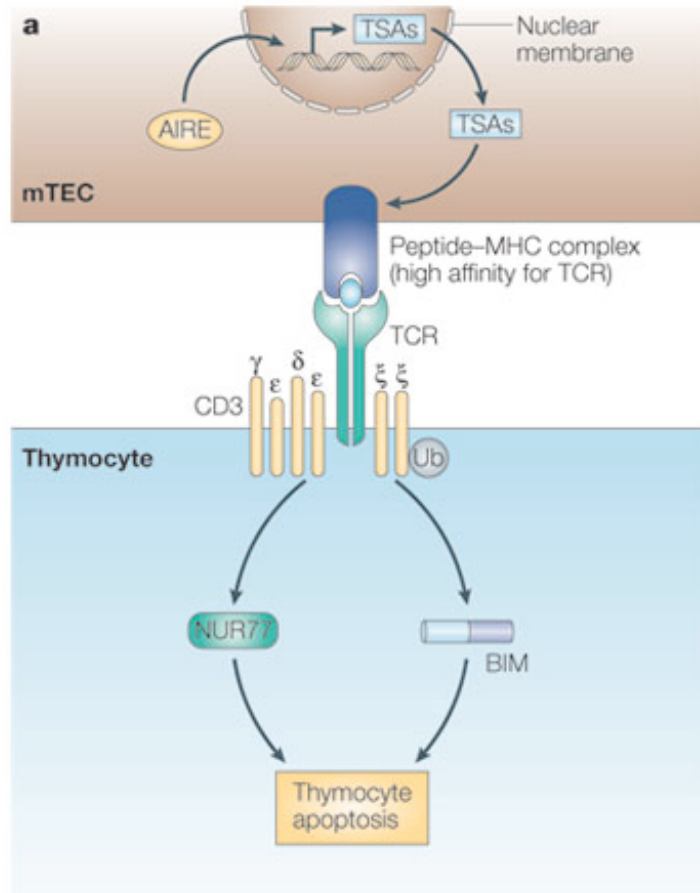
3A9 anti-HEL : TCR transgenic

s-HEL dbl: soluble HEL x 3A9

m-HEL: membrane-bound HEL x 3A9



AIRE: expression intrathymique des Ag spécifiques des tissus



Autoimmune regulator (AIRE), a RING (really interesting new gene)-type E3 ligase, is implicated in expression of tissue-specific antigens (TSAs) by medullary thymic epithelial cells (mTECs), which present antigens (including TSAs) to developing thymocytes to induce central tolerance to those antigens. In developing thymocytes, signalling through the T-cell receptor (TCR) causes negative selection by inducing thymocyte apoptosis through expression of NUR77 and BIM (B-cell-lymphoma-2-interacting mediator of cell death). The E3 ligase CBL (Casitas B-lineage lymphoma) can function as an E3 ligase for BIM. **Liu Nature Reviews Immunol 2005**

EXPRESSION

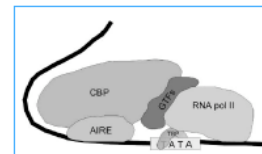
- Chez l'homme : **Thymus**, ganglions lymphatiques, rate, foie foetal
PBL, CD différenciées

- Thymique : une sous-population de **cellules épithéliales médullaires**

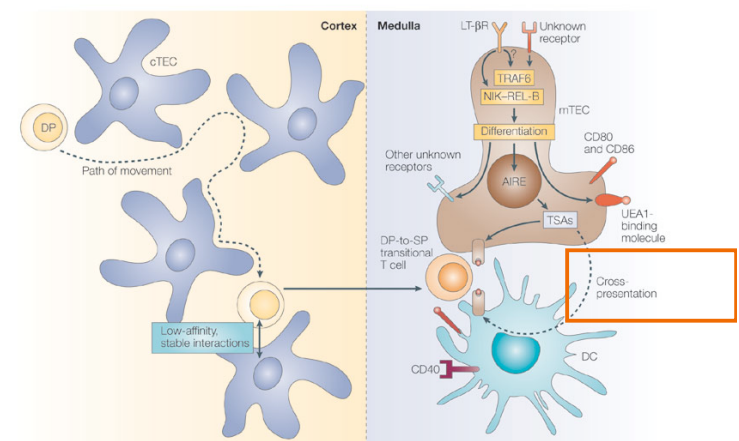
- Chez la souris : + moelle osseuse, tractus urinaire et génital, tractus respiratoire et digestif, cerveau, glandes endocrines

FUNCTION

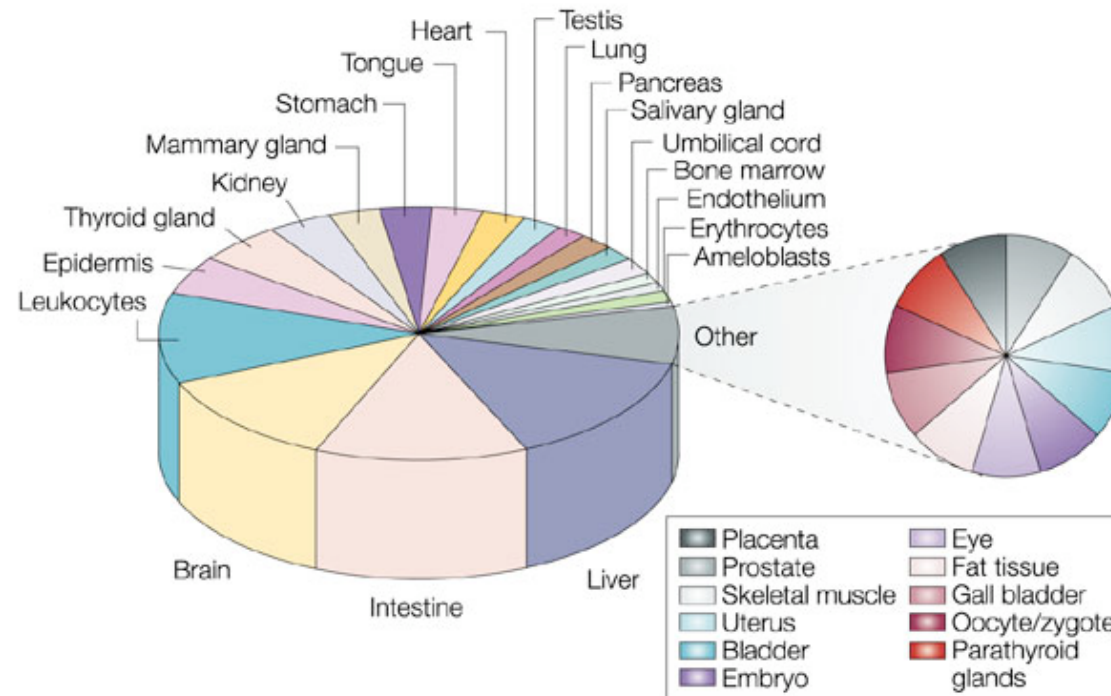
- Les signatures structurales suggèrent un rôle de **facteur de transcription** de AIRE
- Élément d'un complexe multiprotéique dont les partenaires restent à identifier
 - La CBP (CREB-binding protein), colocalisée avec AIRE, fonctionne comme un coactivateur de plusieurs protéines: protéines STAT, Jun, Fos, NFκB...



AIRE (homme&souris) sont de fort **activateur transcriptionnel**



Spécificité tissulaire des gènes exprimés dans les mTECs



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- Genes identified as overexpressed in mouse **mTECs compared with cortical TECs** using gene chip analysis were assigned to tissues according to their predominant expression (where applicable) using combined information from the public databases GNF Gene Expression Atlas and Swissprot (see online links box) and the literature. About one quarter of all mTEC-overexpressed genes could be categorized as tissue-restricted according to this approach and are shown. Note the diversity of tissues that meet these criteria. The fraction of tissue-restricted genes is probably underestimated given their low expression levels in mTECs and the limited sensitivity of the gene array method.

Projection of an Immunological Self Shadow Within the Thymus by the Aire Protein

Mark S. Anderson, Science 2002

Aire-deficient mice develop autoimmunity.

(A) Tissue sections from aire-deficient mice from salivary gland, ovary and retina (knockout; top row) and wild-type mice (bottom row) mice

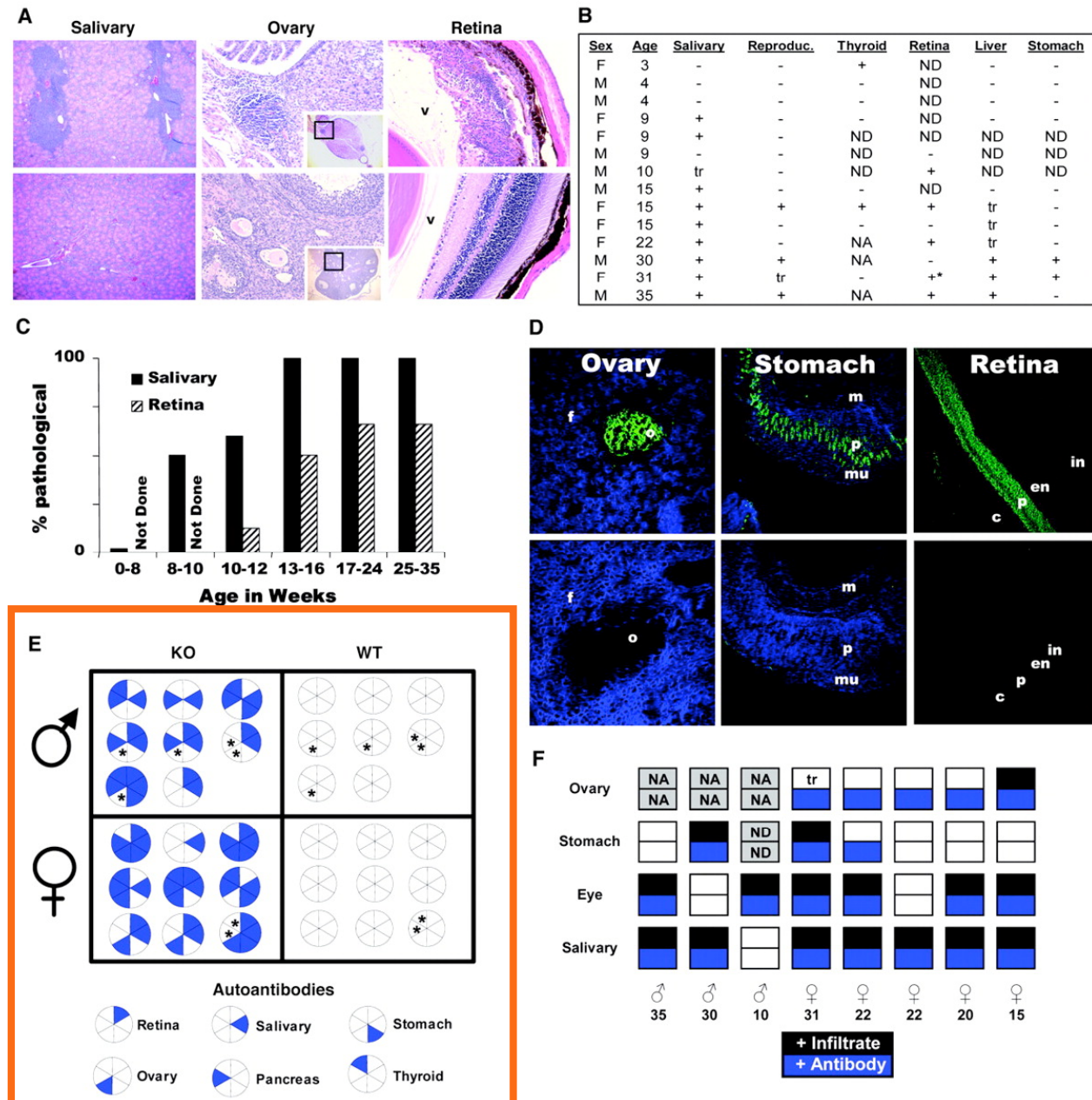
(B) Table summarizes presence (+) or absence (-) of lymphocytic infiltrates observed in knockout mice of various ages and both sexes for the indicated organs.

(C) Salivary gland infiltrates and retinal degeneration develop in a time-dependent manner.

(D) Aire-deficient mice have autoantibodies to multiple organs.

(E) Summary of autoantibodies in aire-deficient mice. Serum from age- and sex-matched mice (9 to 35 weeks old) was collected and tested for the presence of autoantibodies to the indicated organs.

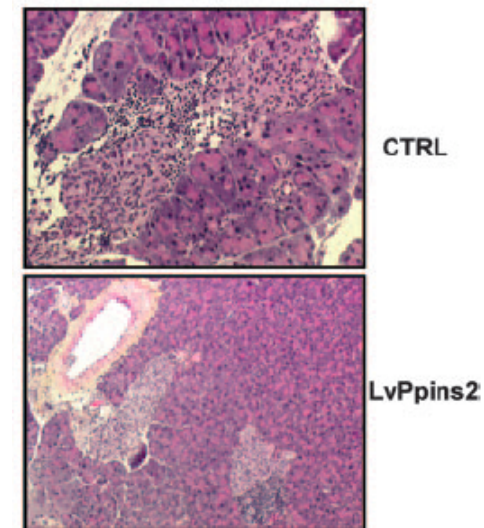
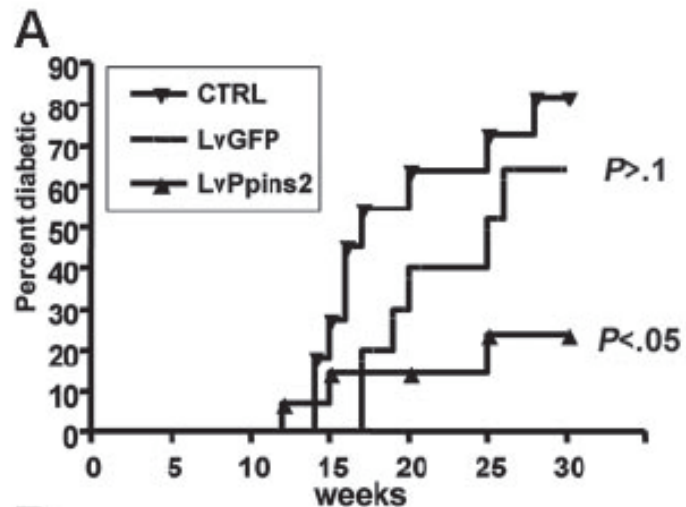
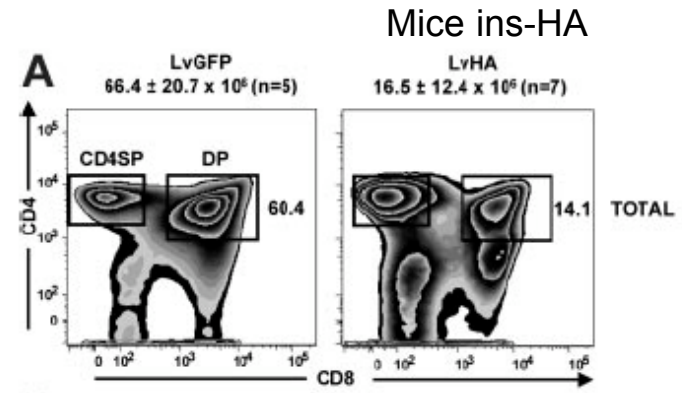
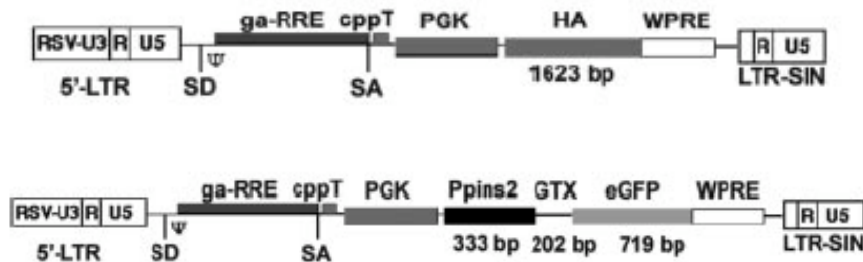
(F) Summary of correlation of autoantibodies to immune infiltrates for ovary, salivary gland, retina, and stomach in seven knockout mice.



Tolérance centrale et Immunointerventions

Induction of antigen-specific tolerance by intrathymic injection of lentiviral vectors

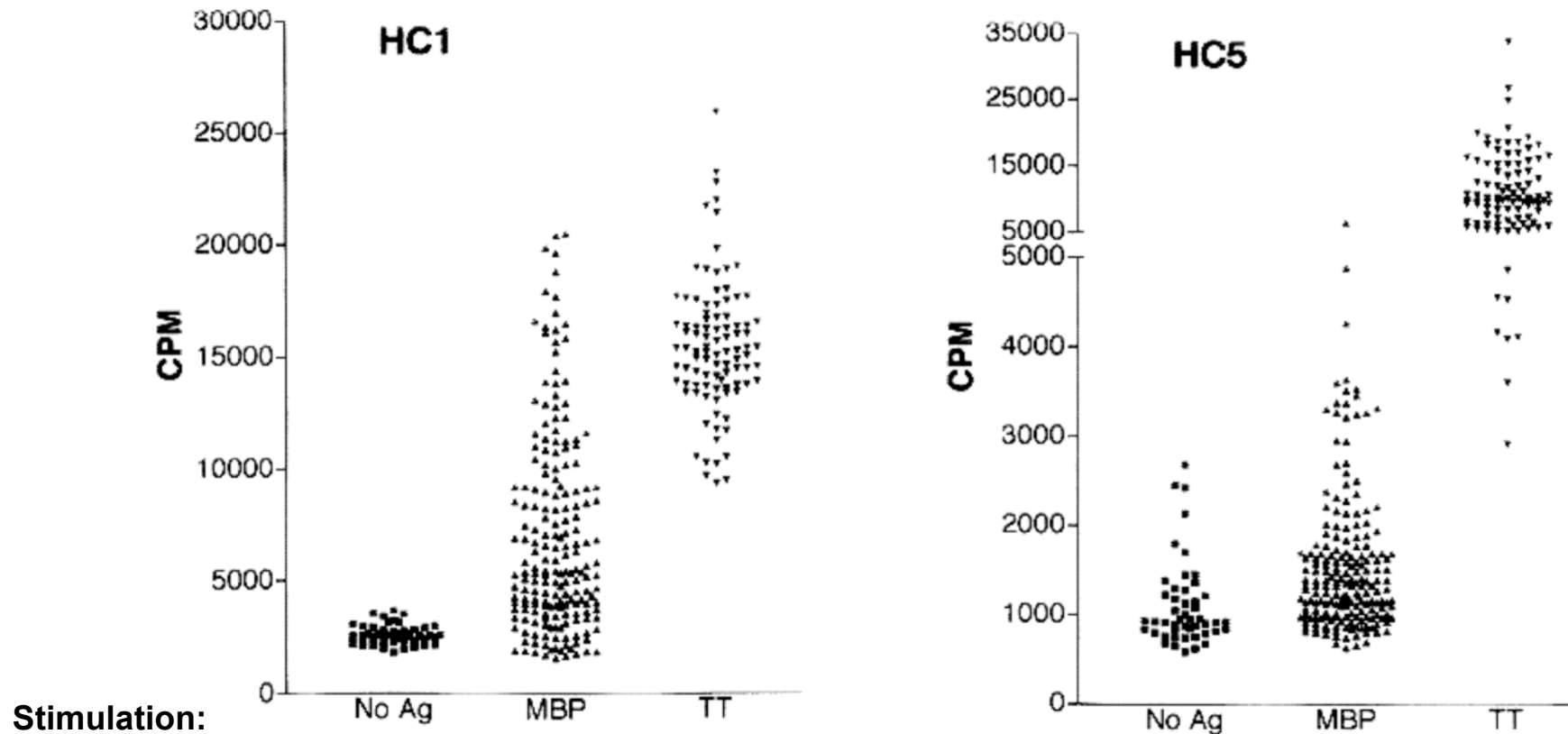
Gilles Marodon, Sylvain Fisson, Béatrice Levacher, Monique Fabre, Benoît L. Salomon, and David Klatzmann



Présence de lymphocytes autoréactifs en périphérie: un danger potentiel



Reconnaissance de la MBP chez des individus sains

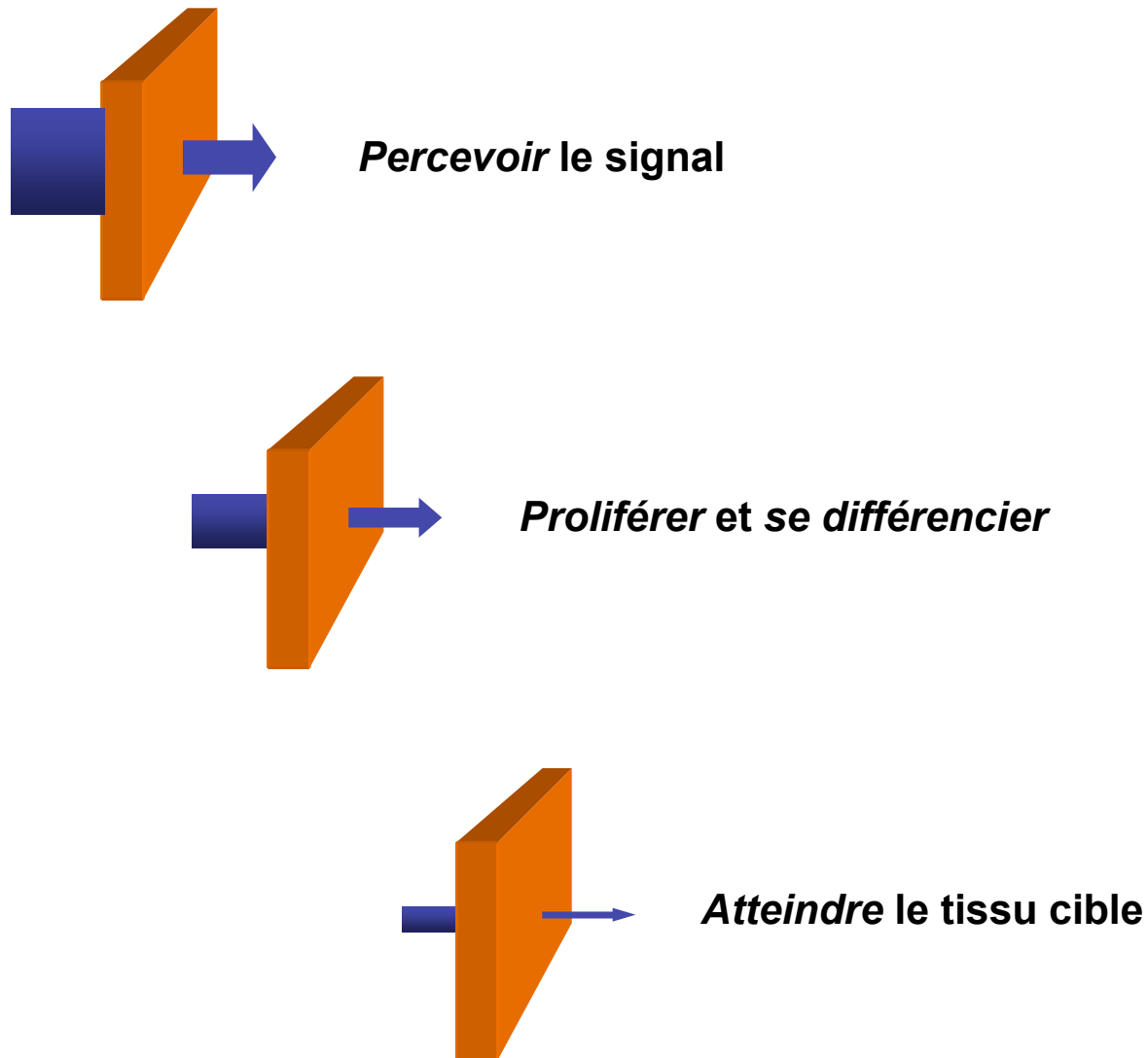


Lovett-Racke *et al.* 1998

Il existe donc dans le sang des sujets sains des lymphocytes T dont le TCR reconnaît des peptides d'une **protéine de la myéline**

...mais absence de réponse des
clones auto-réactives

Etapes limitantes au déclenchement des réponses:

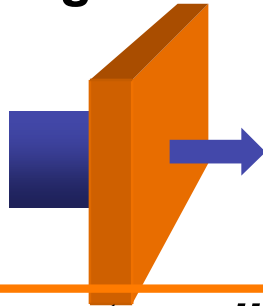


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Tolérance périphérique

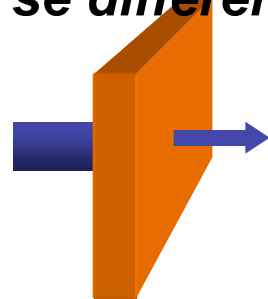
Les mécanismes périphériques de contrôle des LyT auto-réactifs

Percevoir le signal



- Absence d'activation des LyT:
 - Ignorance /anergie
 - Influence des DC
- Déletion clonale en excès d'Ag

Proliférer et se différencier



- Contrôle des réponses inflammatoires (rôle des cytokines)
- Populations suppressives
 - LyT régulateurs

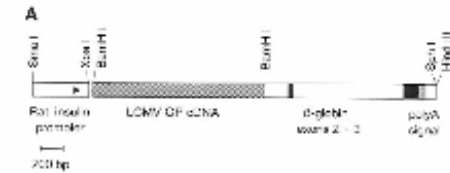
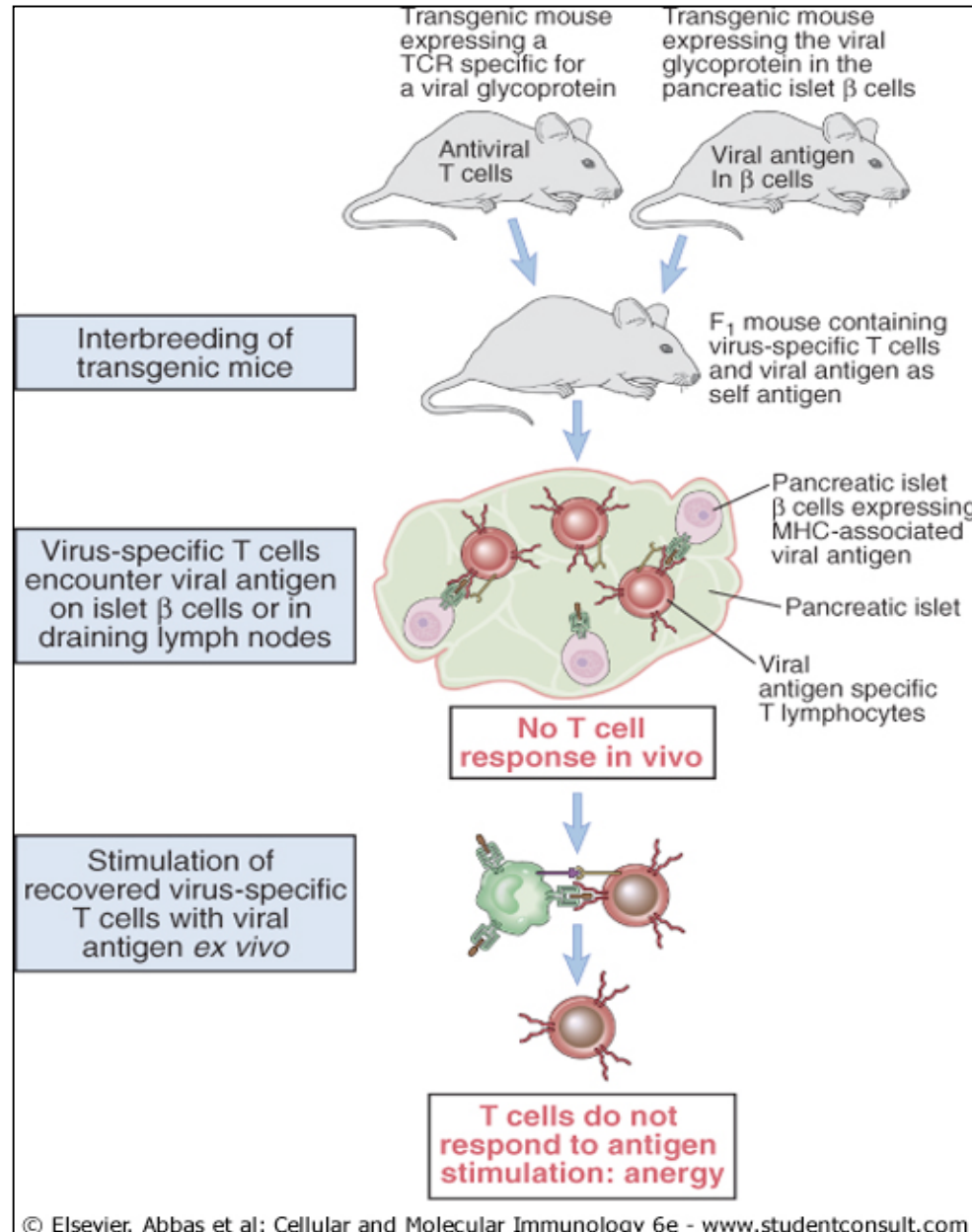
Atteindre le tissu cible



- Accès limité aux tissus

REPONSE

Non-reconnaissance des Ag du soi « ignorance »

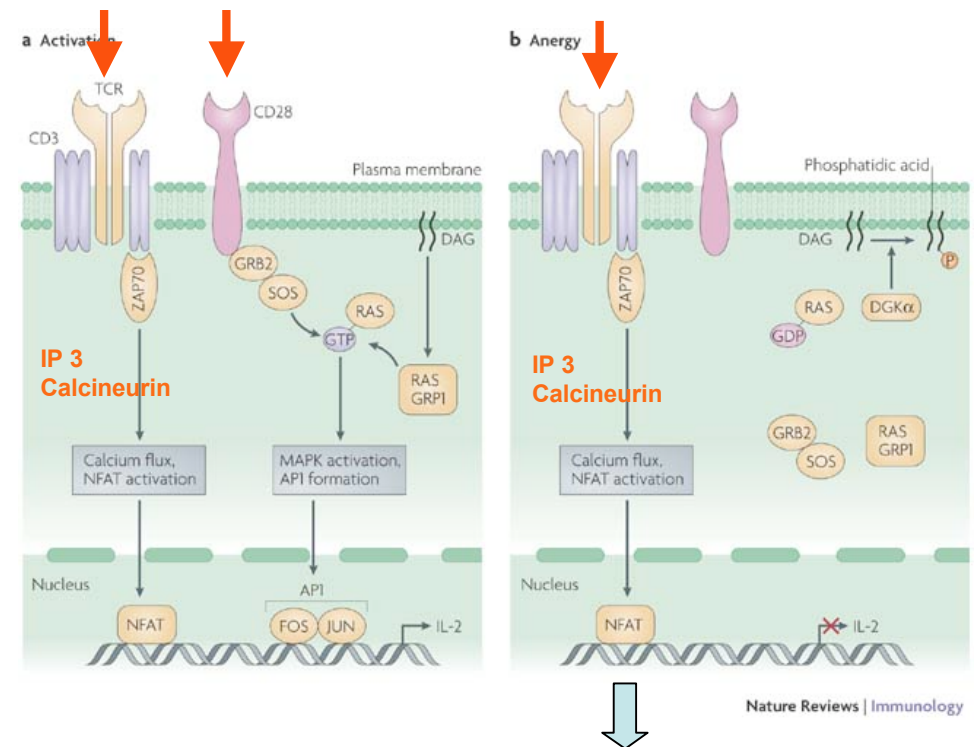
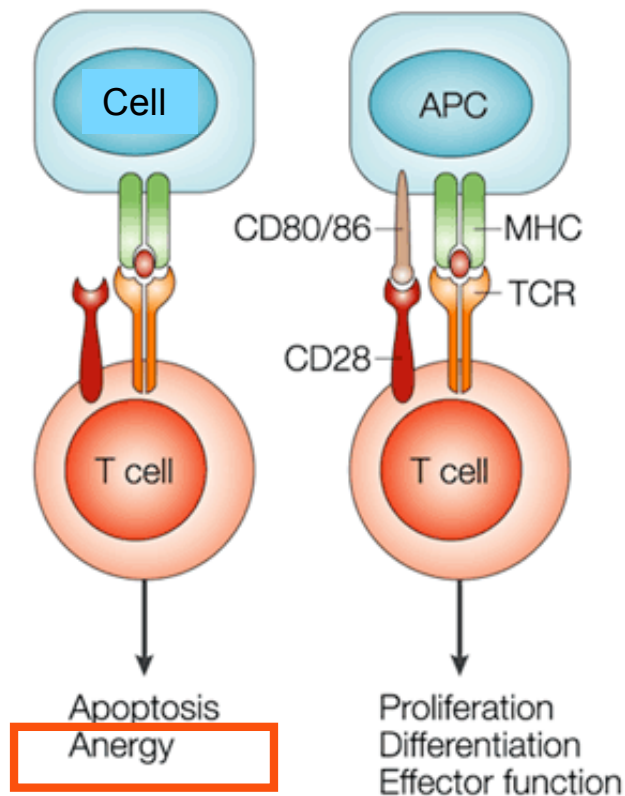


Ignorance

Anergie

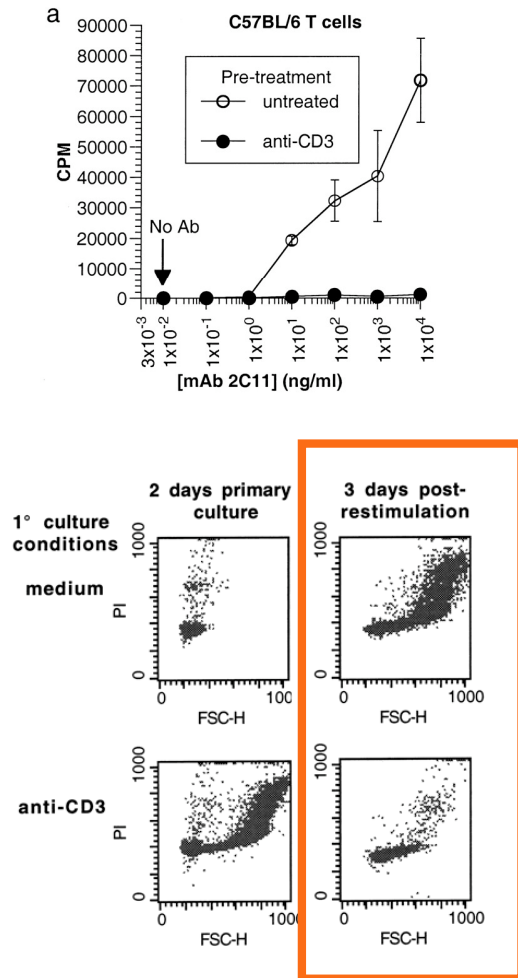
Ignorance des Ag tissulaires & **Anergie**

**Absence d'activation T en l'absence
de molécules de co-stimulation**



Inhibiteurs:
Egr2, Egr3
DGKα

Anergie: Un état de non-réponse réversible par l'IL-2



Anergized cells enter cell cycle,
but arrest in G₁ phase upon restimulation.

Induction of T cell anergy in the absence of CTLA-4/B7 interaction.
J Immunol. 2000 Mar 15;164(6):2987-93.

B7-Blocking Agents, Alone or in Combination with Cyclosporin A, Induce Antigen-Specific Anergy of Human Memory T Cells¹

Zhang Yi-qun,* Katrien Lorré,* Mark de Boer,* and Jan L. Ceuppens^{2*}

The Journal of Immunology

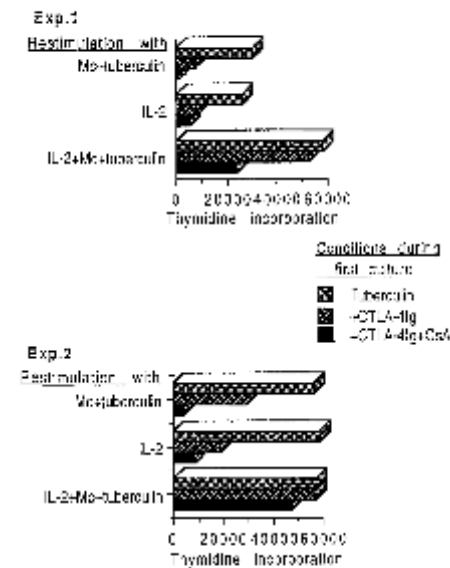
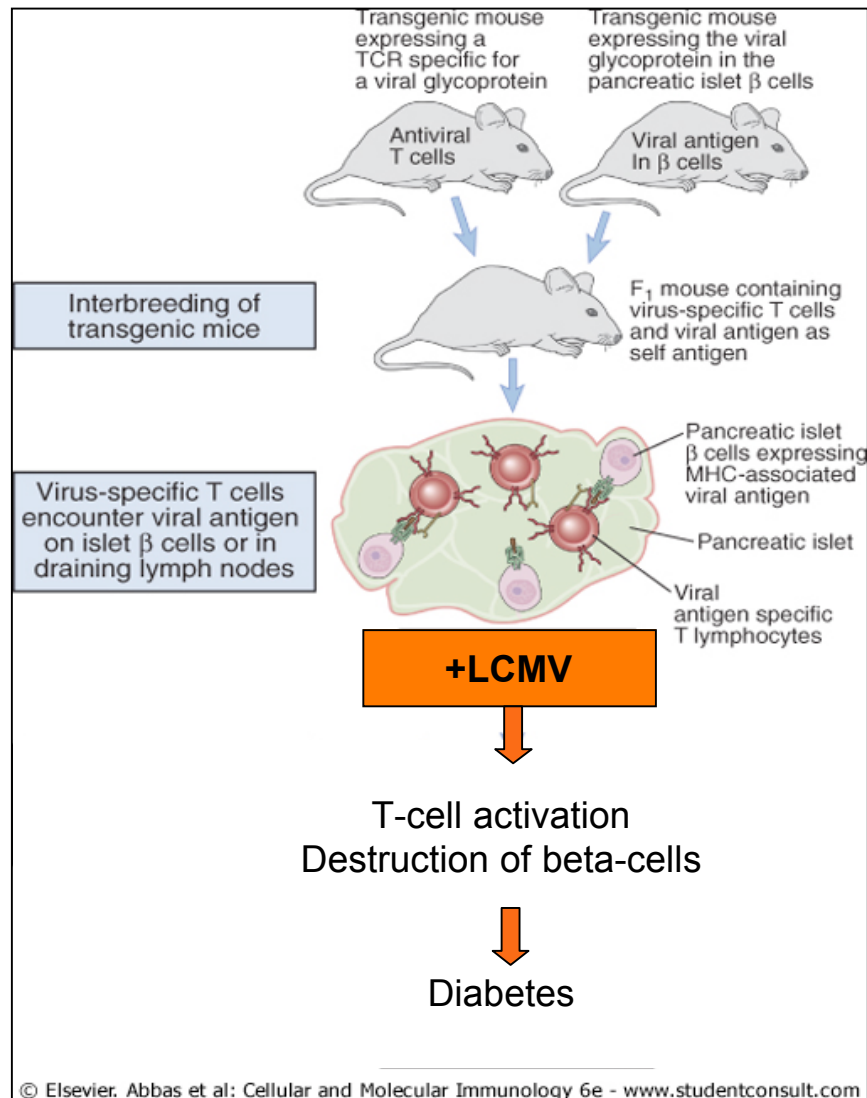


FIGURE 6. IL-2 reverses the state of unresponsiveness induced by CTLA-4lg, or CTLA-4lg and CsA. PBMC (1×10^6 /ml) were cultured for 6 days with tuberculin (200 U/ml), and CTLA-4lg (10 µg/ml), or CTLA-4lg and CsA (200 µg/ml) first culture. The cells were rested and re-challenged with IL-2 (10 U/ml) along with tuberculin in the presence of autologous macrophages (Mφ), or with IL-2 in combination with tuberculin and macrophages. After 3 days, the proliferative responses were determined. The results of two independent experiments are presented. Thymidine incorporation in response to tuberculin in the first culture were $12,376 \pm 1,043$ and $35,036 \pm 1,515$, respectively.

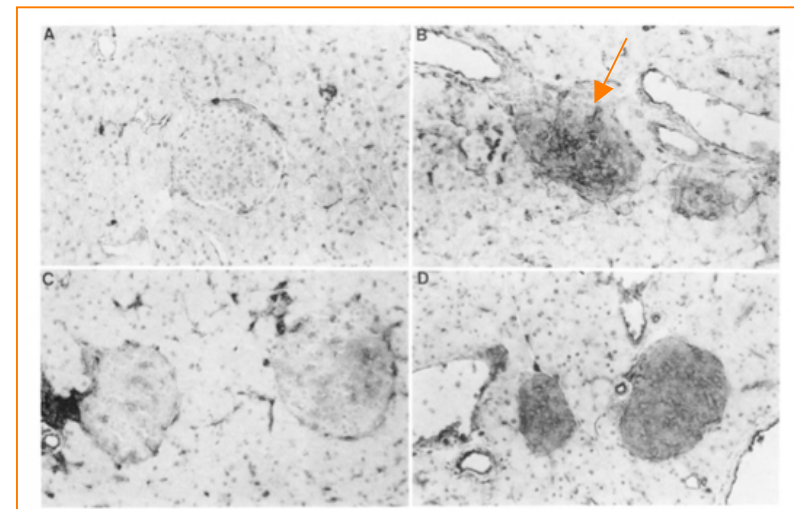
Rupture de la tolérance aux Ag tissulaires

Modèle Diabète induit



Mice	Infectious Agent	MHC Levels ^a	Diabetes
C57BL/6	None	Low	-
C57BL/6	LCMV	High	-
C57BL/6	vacc-gp	Low	-
RIP-gp	None	Low	-
RIP-gp	LCMV	High	+
RIP-gp	vacc-gp	Low	-
TCR	None	Low	-
RIP-gp/TCR	None	Low	-
RIP-gp/TCR	LCMV	High	+
RIP-gp/TCR	vacc-gp	High	+

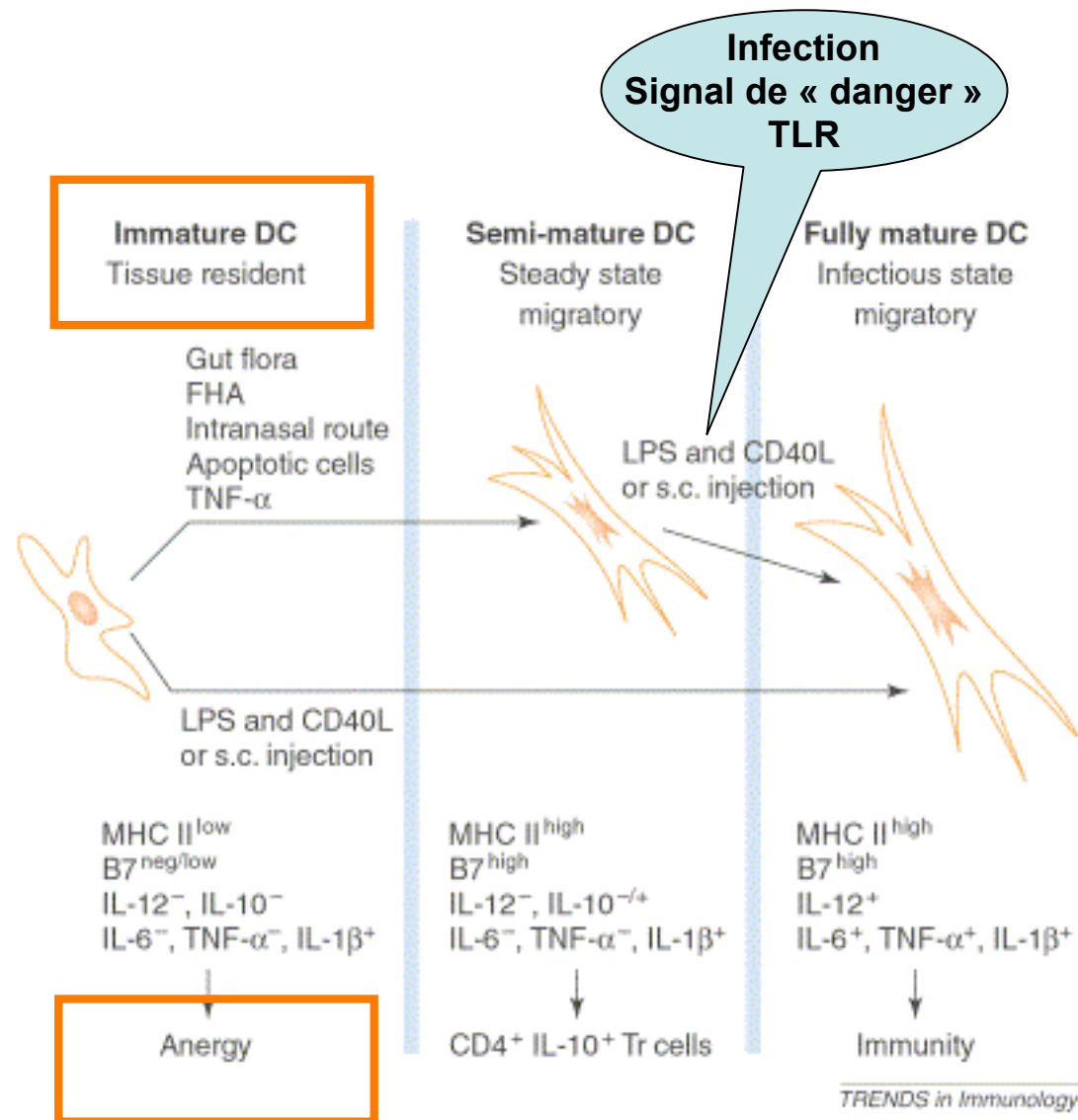
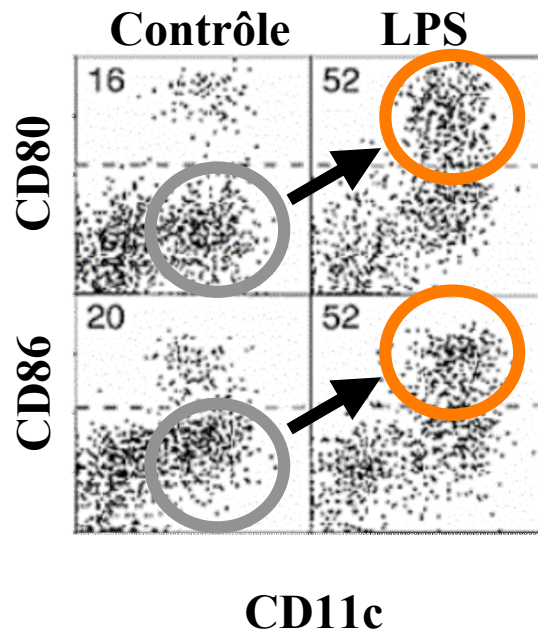
^a Class I MHC expression on β -islet cells.



Facteurs tolérogènes dans les tissus: DC immatures

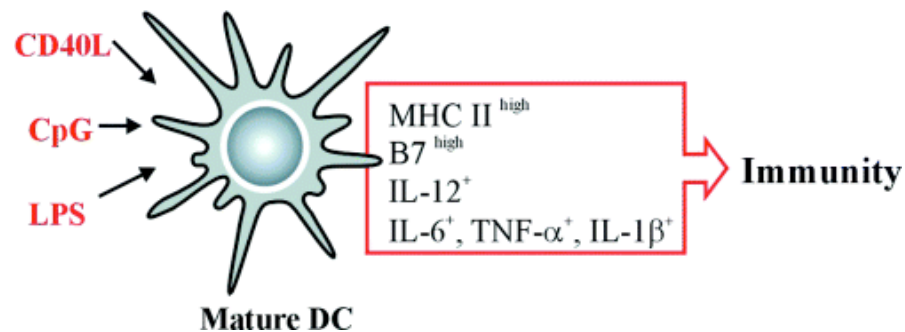
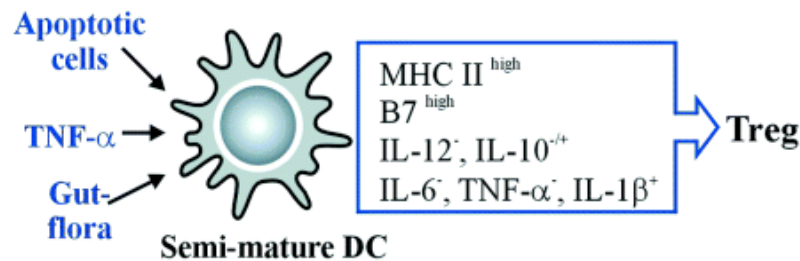
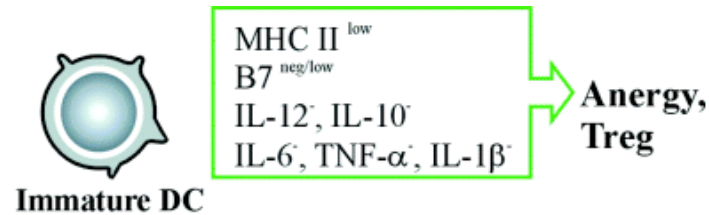
En conditions naturelles, non-pathologiques, la majorité de cellules dendritiques sont tolérogènes

= Ignorance



Rupture de Tolérance au Soi lors d'infections

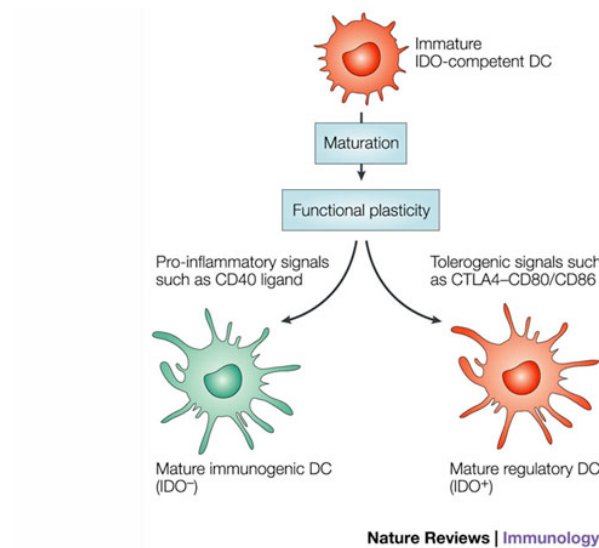
Théorie du danger



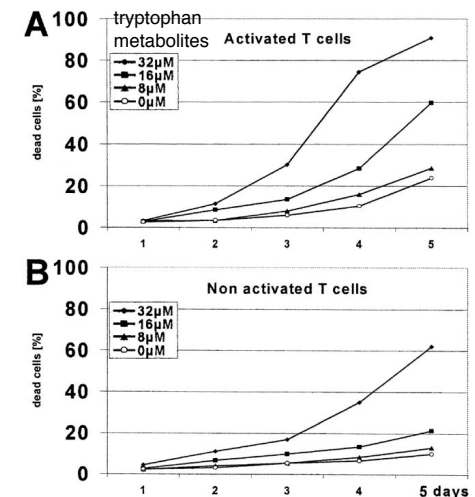
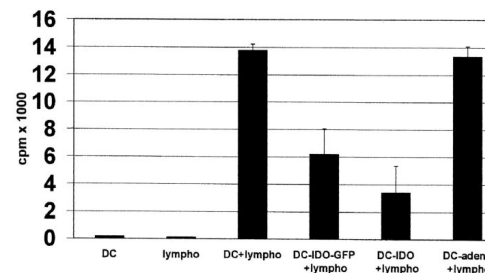
- **Activation due aux PAMPs (Pathogen Associated Molecular Patterns), reconnues par les PRRs, Pattern Recognition Receptors, présents à la surface des cellules du syst imm inné.**
 - les lipopolysaccharides (LPS) des bactéries Gram-
 - les acides lipotéichoïques, le peptidoglycane des bactéries Gram+,
 - des liposaccharides divers des mycobactéries
 - les parois riches en mannane des levures.
 - ADN bactériens riche en CpG
 - existence d'ARN double brins de certains virus.

DC immatures & DC tolérogènes

- **DC immatures**
 - CMHlo CD80- CD86- IL-12lo
- **DC tolérogènes:**
 - Réfractèrent à l'activation
 - IL-10+ IDO+
 - induit apoptose des T activés
- **Indoleamine 2,3-dioxygenase (IDO)**
 - Exprimé par Mph, DC imm et DC tolérogènes
 - Dégradation du Tryptophane dans les ganglions, rate, poumon, cœur, ...
 - Régulation du taux sérique du Trp
 - Trp essentiel pour la prolifération des lymphocytes T



Inhibition of Allogeneic T Cell Proliferation by Indoleamine 2,3-Dioxygenase-expressing Dendritic Cells Mediation of Suppression by Tryptophan Metabolites
Peter Terness et al, J Exp Med. 2002



Tolérance orale: implication physiologique des DC tolérogènes

Research article

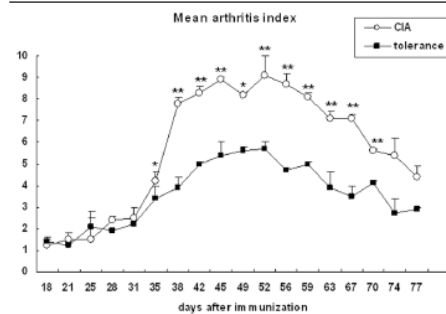
Open Access

Indoleamine 2,3-dioxygenase-expressing dendritic cells are involved in the generation of CD4⁺CD25⁺ regulatory T cells in Peyer's patches in an orally tolerized, collagen-induced arthritis mouse model

Min-Jung Park^{1*}, So-Youn Min^{1*}, Kyung-Su Park^{1,2}, Young-Gyu Cho¹, Mi-La Cho¹, Young-Ok Jung¹, Hyun-Sil Park¹, Soog-Hee Chang¹, Seok Goo Cho², Jun-Ki Min^{1,2}, Sung-Hwan Park^{1,2} and Ho-Youn Kim^{1,2}

Model: Arthritis induction

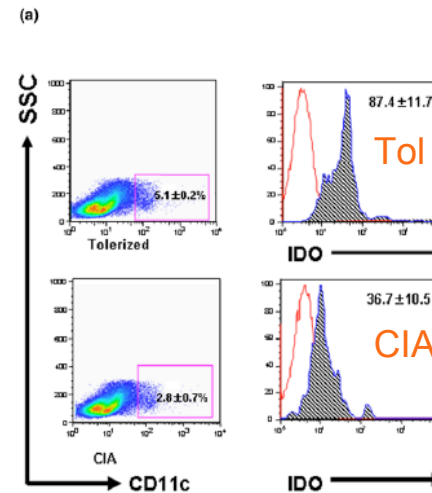
Figure 1



Inhibition of arthritis development in tolerized mice. Mice in the tolerance group were fed 100 µg type II collagen (CII) six times for 2 weeks before immunization. For collagen-induced arthritis (CIA) induction, CII emulsified with complete Freund's adjuvant was injected into the tail of mice in the tolerance group and in the CIA group as a primary immunization. Two weeks later, CII emulsified with incomplete Freund's adjuvant was injected into a hind leg as a booster injection. The mean arthritis index was significantly lower in the tolerance group than in the CIA group throughout the examination period. Values are presented as the mean ± standard deviation of three independent experiments involving 20 tolerized mice and 20 CIA mice per group. *P < 0.05, **P < 0.005.

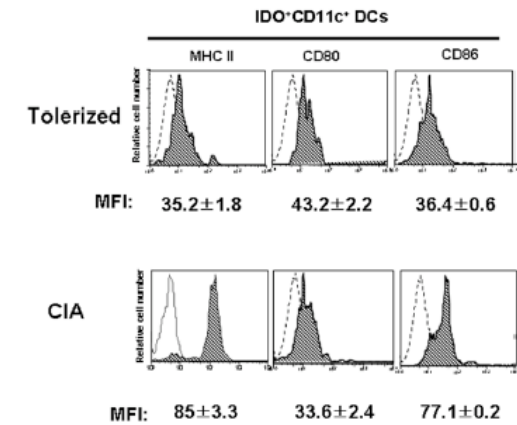
Expression of IDO in Payer's Patches DC

Figure 2



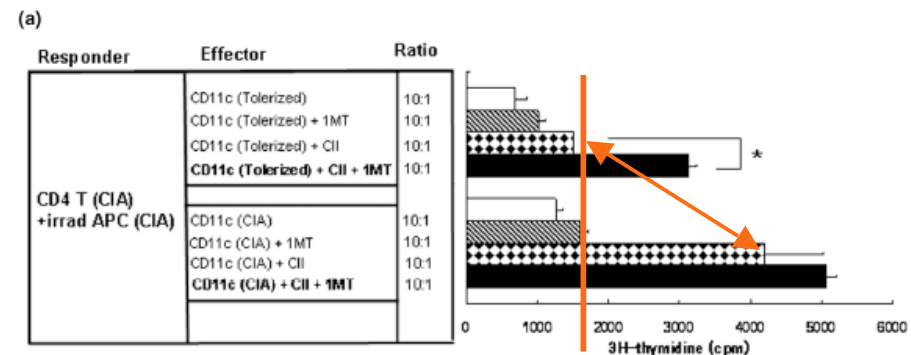
Immature phenotype of DC

Figure 3



IDO-dependent suppression of CII specific T cells

Figure 4



Et en cas de réponse, ...

Excès d'antigène induit la délétion clonale Mort cellulaire par activation (AICD)

- Fortes doses d'Ag induisent la tolérance spécifique des T et éventuellement celle des B

T Cell Priming Versus T Cell Tolerance Induced by Synthetic Peptides

By Peter Aichele, Karin Brduscha-Riem, Rolf M. Zinkernagel, Hans Hengartner, and Hanspeter Pircher

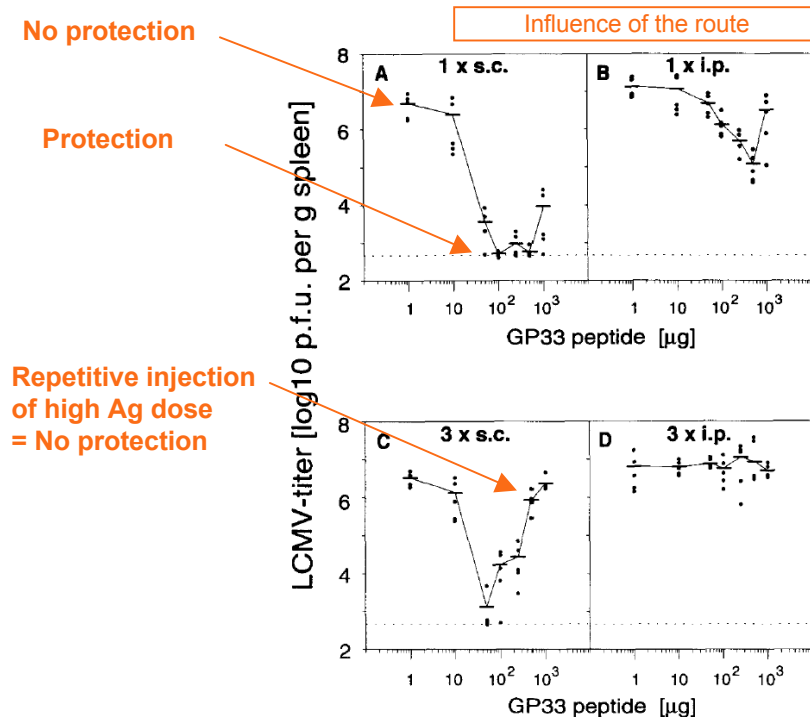


Figure 1. Parameters defining peptide-induced priming or tolerance of CTL. C57BL/6 mice were immunized with different amounts of GP33 peptide emulsified in IFA once s.c. (A) or i.p. (B) or three times at 3-d intervals s.c. (C) or i.p. (D). 10 d after the last peptide administration, mice were challenged i.v. with 200 PFU LCMV-WE, and virus titer in the spleen was analyzed 4 d later. Dots represent values from individual mice (five animals per group). Detection level is indicated as a dotted line.

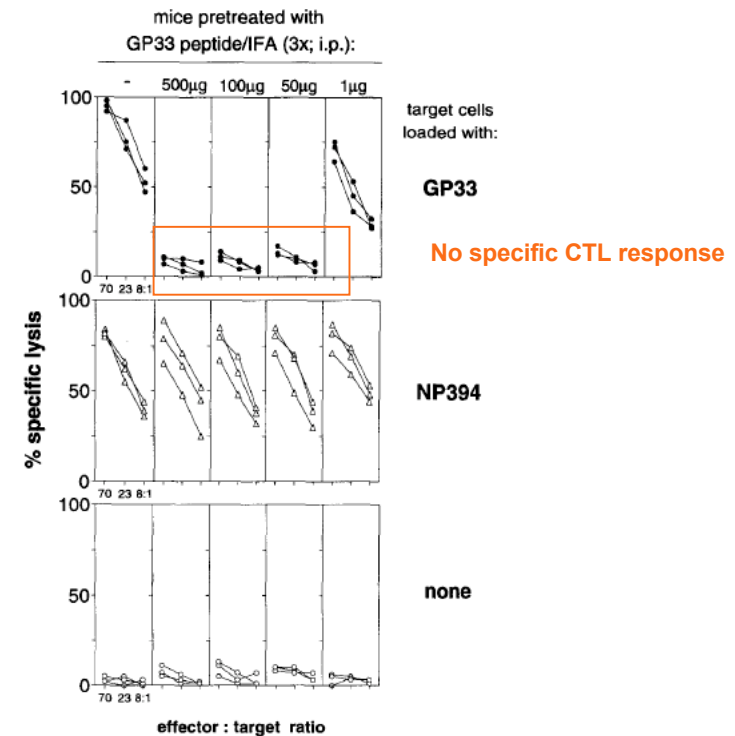
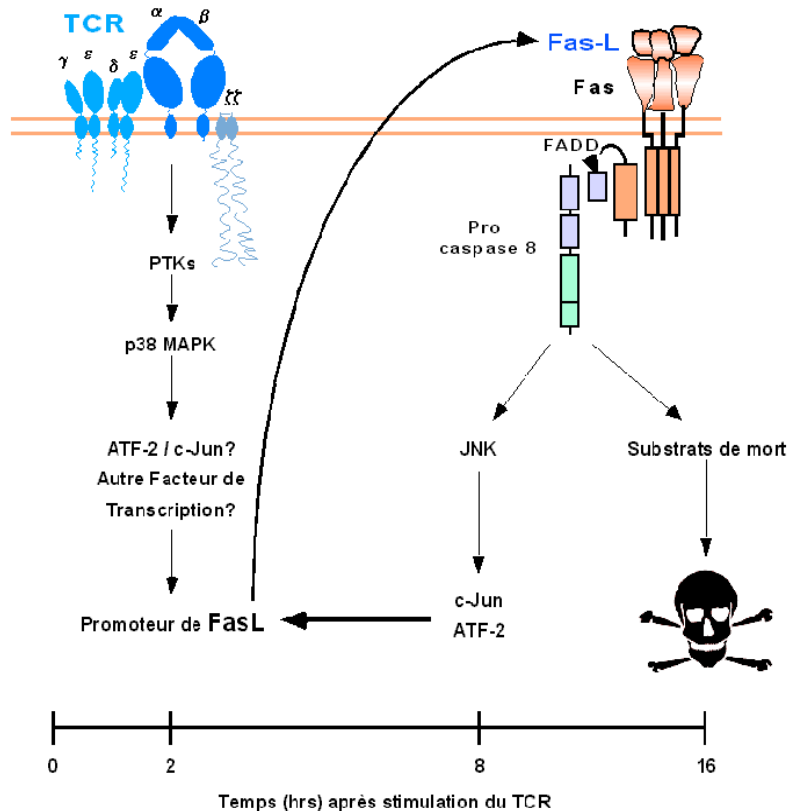


Figure 2. Peptide-induced tolerance of CTL. C57BL/6 mice were treated three times at 3-d intervals with 1–500 μg GP33 peptide in IFA i.p. or with IFA alone. Lines represent individual animals. 10 d after the last peptide application, mice were infected i.v. with 200 PFU LCMV-WE, and the ex vivo CTL activities of splenocytes were assayed 8 d later in a ^{51}Cr release assay. MC57G fibroblast target cells were either loaded with 10^{-6} M GP33 peptide (●) or with 10^{-6} M NP394 peptide (Δ) or left untreated (○).

Mécanisme d'AICD



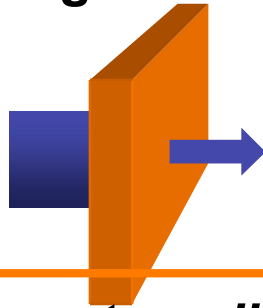
Déletion clonale
Mécanisme actif de tolérance périphérique

- L'engagement du TCR conduit à l'activation des kinases de la famille Src (SFks) ce qui aboutit à l'activation de p38MAPK. p38MAPK phosphoryle divers facteurs de transcription (par exemple ATF-2), qui pourrait avoir la possibilité de se fixer sur le promoteur de FasL et ainsi activer la transcription du gène. Une fois FasL traduit, il est adressé à la membrane plasmique où il va interagir avec son récepteur (Fas). L'interaction Fas/FasL aboutit à l'activation des caspases qui vont, entre autre, activer les JNK. Les JNK activées vont promouvoir l'AICD en régulant l'expression de FasL et ainsi amplifier le signal. Il semble que p38MAPK et JNK puissent réguler l'expression de FasL à des étapes différentes suite à l'activation. D'après Zhang et al., 2000.

- Rôle physiologique est mis en évidence dans des situations où des **mutations Fas ou FasL**, ou l'expression de molécules bloquant l'apoptose des lymphocytes B et T (c-FLIP) conduisent à une expansion lymphocytaire anormale (**lymphoprolifération**) la production d'auto-anticorps et des atteintes rénales de type lupique.

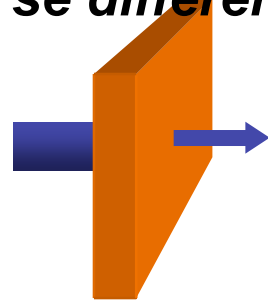
Les mécanismes périphériques de contrôle des LyT auto-réactifs

Percevoir le signal



- Déletion clonale
- Activation des LyT: un processus contrôlé
 - Molécules co-stimulation
 - Influence des DC

Proliférer et se différencier



- Contrôle des réponses inflammatoires (rôle des cytokines)
- Populations suppressives
 - LyT régulateurs

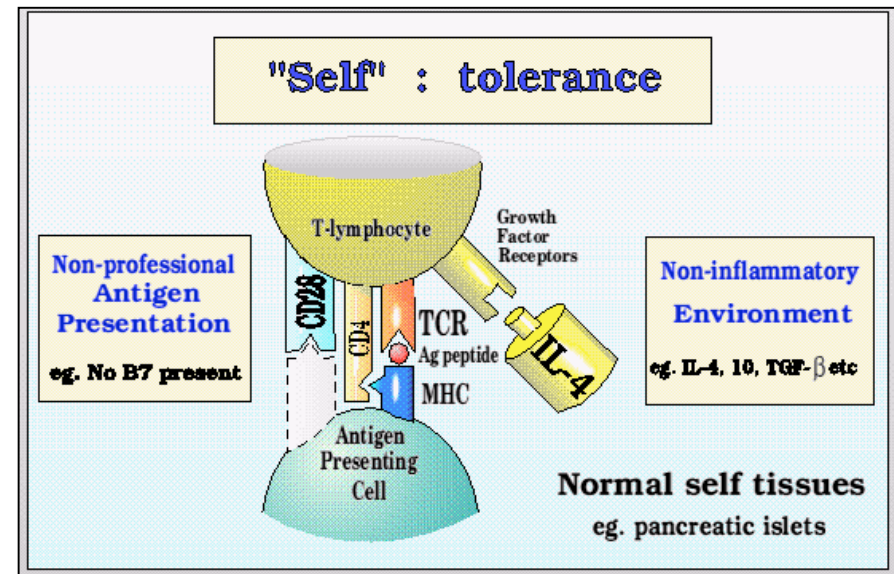
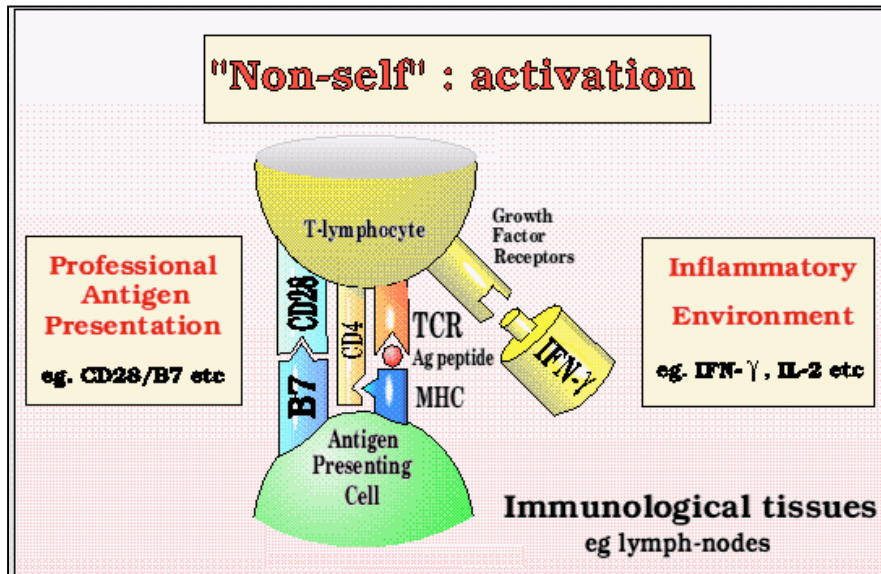
Atteindre le tissu cible



- Accès limité aux tissus
- Sites immunoprivilégiés

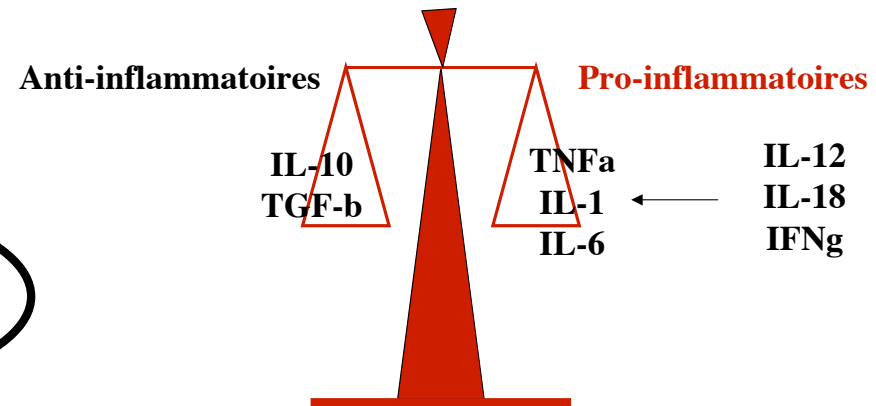
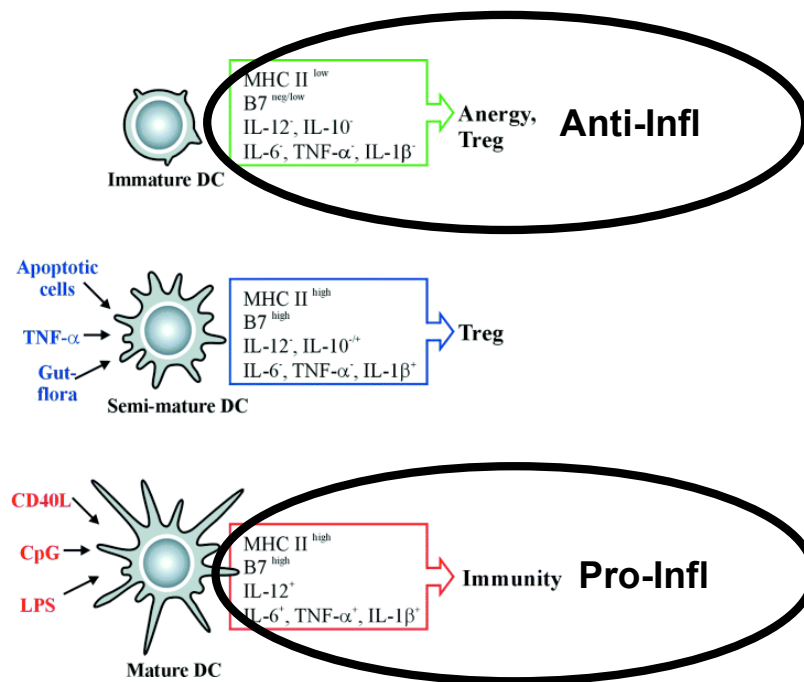
REPONSE

Cytokine: le 3eme signal d'activation

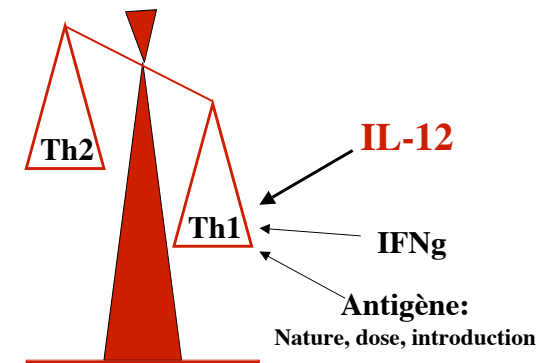


Cytokines

Pro-inflammatoire / Immunosuppressive



Hypersensibilité de type IV Maladies auto-immunes



- Dermatite de contact
- Maladies granulomateuses (Crohn, Vascularites)

Tolérance et cytokines

Neonatal tolerance

Induction of neonatal tolerance is associated with expression in vivo of **IL-4** and **IL-10**. (Fig 1)

Mucosal tolerance

Tolerance to ubiquitous environmental antigens, : high frequency of **TGF- β** + cells (Th3) that have suppressive functions

Autoimmunity

-IL-10-knockout mice develop an autoimmune colitis spontaneously, and TGF- β -knockout mice that develop a more extensive, multifocal autoimmunity

Default of IL-10 production in AID:

Colitis, Crohn disease: presence of neutralizing Ab (Fig 2)

-IL-4: Systemic injection of IL-4, gene therapy with IL-4 and transgene expression of IL-4 in β cells of the islets of Langerhans each prevent the onset of diabetes in NOD mice

More complex...

TGF- β : treatment with TGF- β ameliorates EAE. TGF- β is, however, known to be pro-fibrogenic and is implicated in the pathogenesis of several fibrotic conditions such as scleroderma and pulmonary fibrosis. Transgenic expression of TGF- β resulted in chronic pancreatitis and fibrosis .

IL-10 : Under some circumstances, IL-10 is protective; in EAE. By contrast, diabetes is exacerbated following increased pancreatic expression of IL-10 in a transgenic model

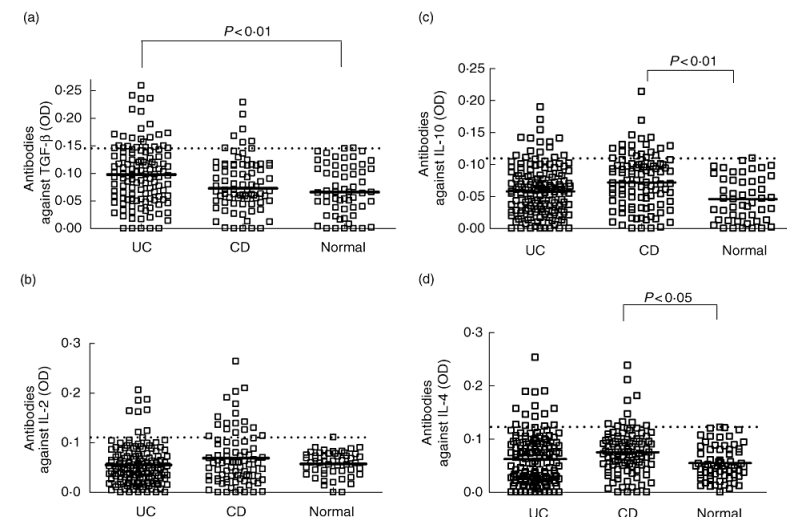
Groups ^a	Days of graft survival	MST ^b
Neonatal	20, 21, >40, >40, >40, >40, >40	>40
Neonatal + IFN- γ	14, 14, 14, 15, 16	14
Neonatal + anti-IL-4	10, 15, 16, 18, 20	16
Naive	11, 13, 14, 14, 16, 17, 17	14

^a Four-week-old neonatal primed, neonatal primed + IFN- γ , neonatal primed + anti-IL-4, and age-matched naive control mice were grafted with A/J tail skin and graft survival was followed from day 10.

^b Median survival time.

Chen N, Transplantation. 1996

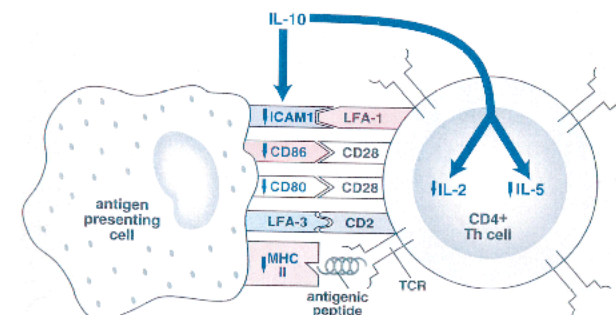
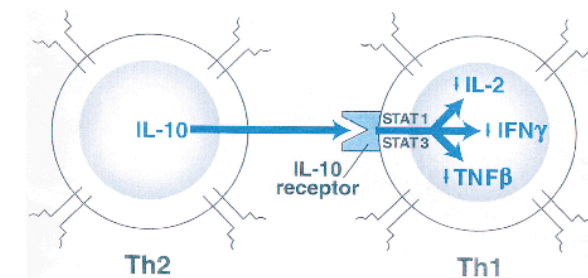
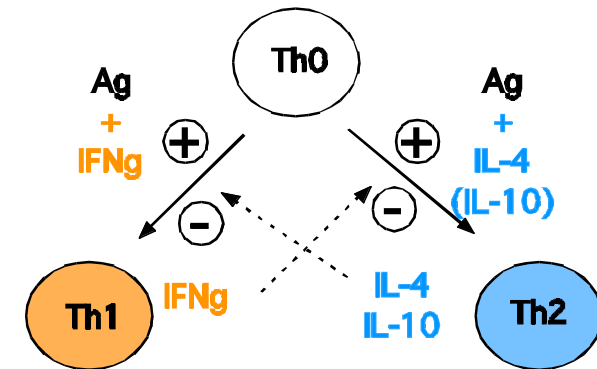
Patients with inflammatory bowel disease may have a transforming growth factor- β , interleukin (IL)-2- or IL-10-deficient state induced by intrinsic neutralizing antibodies. E. C. Ebert, Clin Exp Immunol. 2009



Cytokines anti-inflammatoires

IL-10, TGFb

- **Production**
 - IL-10: Mono, Macro, LyB, LyT Tr1
 - TGFb: tout type cellulaire, Th3
- **Cytokines anti-Th1:**
 - IL-10
 - IL-4
- **Actions:**
 - Inhibition de production des cytokines pro-inflammatoires
 - Inhibition activité microbienne du Macro, PNneutro
 - Induit baisse d'expression MHC II et B7, ICAM-1
- **Génération de Lymphocytes suppresseurs:**
 - Treg induits: Tr1
 - Th3



IL-10 : cytokine immunosuppressive

Altered Immune Responses in Interleukin 10 Transgenic Mice

By Amy Hagenbaugh,^{**} Sherven Sharma,[†] Steven M. Dubinett,[†]
Sherry H.-Y. Wei,^{§§} Richard Aranda,[§] Hilde Cheroutre,[‡]
Deborah J. Fowell,^{**} Scott Binder,^{††} Betty Tsao,^{*} Richard M. Locksley,^{**}
Kevin W. Moore,^{§§} and Mitchell Kronenberg^{*†§}

Table 1. T and B Lymphocyte Differentiation Is Normal in IL-10 Transgenic Mice

Mouse type	Cell no.	CD4 ⁺	CD8 ⁺	CD4 ⁺ / CD8 ⁺	B220/IgM ⁺
Thymus		%	%	%	%
Control No. 1	1 • 10 ⁶	9.8	2.6	83.7	—
Control No. 2	1.2 • 10 ⁶	10.9	2.6	82.7	—
IL-10 Tg No. 1	9.8 • 10 ⁷	10.4	3.2	82.1	—
IL-10 Tg No. 2	1.6 • 10 ⁸	15.3	3.4	77.4	—
Spleen					
Control No. 1	4.8 • 10 ⁷	26.6	17.5	• 1	60.8
Control No. 3	5.5 • 10 ⁷	22.8	9.7	• 1	61.8
Control No. 4	5.4 • 10 ⁷	19.5	14.4	• 1	60.3
IL-10 Tg No. 1	3.0 • 10 ⁷	22.8	13.2	• 1	62.8
IL-10 Tg No. 2	3.0 • 10 ⁷	24.9	13.2	• 1	58.4
IL-10 Tg No. 3	6.5 • 10 ⁷	16.5	10.3	• 1	63.7
IL-10 Tg No. 4	6.5 • 10 ⁷	23.4	14.6	• 1	50.5

Cells from spleen and thymus of line 9 IL-10 transgenic (Tg) mice were prepared, counted, and stained with the various cell-surface markers. Stained cells were analyzed by flow cytometry as described in Materials and Methods. Values are given as percentages of total gated lymphocyte populations, based on light scatter. —, not done.

Tumor growth

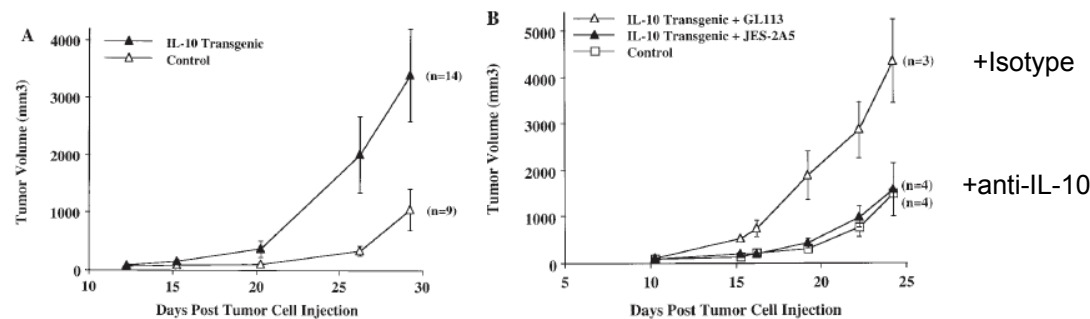


Figure 7. Uncontrolled tumor growth in IL-10 transgenic mice. (A) Groups of 9–14 IL-10 transgenic and control littermate mice were injected subcutaneously with 3LL carcinoma cells. Tumor growth was recorded on a daily basis using metric calipers. Data are shown as the mean tumor volume for each group of mice $\pm$ SE at each indicated time point. This is representative of one of three experiments, which all yielded similar results. (B) 1.5 mg of IL-10 neutralizing antibody, JES-2A5, or control isotype antibody, GL113-5E7, were administered intraperitoneally at 24 h before and 4 d after 3LL cell injection to IL-10 transgenic mice. Control littermates were injected with 3LL cells as a negative control for tumor growth. Groups of three to four mice were analyzed. Data is shown as mean tumor volume $\pm$ SE.

Colitis:

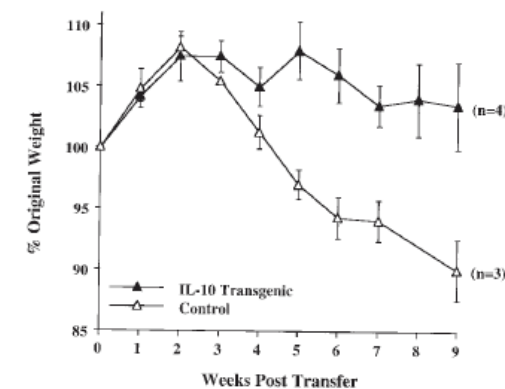
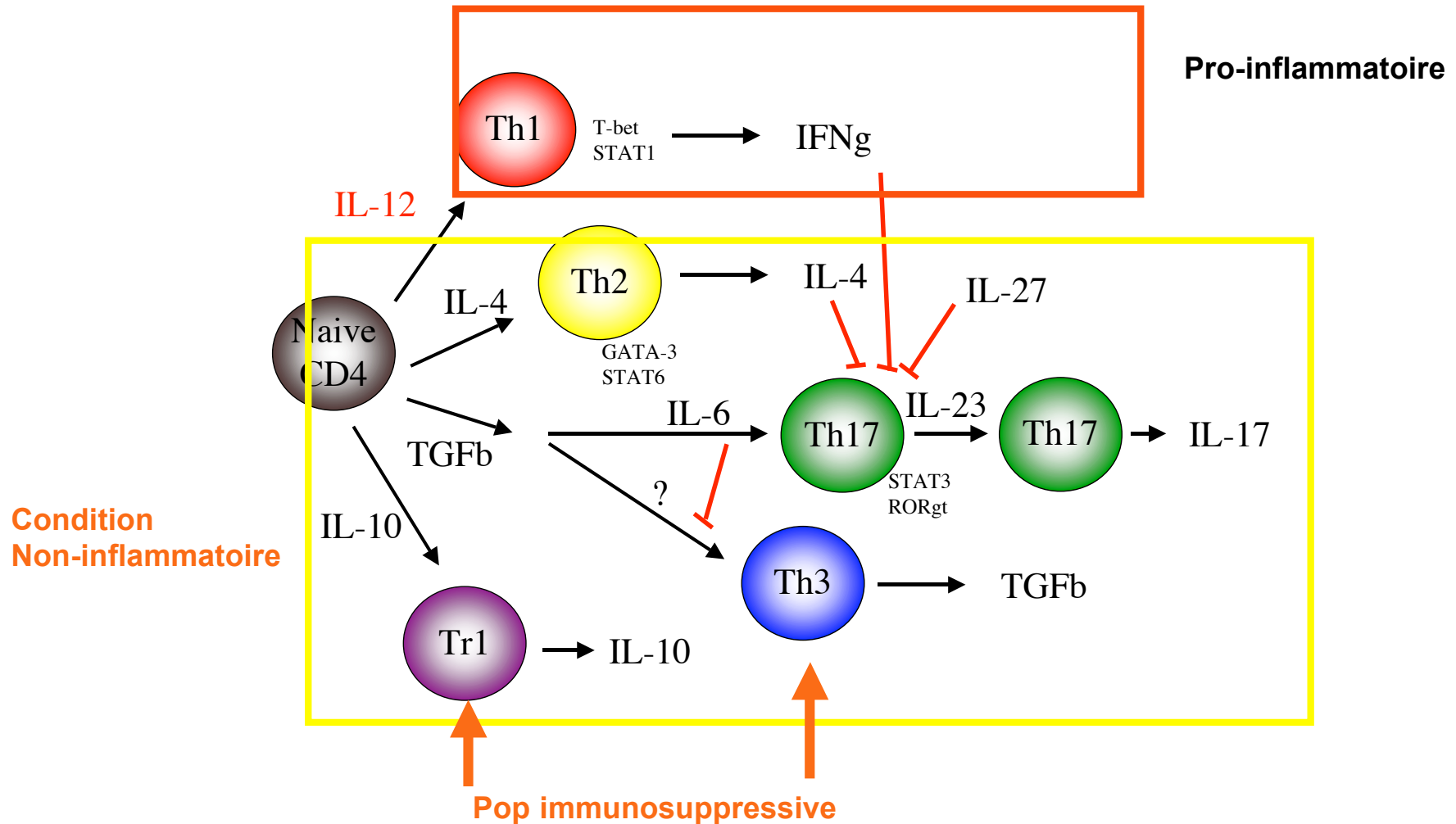


Figure 3. CD4⁺ CD45RB^{high} cells from IL-10 transgenic mice fail to induce colitis in SCID recipients. 4–5 • 10⁵ FACS⁺ sorted CD4⁺ CD45RB^{high} cells from IL-10 transgenic or control littermate mice were injected into SCID mice. Weight gain/loss was scored weekly for 9 wk. Values are shown as a percentage of original weight at day 0, the time of cell injection. Number of mice in each group is indicated in parentheses. Regression analysis of the two curves shown indicated the data to be highly significant, $P = 0.0012$. (Similar results were obtained using Rag-2^{-/-} mice as T cell recipients.)

Déviation immunitaire et tolérance

Environnement cytokinique est déterminant lors de l'activation lymphocytaire
>> Différenciation Th1, Th2, Th3, Th17,



Lymphocytes T suppresseurs

	Th1	Th2	Th3	Tr1	CD25 ⁺ (high)
Interferon gamma	++++	-	+/-	+	+/-
IL-4	-	++++	+/-	-	+/-
TGF-beta	+/-	+/-	++++	++	+/-
IL-10	-	++	+/-	++++	+/-
Growth	IL-2	IL-2/IL-4	IL-4/TGF-β	IL-10, Ifna	IL2+++
B helper	IgG2a	IgG1/IgE	IgA	-	-
Suppression	Th2	Th1	Th1 ou 2	Th1	Th1 or 2
Proliferation	+++	+	+	+/- (IL-15)	IL-2
Affinity for self	+/-	+/-	+	+	+++++

Treg naturels

• Th3

sous-groupe de lymphocytes TCD4⁺ qui secrètent du TGF- (ainsi que de l'IL-4 et de l'IL-10), favorisent la sécrétion d'IgA par les plasmocytes, régulent à la baisse les Th1 et les Th2. Leur maturation dépend de la présence de TGF-, d'IL-4, d'IL-10 et du blocage de l'IL-12, ainsi que de l'expression à leur surface de CD86 et de CTLA-4.

Ces clones Th3 émergent surtout après pénétration de l'antigène par voie orale.

Leurs cibles ne sont pas les T effecteurs mais plutôt les cellules présentatrices en contact avec ces derniers, la libération de TGF- induisant un mécanisme de suppression paracrine sur celles-ci.

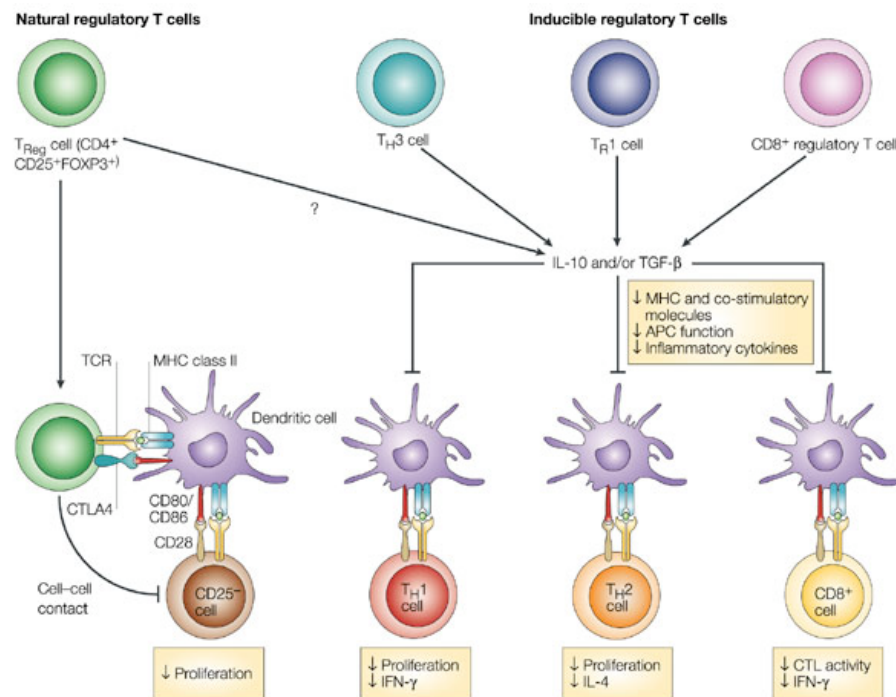
• Tr1

La production de taux élevés d'IL-10 suppriment la réponse immune par contacts cellulaires directs et/ou par sécrétion de cytokines.

faible capacité à proliférer (du fait des hautes concentrations d'IL-10).

se différencient après contacts avec des cellules présentatrices synthétisant de grandes quantités d'IL-10.

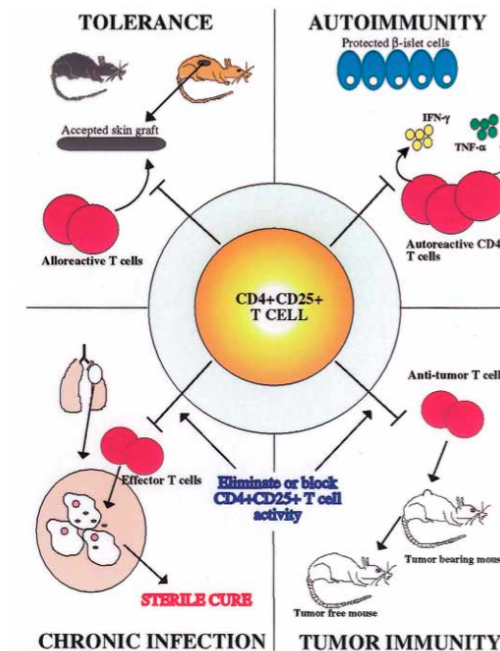
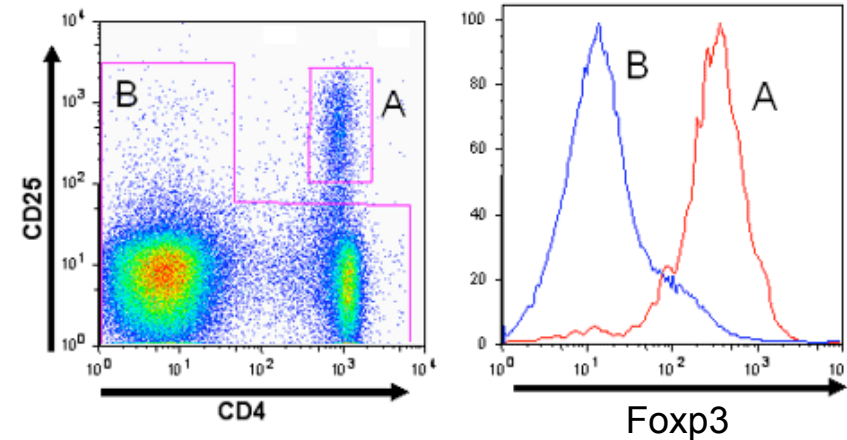
Cette situation est surtout rencontrée lorsque la cellule dendritique est encore assez « naïve », que l'antigène est en *faible concentration*, et que les contacts entre cellule présentatrice et lymphocyte TCD4⁺ sont *répétés*.



Lymphocytes T régulateurs CD4+ CD25+

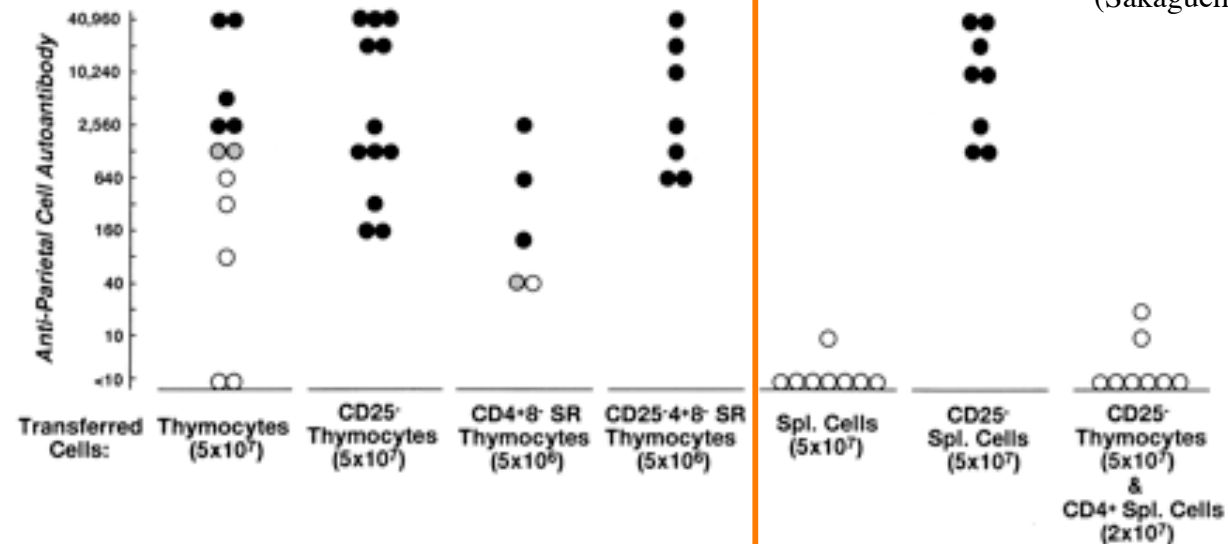
- **Ly T régulateurs:**

- (re)découverts par Sakagushi
- Rôle dans maintien de la tolérance périphérique
- Préviennent l'apparition des MAI
- Phénotype:
 - **CD4+ CD25+**
 - **Foxp3+**
- 5-10% des T périphériques
- Cellules anergiques, dépendantes de l'IL-2 et de CD28 pour leur survie
- Action suppressive de proximité
 - Contact-dépendent
- Rôle multiple
- Suppression des réponses
 - auto-immunes
 - anti-tumorales
 - allogéniques
 - anti-infectieuses



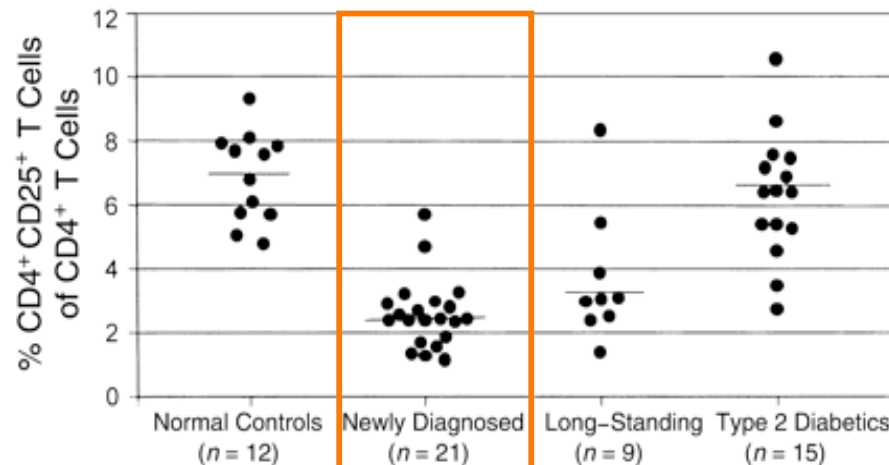
Lymphocytes T régulateurs: mise en évidence de leur implication dans MAI

- Chez la souris nude après transfert adoptif



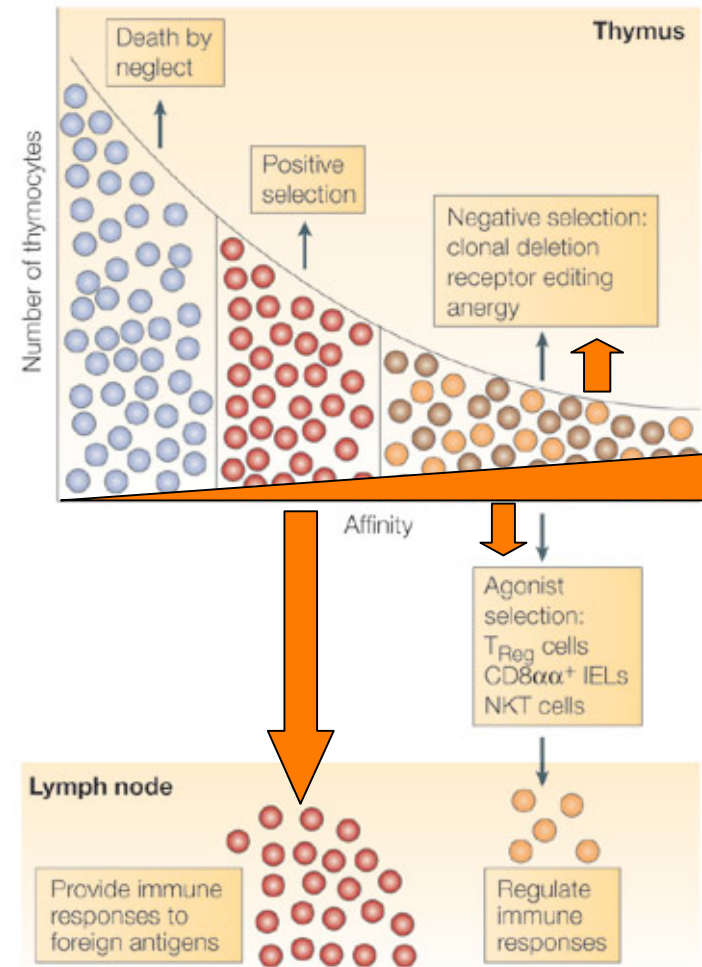
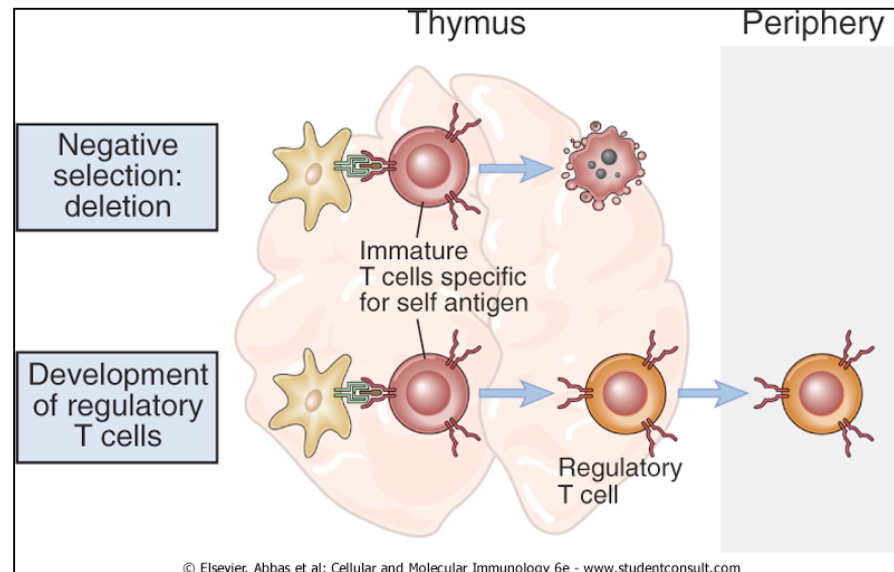
(Sakaguchi; 1999)

- Chez les patients diabétiques insulino-dépendant (type 1)



Ontogénie et Spécificité des Treg

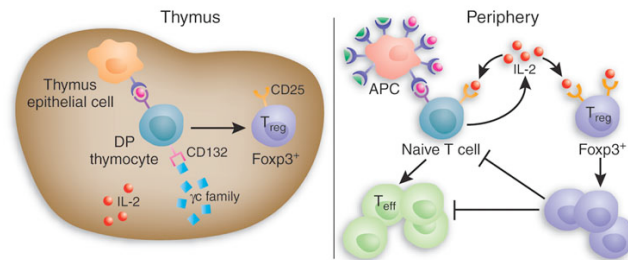
- Treg reconnaissent le soi
- Différenciation dans le thymus après contact avec Ag du soi
- Affinité critique pour CMH-Ag_{soi}



Treg: nouvel outil thérapeutique

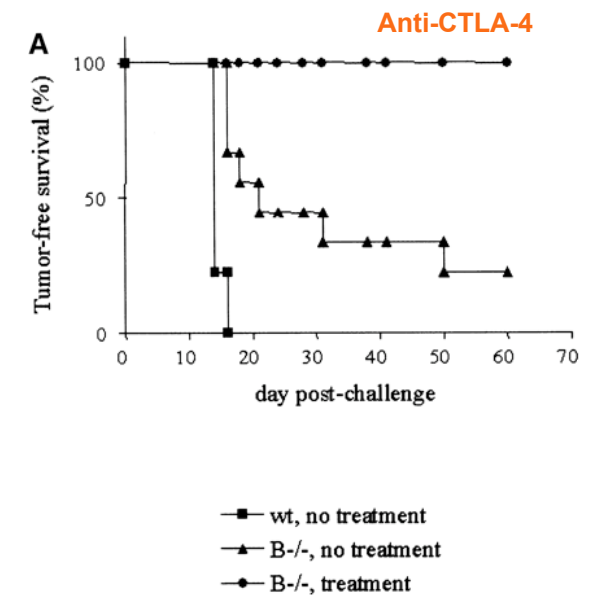
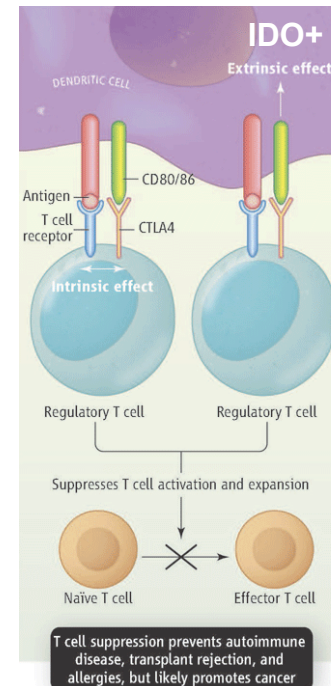
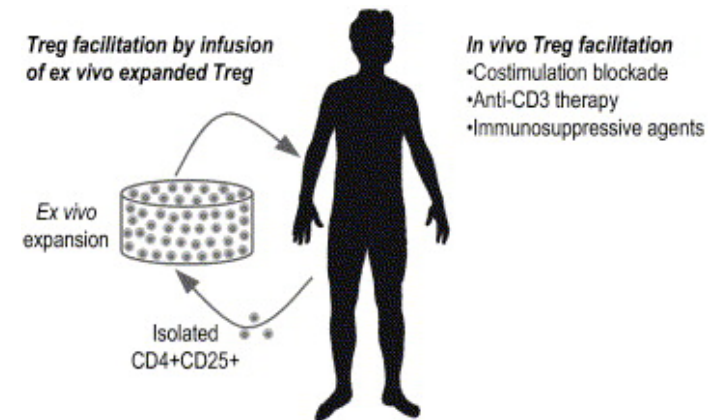
• Suppression

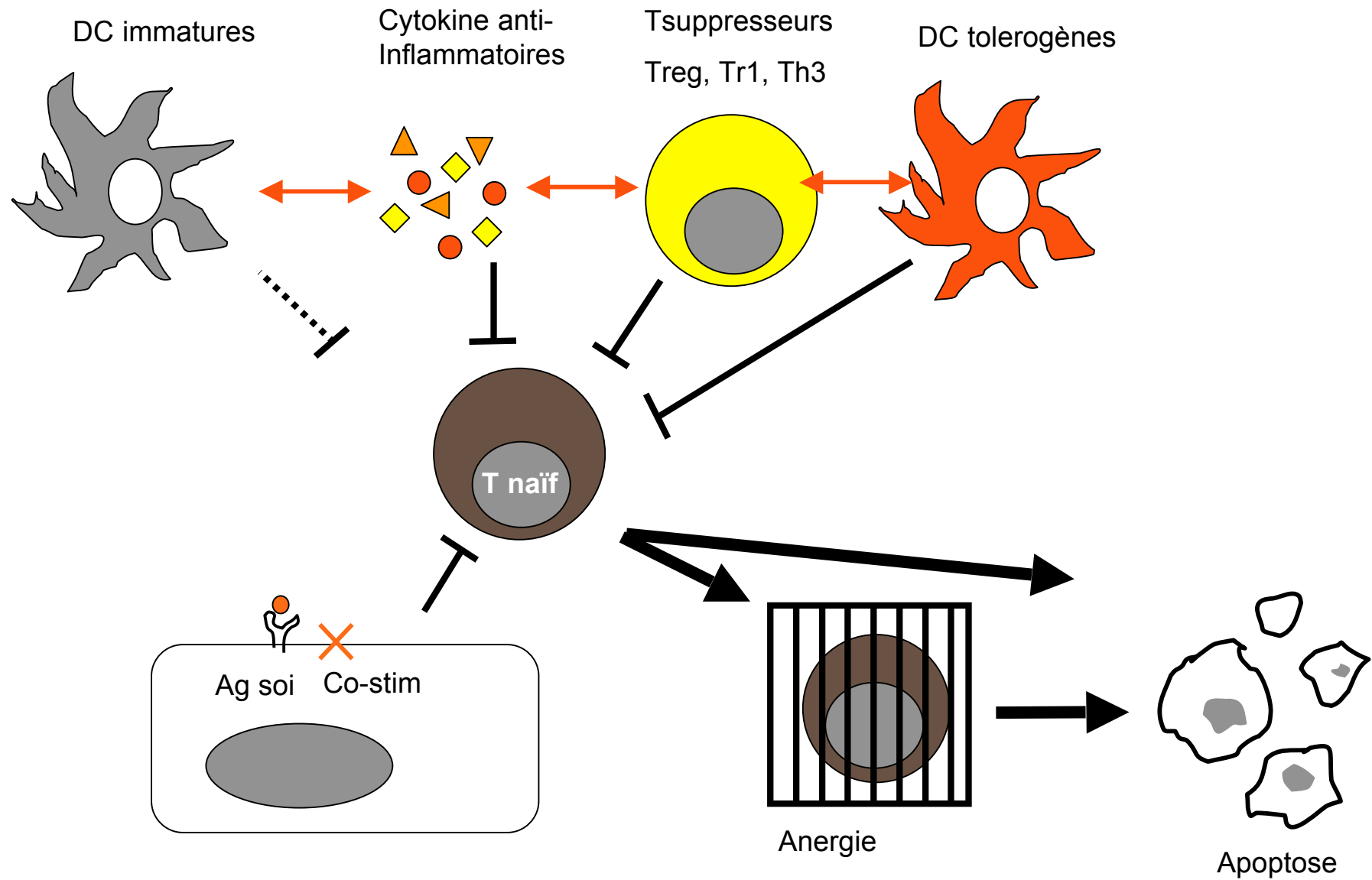
- Modification du ratio Teff: Treg
 - Expansion in vitro et injection
 - Expansion in vivo
 - Rôle de l'IL-2



• Activation:

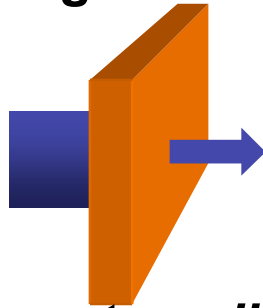
- Déplétion des Treg :
Anticorps monoclonaux anti-CD25
- Bloque l'activation des Treg
 - Anti-CTLA4



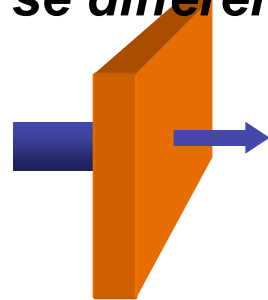


Les mécanismes périphériques de contrôle des LyT auto-réactifs

Percevoir le signal



Proliférer et se différencier



Atteindre le tissu cible



REPONSE

- **Activation des LyT**: un processus contrôlé
 - Molécules co-stimulation
 - Influence des DC
- **Contrôle des réponses inflammatoires** (rôle des cytokines)
- **Populations suppressives**
 - LyT régulateurs
- **Accès limité aux tissus**

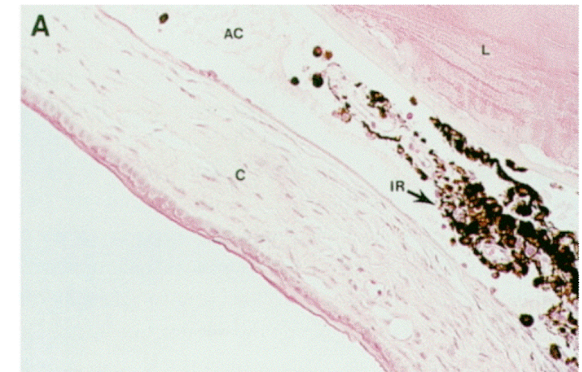
Tissus « immunoprivilégiés »

- Interdire l'accès de certains tissus aux lymphocytes T activés et potentiellement autoréactifs
- **FasL+**
 - Œil, cerveau (astrocytes), testicules, l'utérus et le placenta (Rôle dans la tolérance du fœtus)

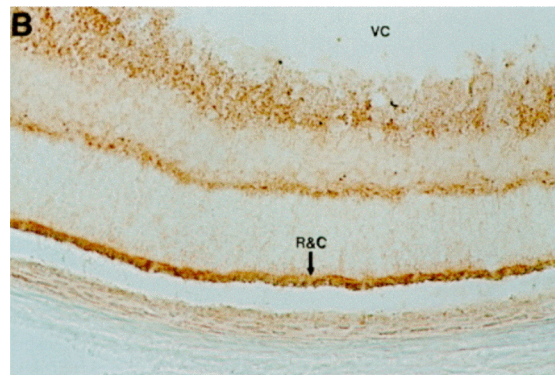
Les anomalies de l'apoptose lymphocytaire induite par Fas peuvent favoriser l'irruption de cellules immunitaires dans des sanctuaires immunoprivilégiés

Infiltration lymphocytaire en réponse à une inoculation virale dans la chambre antérieure

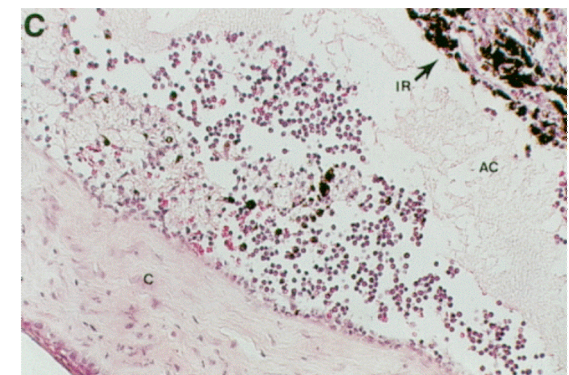
WT



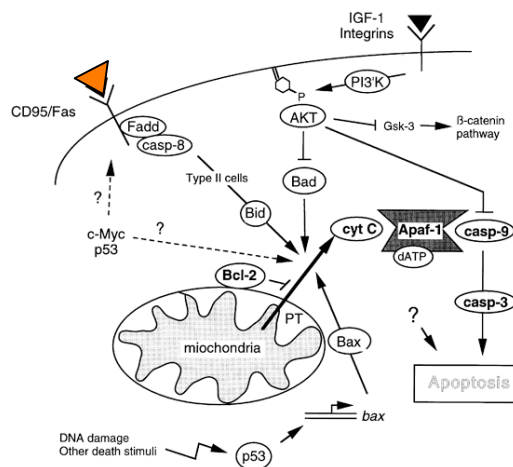
Oeil



FasL^{-/-}



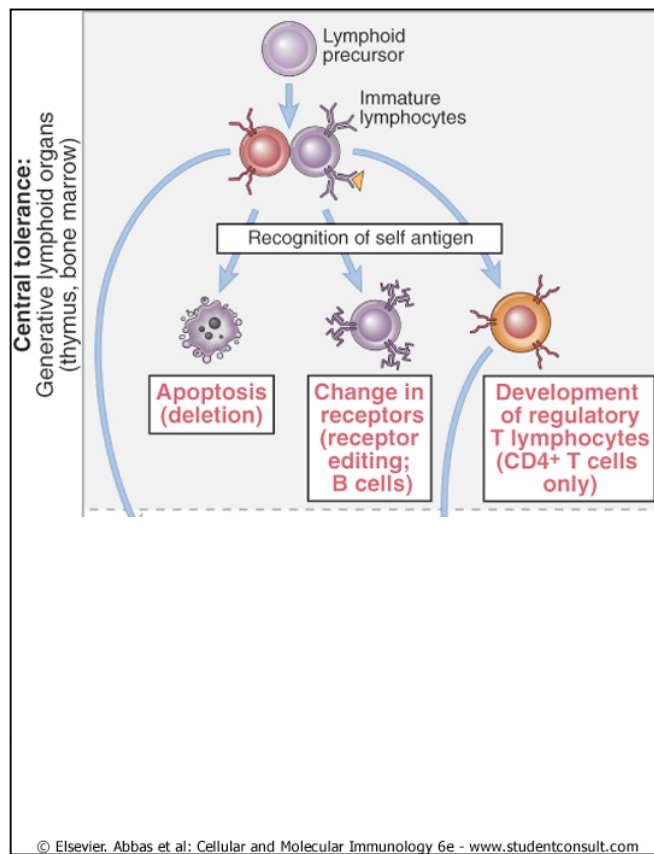
Ferguson et al. 1997



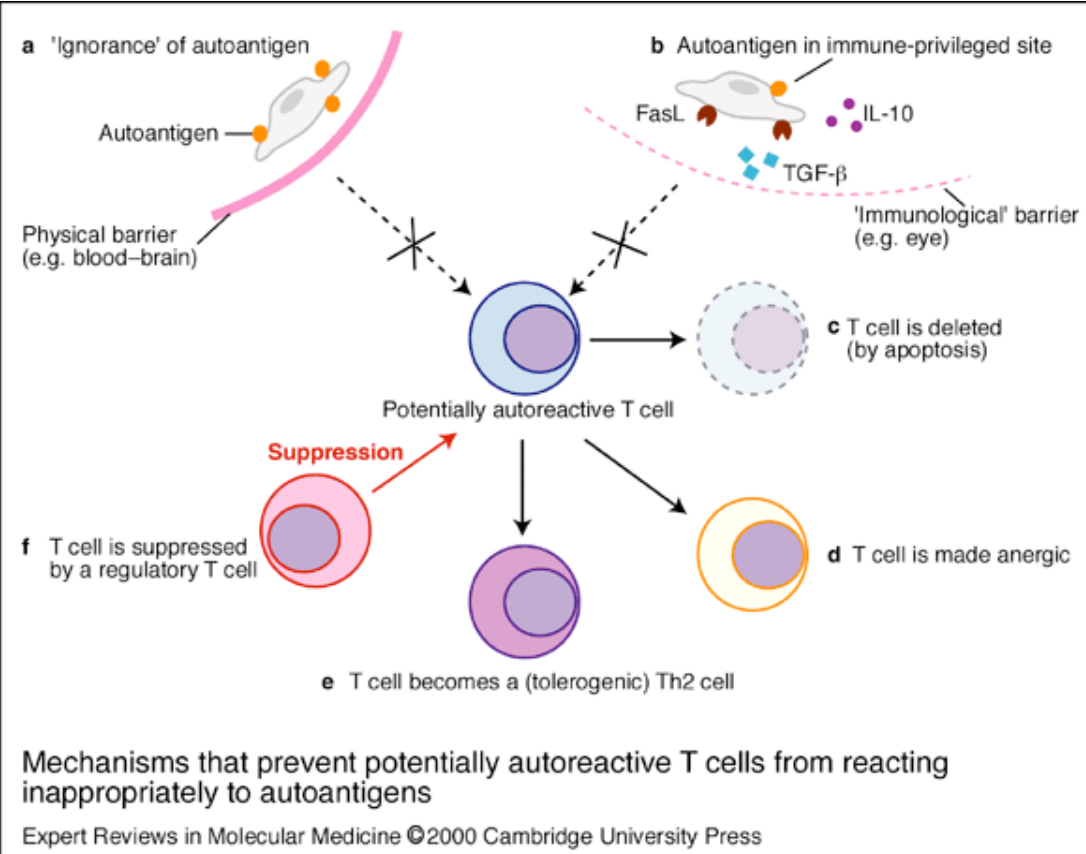
Conclusion

Mécanismes à l'origine de la tolérance

Tolérance centrale



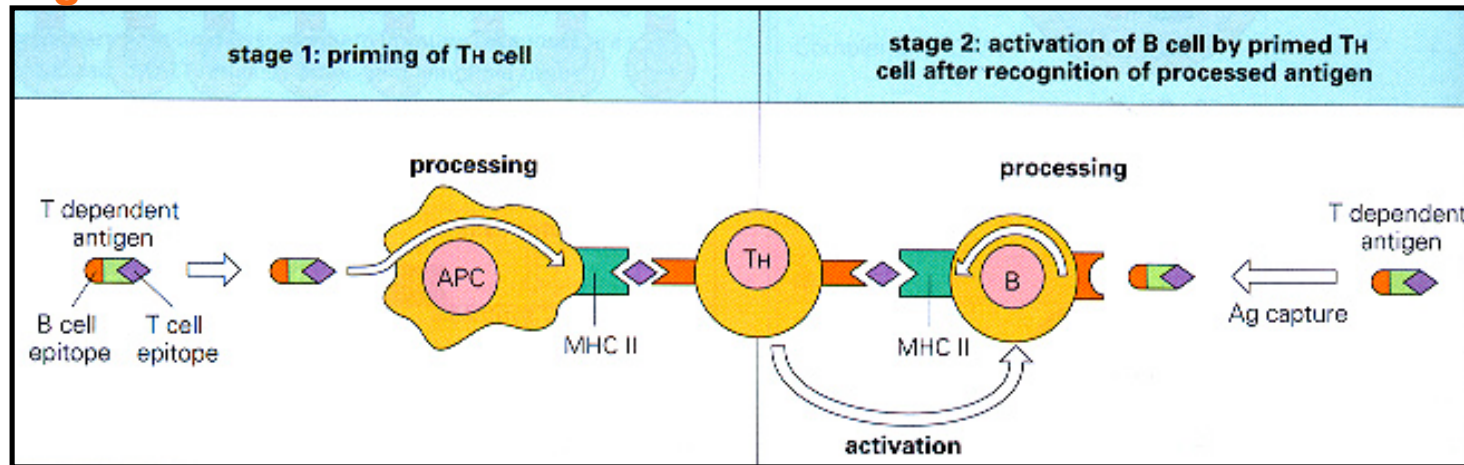
Tolérance périphérique



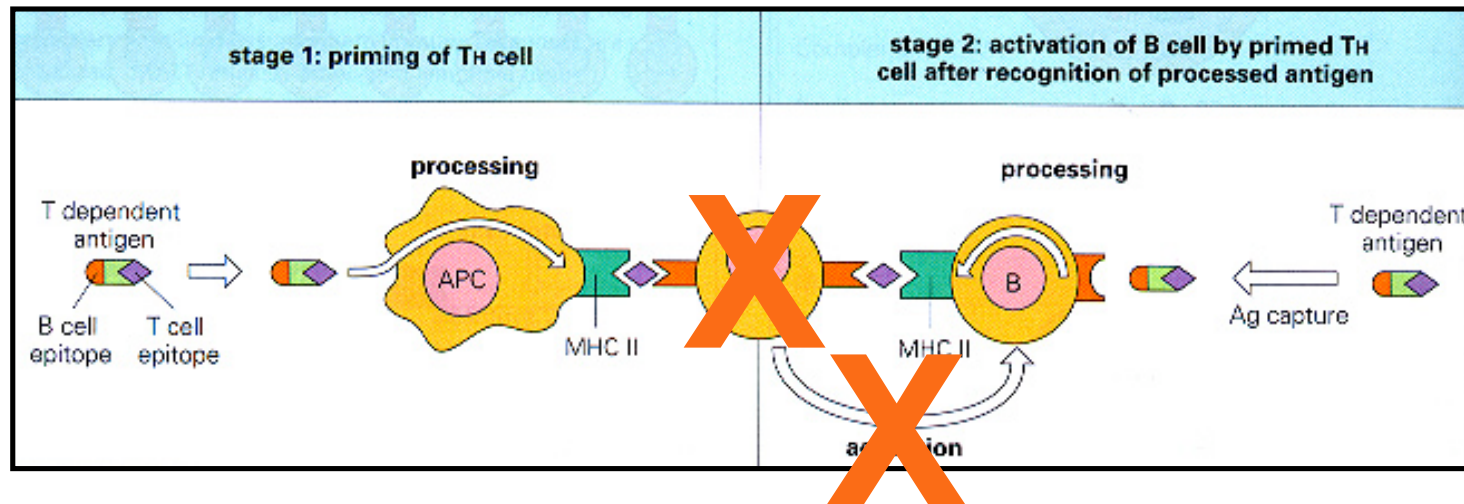
Importance de la tolérance T pour maintenir la tolérance B

B cells need T cell “help” to make high affinity antibody

Non-self Ag



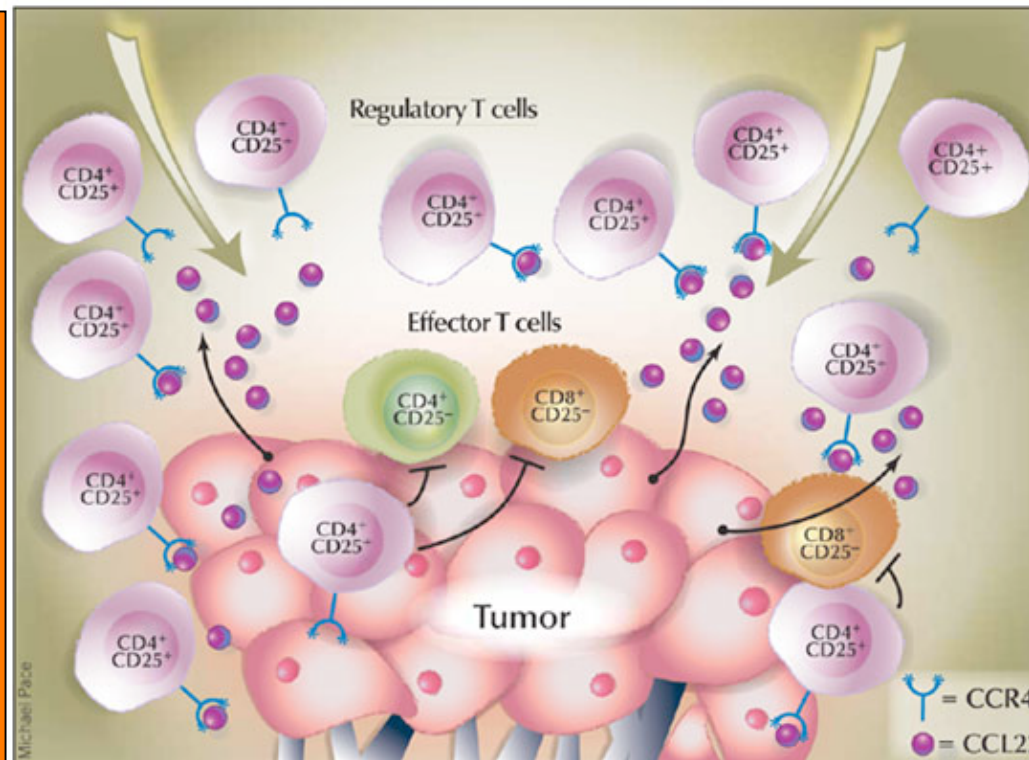
Self Ag



Difficulté de l'établissement des réponses anti-tumorales

Tumeur = soi modifié

Ignorance
Anergie
Déviation immunitaire
Suppression
Treg
Echappement



Développement des maladies auto-immunes

Théorie du mimétisme moléculaire

