UPMC PARIS UNIVERSITAS
B. Bellier

UPMC BMC423 IF

Tolérance Immunitaire

Bertrand Bellier
UPMC CNRS
Immunology Immunopathology Immunotherapy
Pitié-Salpétrière - Paris - France
bertrand.bellier@upmc.fr

UPMC PARIS UNIVERSITAS
B. Bellier

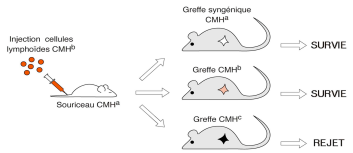
Tolérance immunitaire

- **Propriété essentielle du sytème immuniatire**
- **Assure la discrimination du soi / non-soi**
- **Inné (Eléments 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

UPMC PARIS UNIVERSITAS
B. Bellier

Tolérance: Premiers témoignages Ehrlich

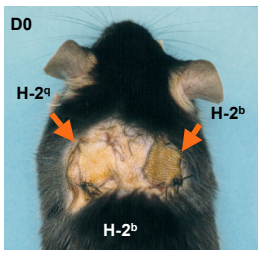
- **Tolérance vis-à-vis d'antigènes du soi:**
  - Debut XXeme siècle: **Ehrlich and Morgenroth** (1901)
    Absence de réponse immunitaire de la chèvre vis à vis de ses propres hématies; par contre production d'Ac hémolytiques contre des hématies d'un animal tiers
- **Les périodes fœtale et néonatale 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 :

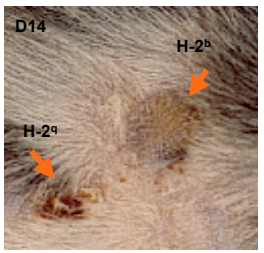


UPMC PARIS UNIVERSITAS
B. Bellier

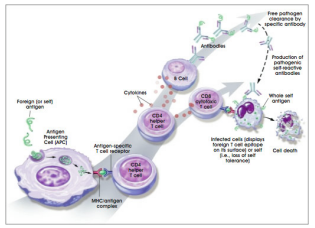
Conséquences physiologiques:
Tolérance au soi / Rejet du non-soi

- **Greffes:**



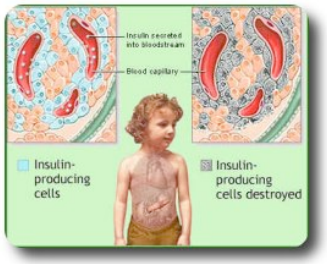


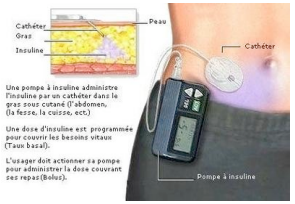
- **Infections:**



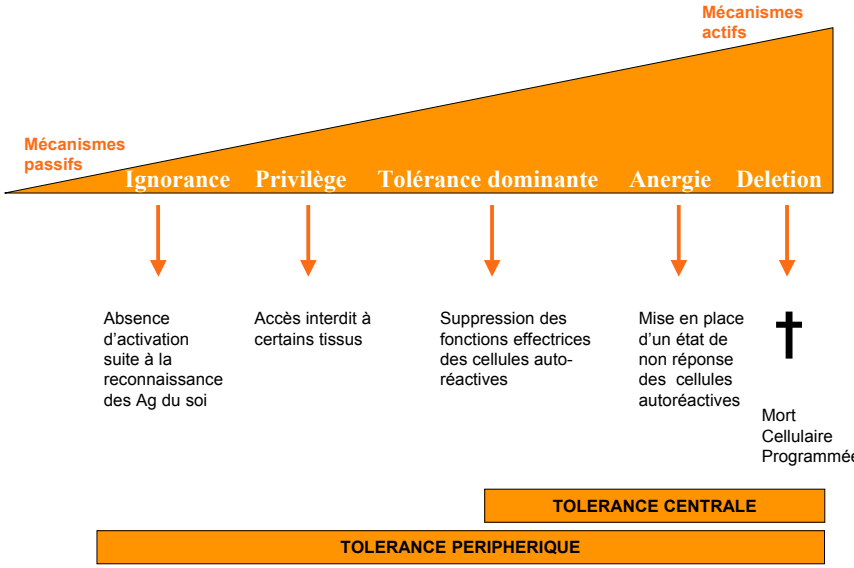
Conséquences physiologiques:
Rupture de Tolérance / Rejet du soi

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



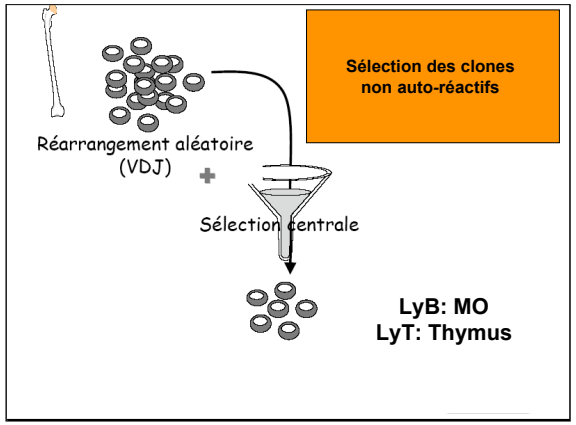


Cette absence de réponse aux Ag du soi est assurée par différents mécanismes



Tolérance centrale

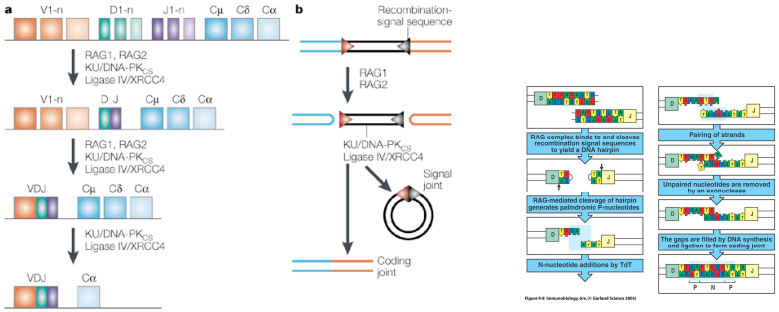
Tolérance centrale:
Sélection lymphocytaire dans les organes lymphoïdes primaires



UPMC PARIS UNIVERSITAS
B. Bellier

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

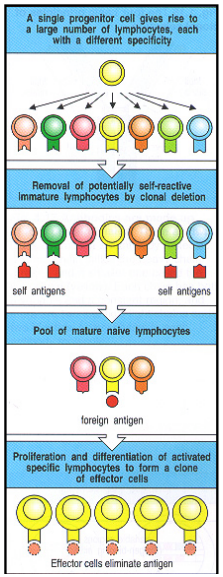
- **Ontogénie T et B:**
  Récepteur TCR et BCR sont générés par recombinaison génétique des locus
  >> Diversité combinatoire
  >> Diversité jonctionnelle

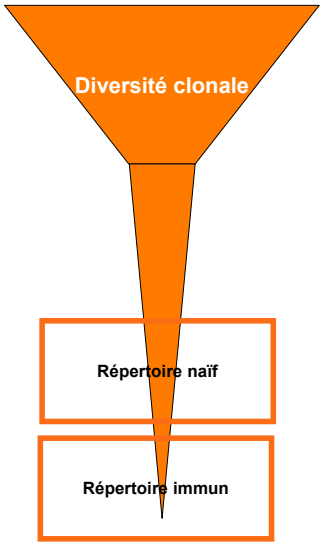


Nature Reviews | Genetics

UPMC PARIS UNIVERSITAS
B. Bellier

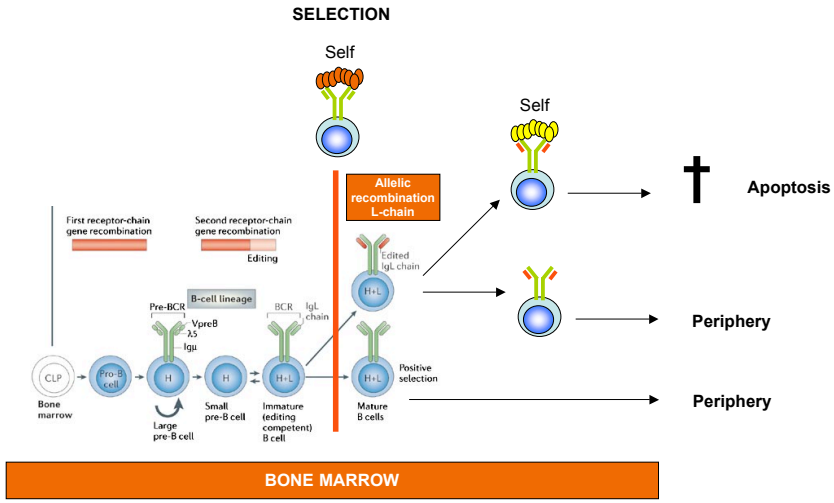
Sélection lymphocytaire : Une nécessité
élimination des clones auto-réctifs





UPMC PARIS UNIVERSITAS
B. Bellier

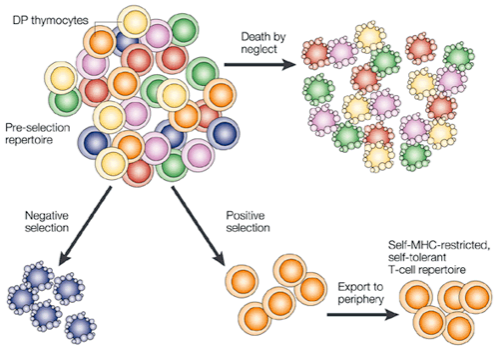
Sélection lymphocytaire B:
Survie après « Editing » ou Mort programmée



UPMC PARIS UNIVERSITAS
B. Bellier

Sélection lymphocytaire T

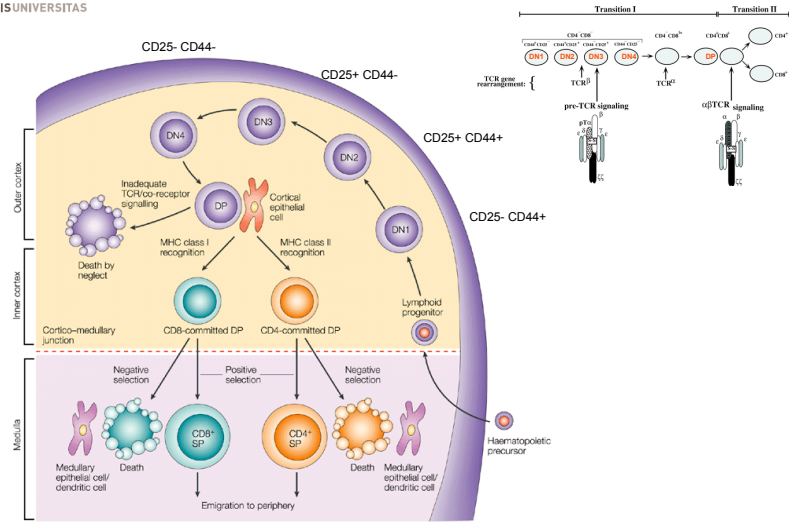
- **Double sélection :**
  - **Sélection positive** des clones T dont le TCR peut interagir avec les molécules de CMH
    >> apporte un **signal de survie**
  - **Sélection négative** des clones T reconnaissant les Ag du soi
    >> délétion par **apoptose**



Nature Reviews | Immunology

UPMC PARIS UNIVERSITAS B. Bellier

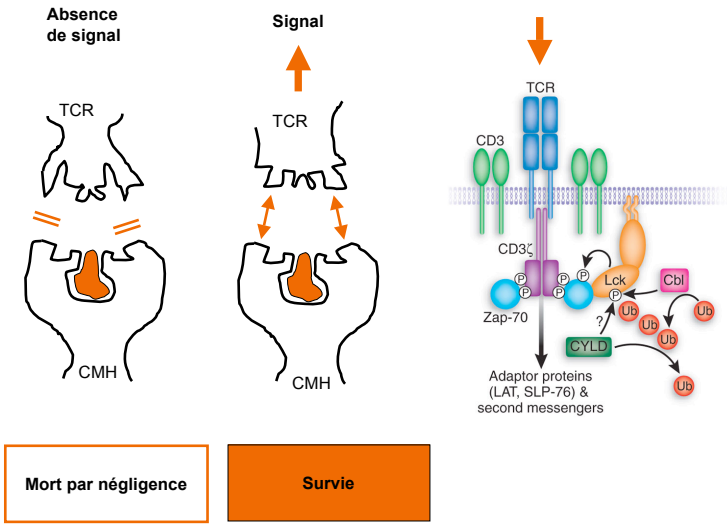
Mécanisme de sélection thymique



Nature Reviews | Immunology

UPMC PARIS UNIVERSITAS B. Bellier

Sélection positive
Signal de survie

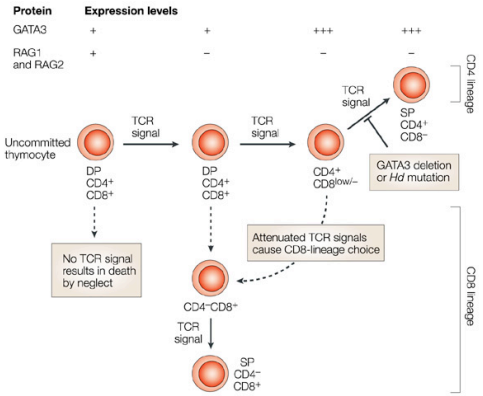


Mort par négligence

Survie

UPMC PARIS UNIVERSITAS B. Bellier

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

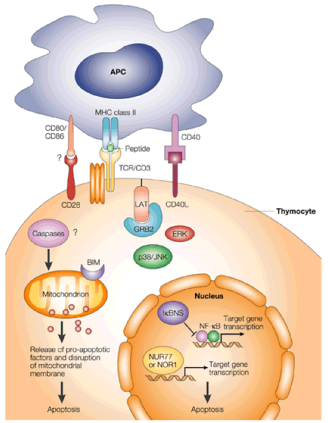


Nature Reviews | Immunology

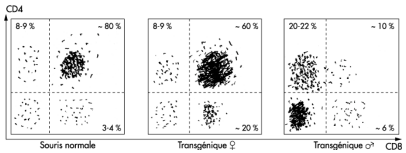
- **T-cell receptor (TCR)-signal-induced and TCR-independent (dotted arrows) developmental stages are shown, based on a hypothetical model of thymocyte development. In the absence of TCR signalling, double-positive (DP) thymocytes die by neglect prior to lineage differentiation. Initial TCR signalling in DP cells causes the termination of recombinase-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 expression31, 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 differentiation31, 80. A terminal TCR signalling step 'proofreads' CD8-lineage differentiation80, 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 transgenes24, 27 or germline deletion of the CD4 silencer154. Hd, helper cell deficient.**

UPMC PARIS UNIVERSITAS B. Bellier

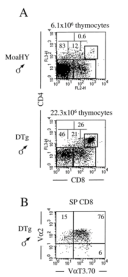
Sélection négative
Signal de mort cellulaire



Nature Reviews | Immunology



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



Souris male TCR-transgénique

Anti-HY

Anti-HY & Anti-GP33

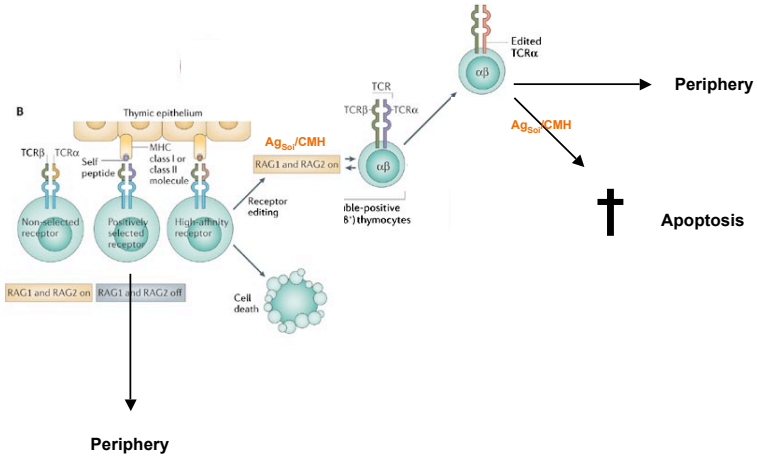
- Although the signalling pathway that mediates negative selection is not fully understood, many of the important signalling molecules have been identified and studied. Future work should identify the other signalling components and establish the pathway, which begins with engagement of the T-cell receptor (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-B; JNK, JUN N-terminal kinase; LAT, linker for activation of T cells; NF-B, nuclear factor-B; NOR1, neuron-derived orphan receptor 1; NUR77, nuclear receptor 77.

Legrand et al., JI 2001

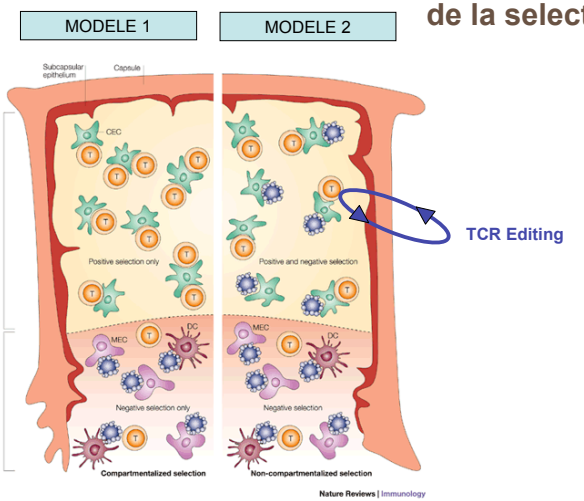
Détournement de la Sélection négative

Editing, une alternative à l'apoptose

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

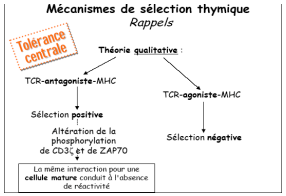


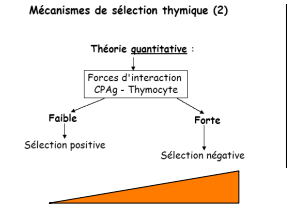
Absence de « compartimentalisation » de la selection

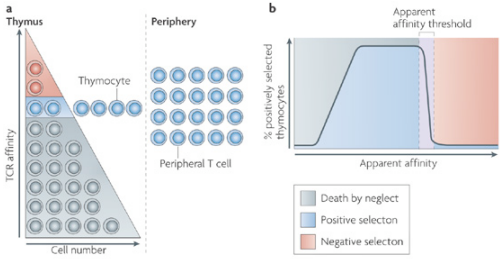


- Epithelial cells in the thymic cortex are the antigen-presenting cells (APCs) that mediate positive selection, whereas the medulla contains epithelial and dendritic cells (DCs) that are efficient APCs for negative selection. For these reasons, positive selection occurs in the cortex, whereas deletion 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.

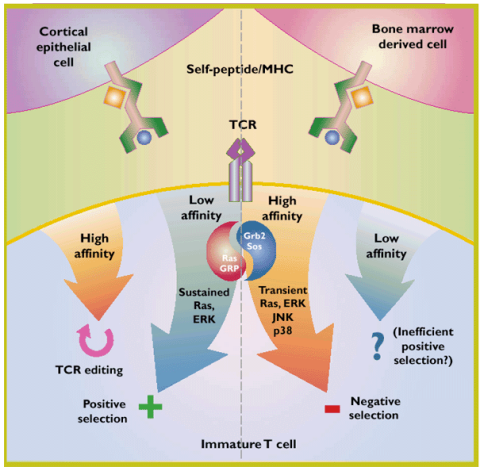
Modèle alternatif de la sélection: « une question d'affinité… »







Modèle intégré

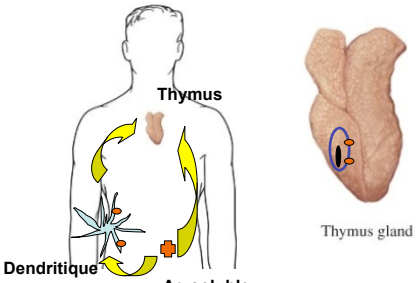


UPMC PARIS UNIVERSITAS
B. Bellier

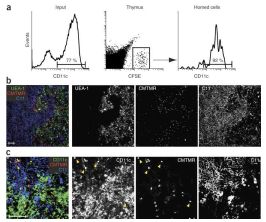
Présentation inrathymique des Ag du soi

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

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



Thymus gland

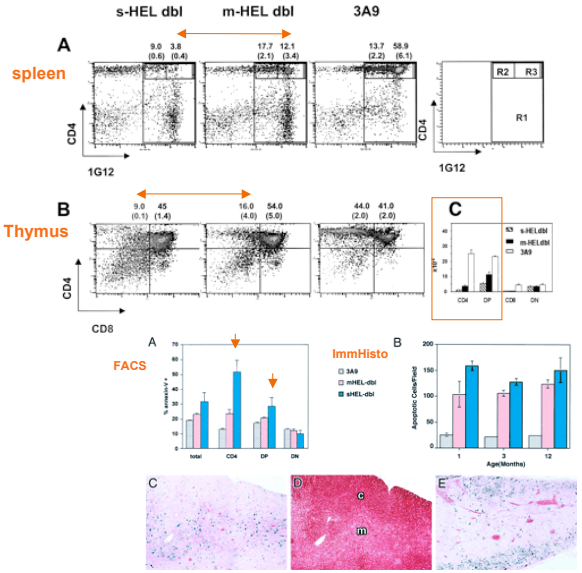


UPMC PARIS UNIVERSITAS
B. Bellier

Ag soluble versus membranaire

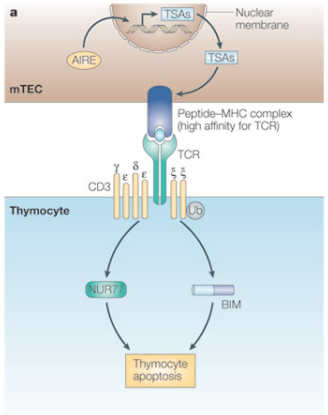
T Cell Tolerance to a Neo-Self Antigen Expressed by Thymic Epithelial Cells: The Soluble Form Is More Effective Than the Membrane-Bound Form
Zhang M, et al, J Immunol 2003

3A9 anti-HEL : TCR transgenic
s-HEL dbl: soluble HEL x 3A9
m-HEL: membrane-bound HEL x 3A9



UPMC PARIS UNIVERSITAS
B. Bellier

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

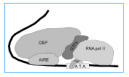
•Chez l'homme : Thymus, ganglions lymphatiques, rate, foie foetal
PBL, CD différenciées

•Thymique : une sous-population de cellules épithéliales médullaires

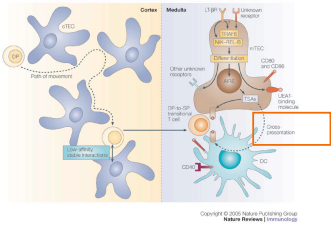
•Chez la souris : + moelle osseuse, tractus urinaire et génital, tractus respiratoire et digestif, cerveau, glandes endocrines

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, NFkB…

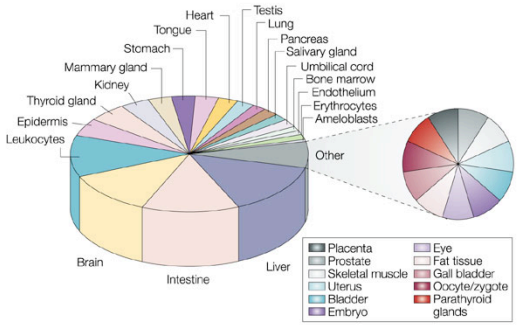


AIRE (homme&souris) sont de fort activateur transcriptionnel



UPMC PARIS UNIVERSITAS
B. Bellier

Spécificité tissulaire des gènes exprimés dans les mTECs



Nature Reviews | Immunology

- 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.

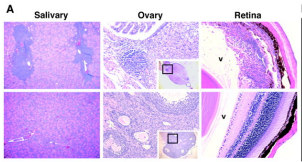
UPMC PARIS UNIVERSITAS
B. Bellier

Aire -/-

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

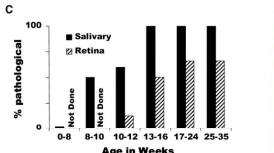
Mark S. Anderson, Science 2002

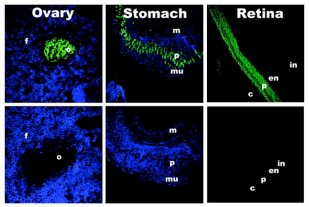
Aire-deficient mice develop autoimmunity.
(A) Tissue sections from aire-deficient mice from salivary gland, ovary and retina (knockout; top row) and wild-type (bottom row) mice
(B) Table summarizes presence (+) or absence (–) of lymphocytic infiltrates observed in knockout mice of various ages and both 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.

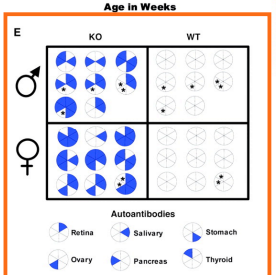


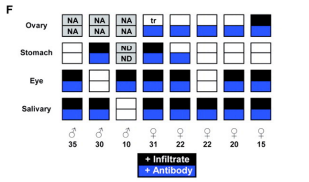
B

Sex	Age	Salivary	Reproduc.	Thyroid	Retina	Liver	Stomach
F	3	-	-	+	ND	-	-
M	4	-	-	-	ND	-	-
M	4	-	-	-	ND	-	-
F	9	+	-	-	ND	-	-
F	9	+	-	ND	ND	ND	ND
M	9	-	-	ND	-	ND	ND
M	10	tr	-	ND	+	ND	ND
M	15	+	-	-	ND	-	-
F	15	+	+	+	+	tr	-
F	15	+	-	-	-	tr	-
F	22	+	-	NA	+	tr	-
M	30	+	+	NA	-	+	+
F	31	+	tr	-	-	+	+
M	35	+	+	NA	+	+	-







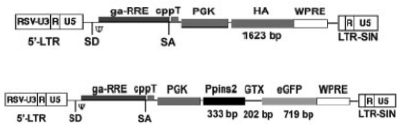


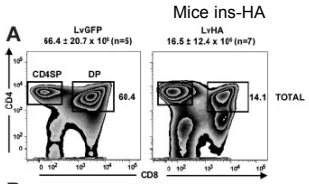
UPMC PARIS UNIVERSITAS
B. Bellier

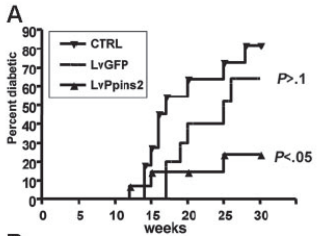
Tolérance centrale et Immunointerventions

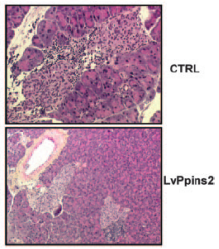
Induction of antigen-specific tolerance by intrathymic injection of lentiviral vectors

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







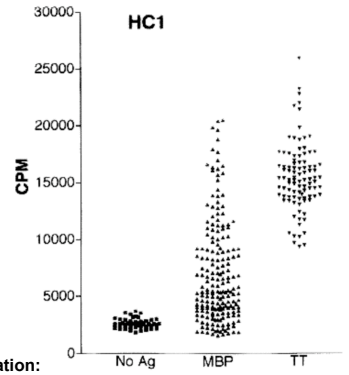


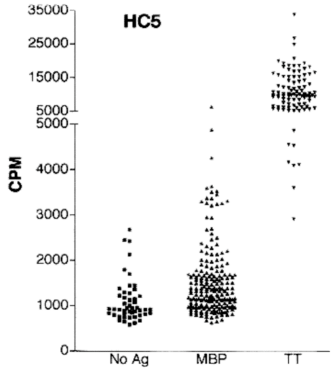
UPMC PARIS UNIVERSITAS
B. Bellier

Présence de lymphocytes autoréactifs en périphérie: un danger potentiel

UPMC PARIS UNIVERSITAS
B. Bellier

Reconnaissance de la MBP chez des individus sains





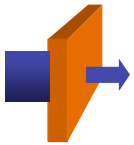
Stimulation:

Lovett-Racke *et al.* 1998

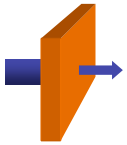
Il existe donc dans le sang des sujets sains des lymphocytes T dont le TCR reconnaît des peptides d'une **protéine de la myéline**

…mais absence de réponse des clones auto-réactives

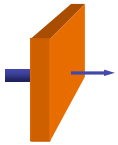
Etapes limitantes au déclenchement des réponses:



***Percevoir* le signal**



Proliférer* et *se différencier



***Atteindre* le tissu cible**

R E P O N S E

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

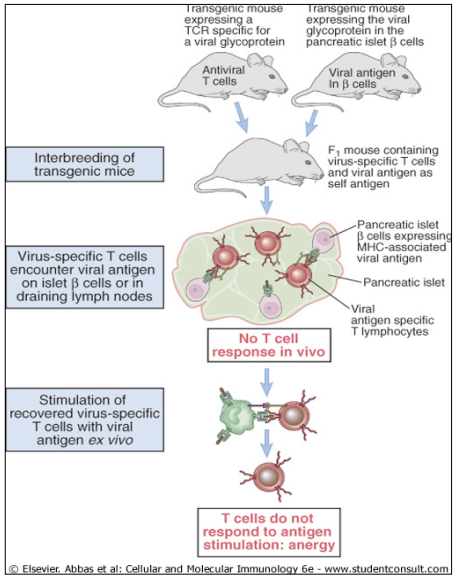
- Contrôle des réponses inflammatoires (rôle des cytokines)
- Populations suppressives
  - **LyT régulateurs**

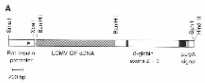
***Atteindre* le tissu cible**

- Accès limité aux tissus

REPONSE

Non-reconnaissance des Ag du soi « ignorance »





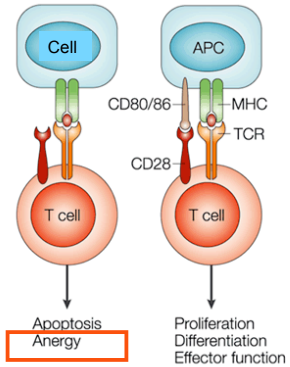
Ignorance

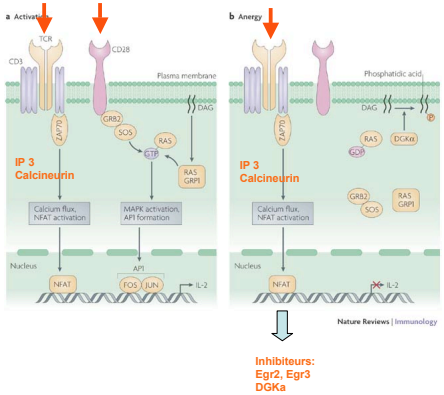
Anergie

UPMC PARIS UNIVERSITAS
B. Bellier

Ignorance des Ag tissulaires & Anergie

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



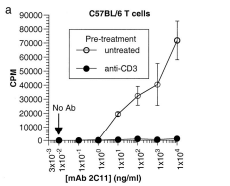


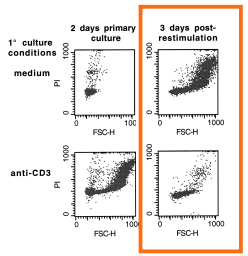
Inhibiteurs:
Egr2, Egr3
DGKa

Nature Reviews | Immunology

UPMC PARIS UNIVERSITAS
B. Bellier

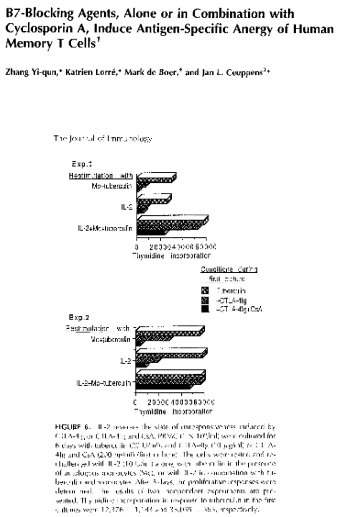
Anergie: Un état de non-réponse réversible par l'IL-2





Anergized cells enter cell cycle, but arrest in G_1 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.

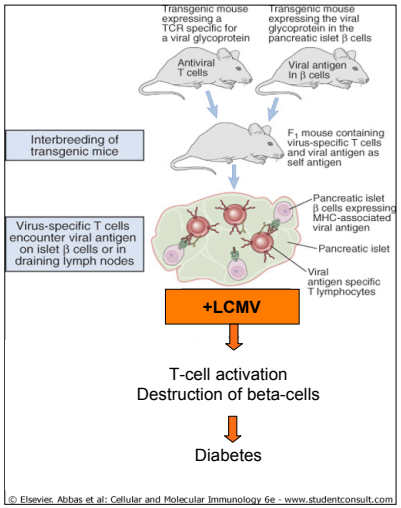

B7-Blocking Agents, Alone or in Combination with Cyclosporin A, Induce Antigen-Specific Anergy of Human Memory T Cells†

Zhang Yi-qun,* Katrien Lorré,* Mark de Boer,* and Jan L. Ceuppens*,+

UPMC PARIS UNIVERSITAS
B. Bellier

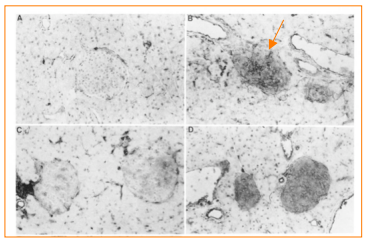
Rupture de la tolérance aux Ag tissulaires

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.

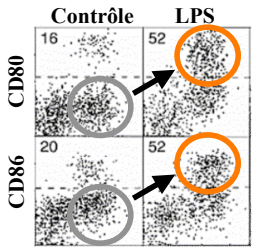


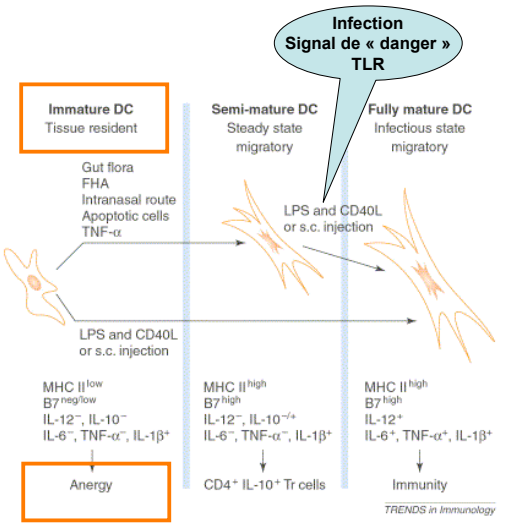
UPMC PARIS UNIVERSITAS
B. Bellier

Facteurs tolérogènes dans les tissus: DC immatures

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

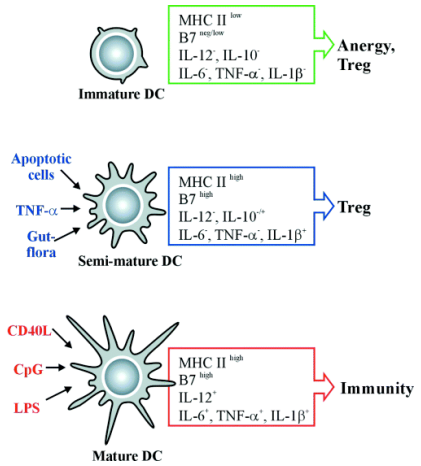
= Ignorance





UPMC PARIS UNIVERSITAS
B. Bellier

Rupture de Tolérance au Soi lors d'infections
Théorie du danger

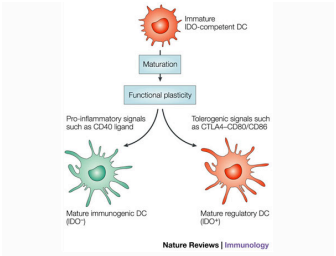


- **Activation due aux** PAMPs **(Pathogen Associated Molecular Patterns), reconnues par les** PRRs**, Pattern Recognition Receptors, présents à la surface des cellules du syst imm inné.**
  - les lipopolysaccharides (LPS) des bactéries Gram-
  - les acides lipotéichoiques, le peptidoglycane des bactéries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

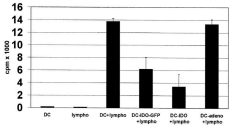
UPMC PARIS UNIVERSITAS
B. Bellier

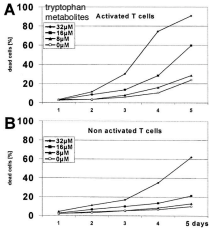
DC immatures & DC tolérogènes

- **DC immatures**
  - CMHIo 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





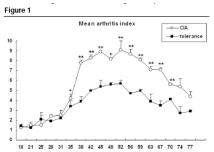
UPMC PARIS UNIVERSITAS
B. Bellier

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 Cho[1], Mi-La Cho[1], Young-Ok Jung[1], Hyun-Sil Park[1], Soog-Hee Chang[1], Baek Goo Cho[3], Jun-Ki Min[1,4], Sung-Hwan Park[1,2] and Ho-Youn Kim[1,2]

Model: Arthritis induction

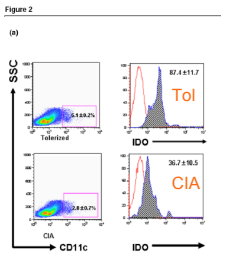
Figure 1



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.

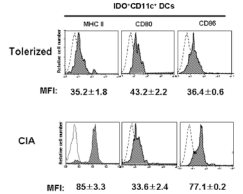
Expression of IDO in Payer's Patches DC

Figure 2



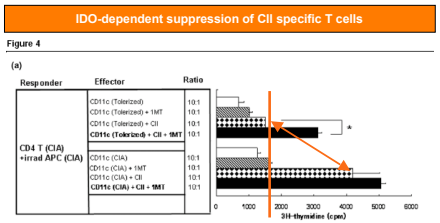
Immature phenotype of DC

Figure 3



IDO-dependent suppression of CII specific T cells

Figure 4



UPMC PARIS UNIVERSITAS
B. Bellier

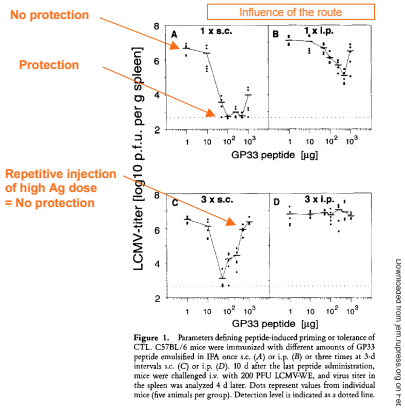
Et en cas de réponse, …

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



Downloaded from jem.rupress.org on the

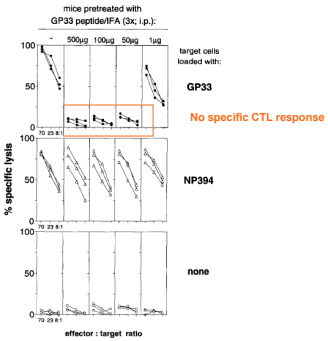
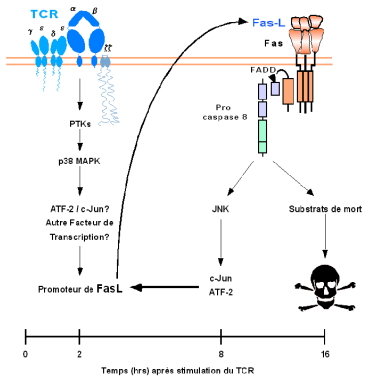


Figure 1. Parameters defining peptide-induced priming or tolerance of CTL. C57BL/6 mice were immunized with different amounts of GP33 peptide emulsified in IFA once s.c. (A) or i.p. (B) or three times at 3-d intervals s.c. (C) or i.p. (D). 10 d after the last peptide administration, mice were challenged 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^{-5} M GP33 peptide (●) or with 10^{-4} M NP394 peptide (△) or left untreated (○).

Mécanisme d'AICD



- 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.

Déletion clonale
Mécanisme actif de tolérance périphérique

- 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

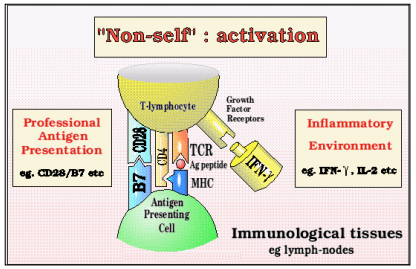
- Contrôle des réponses inflammatoires (rôle des cytokines)
- Populations suppressives
  - **LyT régulateurs**

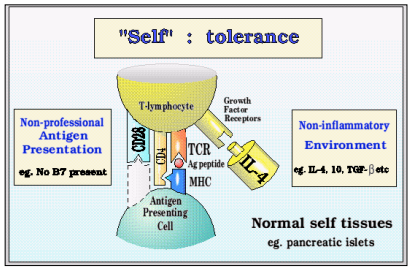
***Atteindre* le tissu cible**

- Accès limité aux tissus
- Sites immunoprivilégiés

REPONSE

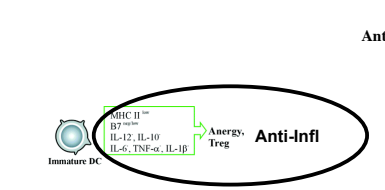
Cytokine: le 3eme signal d'activation

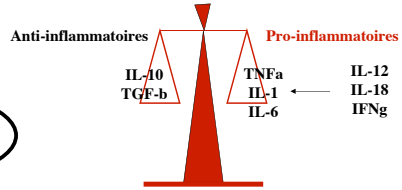




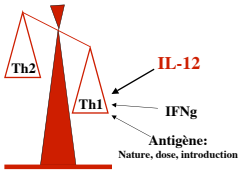
UPMC PARIS UNIVERSITAS
B. Bellier

Cytokines
Pro-inflammatoire / Immunosuppressive





Hypersensibilité de type IV
Maladies auto-immunnes



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

UPMC PARIS UNIVERSITAS
B. Bellier

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 .

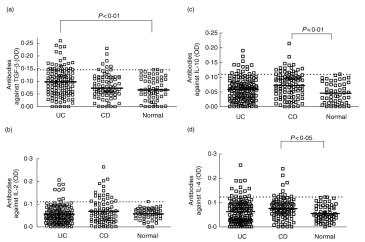
IL-10 : Under some circumstances, IL-10 is protective; in EAE. By contrast, diabetes is exacerbated following increased pancreatic expression of IL-10 in a transgenic model

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

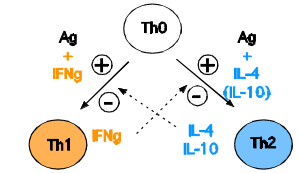
Patients with inflammatory bowel disease may have a transforming growth factor-_, interleukin (IL)-2- or IL-10-deficient state induced by intrinsic neutralizing antibodiesE. C. Ebert, Clin Exp Immunol. 2009

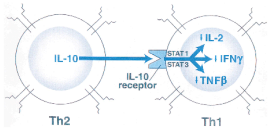


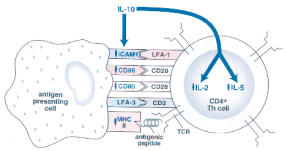
UPMC PARIS UNIVERSITAS
B. Bellier

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







UPMC PARIS UNIVERSITAS
B. Bellier

IL-10 : cytokine immunosuppressive

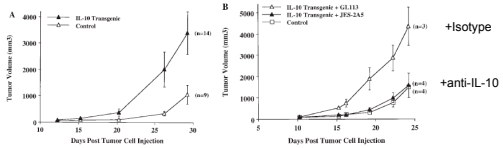
Altered Immune Responses in Interleukin 10 Transgenic Mice
By Amy Hagenbaugh,*‡ Sherven Sharma,§ Steven M. Dubinett,§ Sherry H.-Y. Wei,§‖ Richard Aranda,§ Hilde Cheroutre,‡ Deborah J. Fowell,** Scott Binder,‡‡ Betty Tsao,* Richard M. Locksley,** Kevin W. Moore,§‖ and Mitchell Kronenberg*‡‖

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 • 10^8	9.8	2.6	83.7	–
Control No. 2	1.2 • 10^8	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 6 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



+Isotype

+anti-IL-10

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 • 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, IES-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 • SE.

Colitis:

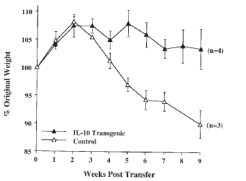
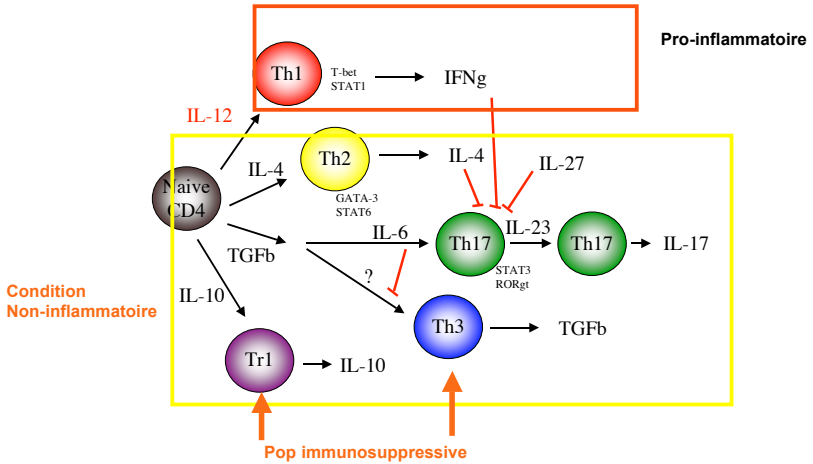


Figure 3. CD4+ CD45RB^{high} cells from IL-10 transgenic mice fail to induce colitis in SCID recipients. 4–5 • 10^5 FACS+ sorted CD4+ CD45RB^{high} cells from IL-10 transgenic or control littermate mice were injected into SCID mice. Weight gain/loss was scored weekly for 9 wk. Values are shown as a percentage 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.)

UPMC PARIS UNIVERSITAS
B. Bellier

Déviation immunitaire et tolérance

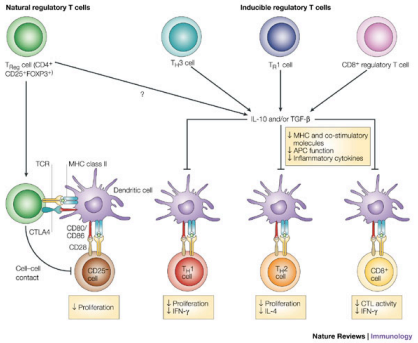
Environnement cytokinique est déterminant lors de l'activation lymphocytaire
>> Différenciation Th1, Th2, TH3, Th17, ….



UPMC PARIS UNIVERSITAS
B. Bellier

Lymphocytes T suppresseurs

	Th1	Th2	Treg naturels Th3	Tr1	CD25+(hi/gh)
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	+/–	+/–	+	+ ?	+++++



- **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.

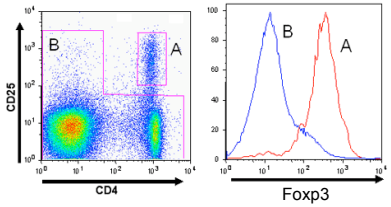
- **Tr1**

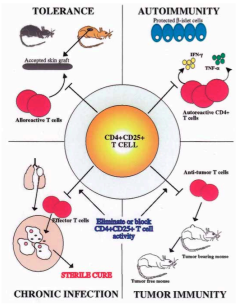
La production de taux élevés d'IL-10
suppriment la réponse immune par contacts cellulaires directs et/ou par sécrétion de cytokines.
faible capacité à proliférer (du fait des hautes concentrations d'IL-10.
se différencient après contacts avec des cellules présentatrices synthétisant de grandes quantités 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*.

UPMC PARIS UNIVERSITAS
B. Bellier

Lymphocytes T régulateurs CD4+ CD25+

- **Ly T régulateurs:**
  - (re)découverts par Sakagushi
  - Rôle dans maintien de la tolérance périphérique
  - Prévient 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

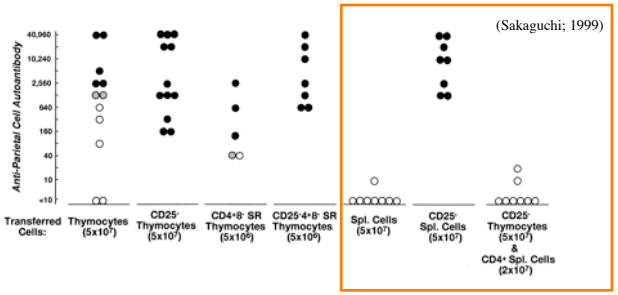




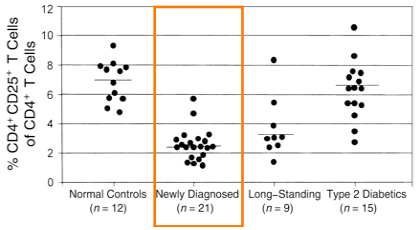
UPMC PARIS UNIVERSITAS
B. Bellier

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

- Chez la souris nude après transfert adoptif



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



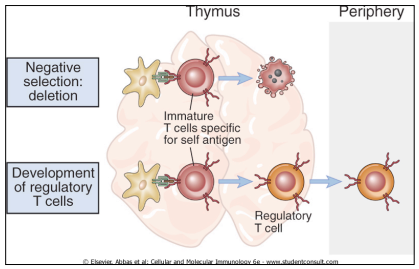
UPMC PARIS UNIVERSITAS
B. Bellier

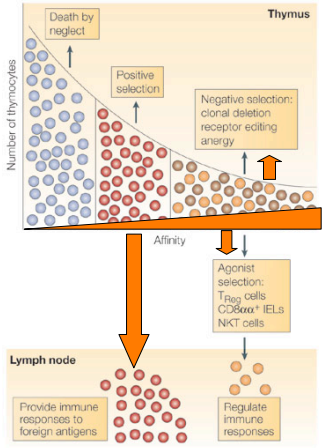
Ontogénie et Spécificité des Treg

-Treg reconnaissent le soi

-Différenciation dans le thymus après contact avec Ag du soi

-Affinité critique pour CMH-Ag_{soi}



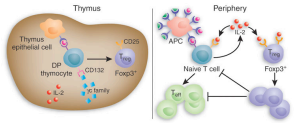


Copyright © 2005 Nature Publishing Group
Nature Reviews | Immunology

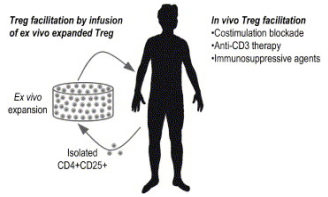
UPMC PARIS UNIVERSITAS
B. Bellier

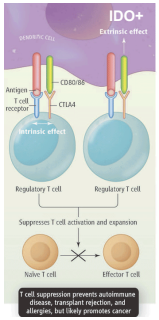
Treg: nouvel outil thérapeutique

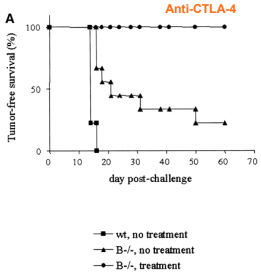
- **Suppression**
  - Modification du ratio Teff: Treg
    - Expansion in vitro et injection
    - Expansion in vivo
      - Rôle de l'IL-2



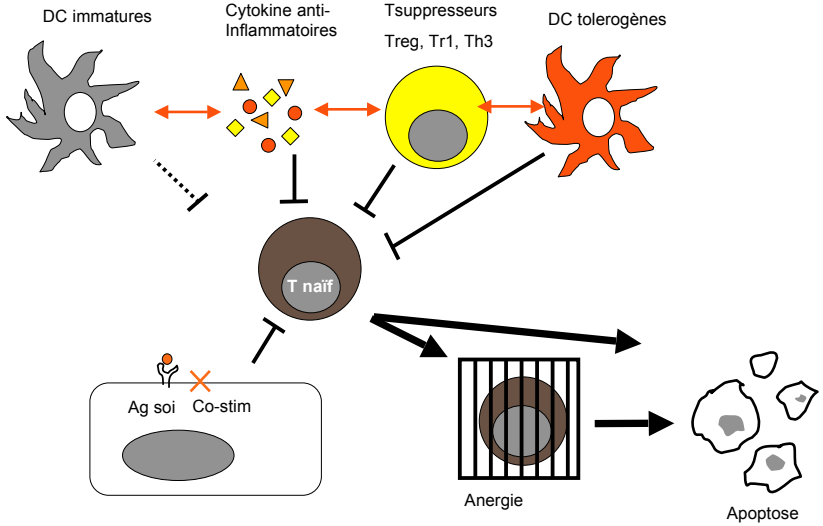
- **Activation:**
  - Déplétion des Treg :
    Anticorps monoclonaux anti-CD25
  - Bloque l'activation des Treg
    - Anti-CTLA4







UPMC PARIS UNIVERSITAS
B. Bellier



UPMC PARIS UNIVERSITAS
B. Bellier

Les mécanismes périphériques de contrôle des LyT auto-réactifs

***Percevoir* le signal**

- 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**

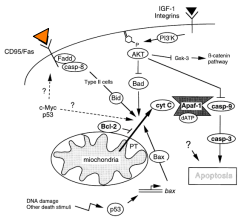
- Accès limité aux tissus

REPONSE

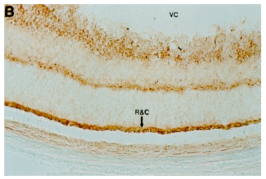
Tissus « immunoprivilégiés »

- **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

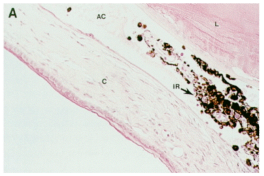


Oeil

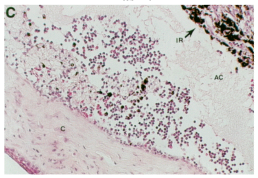


Infiltration lymphocytaire en réponse à une inoculation virale dans la chambre antérieure

WT



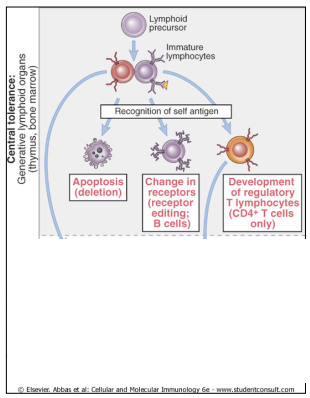
FasL-/-

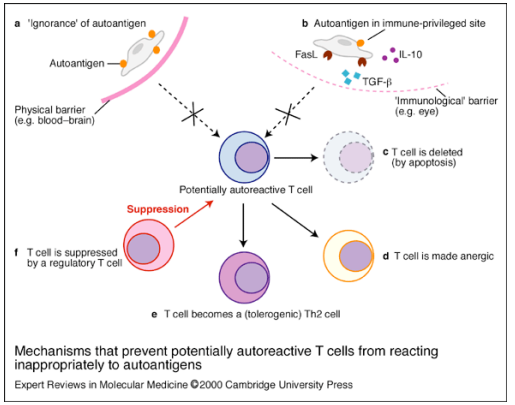


Ferguson *et al.* 1997

Conclusion
Mécanismes à l'origine de la tolérance

Tolérance centrale	Tolérance périphérique

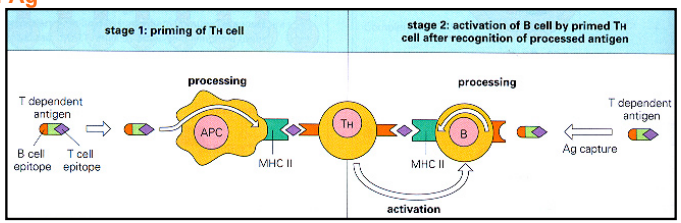




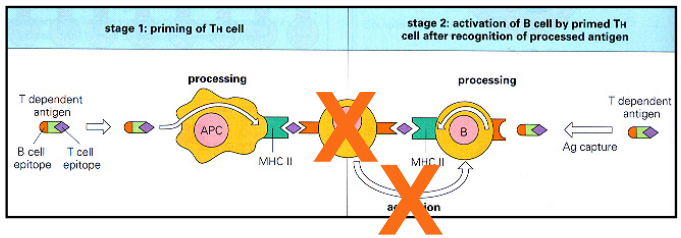
Importance de la tolérance T pour maintenir la tolérance B

B cells need T cell "help" to make high affinity antibody

Non-self Ag

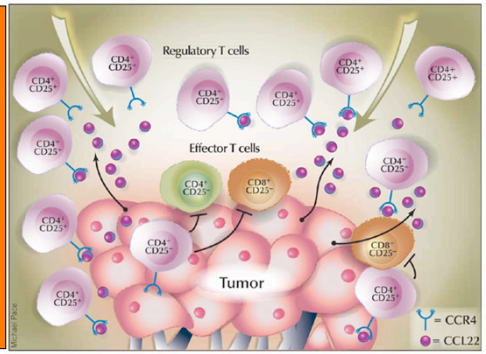


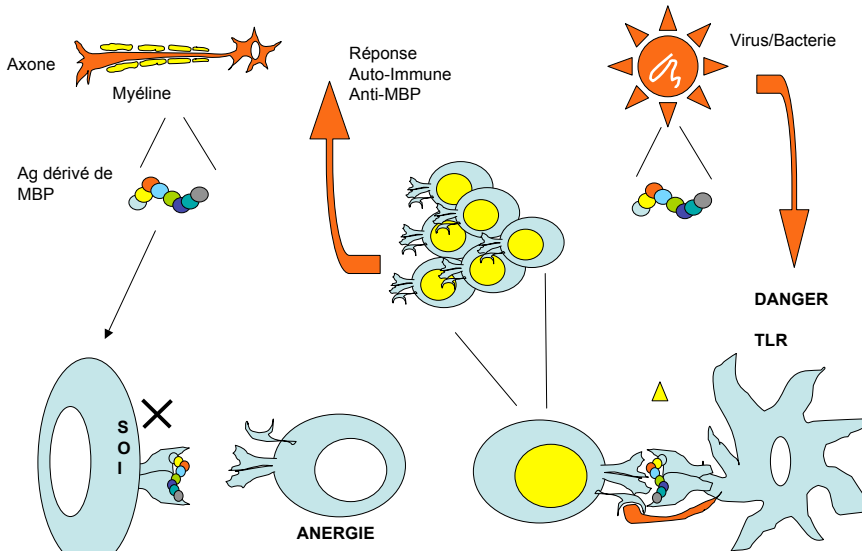
Self Ag



Difficulté de l'établissement des réponses anti-tumorales
Tumeur = soi modifié

Ignorance
Anergie
Déviation immunitaire
Suppression
Treg
Echappement



UPMC PARIS UNIVERSITAS
B. Bellier

Développement des maladies auto-immunes
Théorie du mimétisme moléculaire