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T cell Tolerance: an overview

Immunity and Infection BSc.
Module 3

Dr Nicola Rogers
January 3rd 2013

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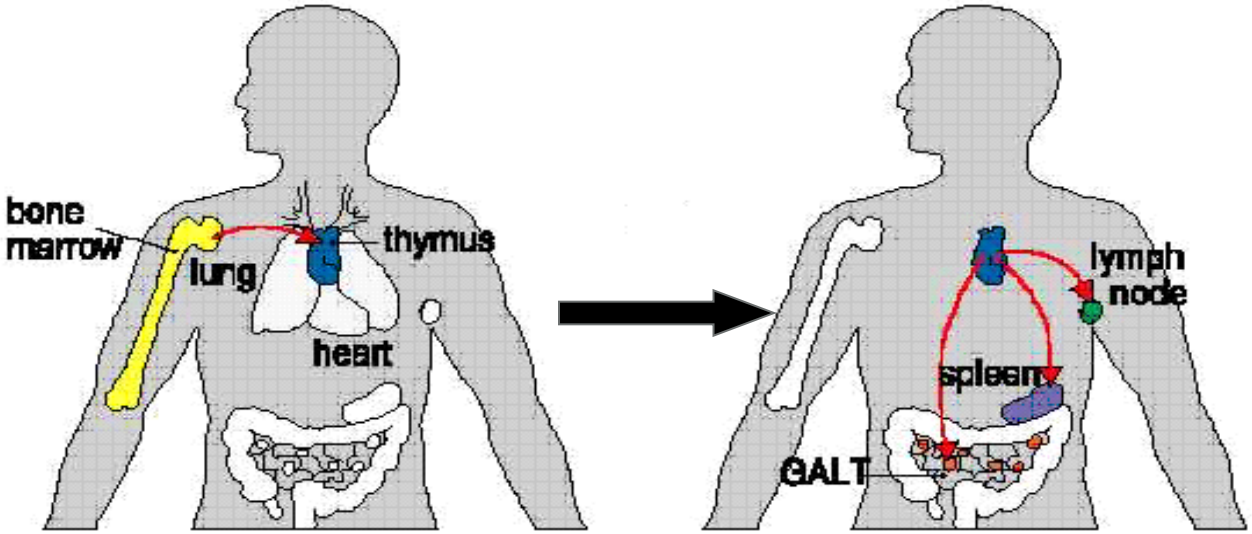
Autoreactive T cells are present in normal immune system



- Originally believed that T cells developing in the thymus are deleted if they recognise “self” – thus preventing autoimmunity
- Thymic negative selection is not 100%

Healthy individuals

Whilst many autoreactive cells are deleted, numerous self-reactive T cells are present in healthy subjects.

Eg: MBP-specific $CD4^+$ in healthy individuals as well as MS patients

- activation status different

Eg: Up to 30% of population thought to carry self-reactive antibodies

- <1% develop SLE

Breakdown in tolerance - consequences

Failure of these tolerance mechanisms may result in a response to:

- Innocuous non-self antigens
  - » Allergy

- Self
  - » Autoimmunity

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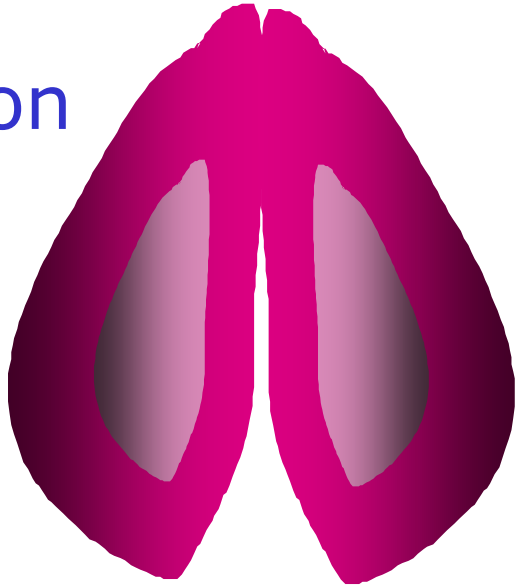
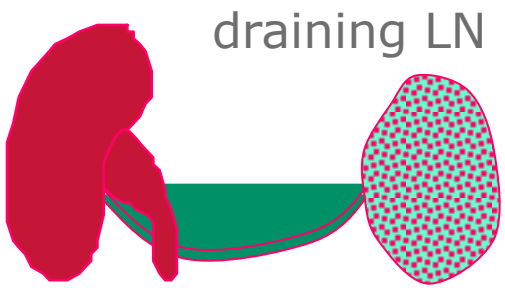
Central and Peripheral tolerance

Central - due to thymic deletion



Peripheral - due to mechanisms operative in lymphoid and peripheral tissues



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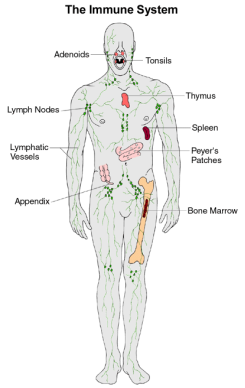
Tolerance mechanisms

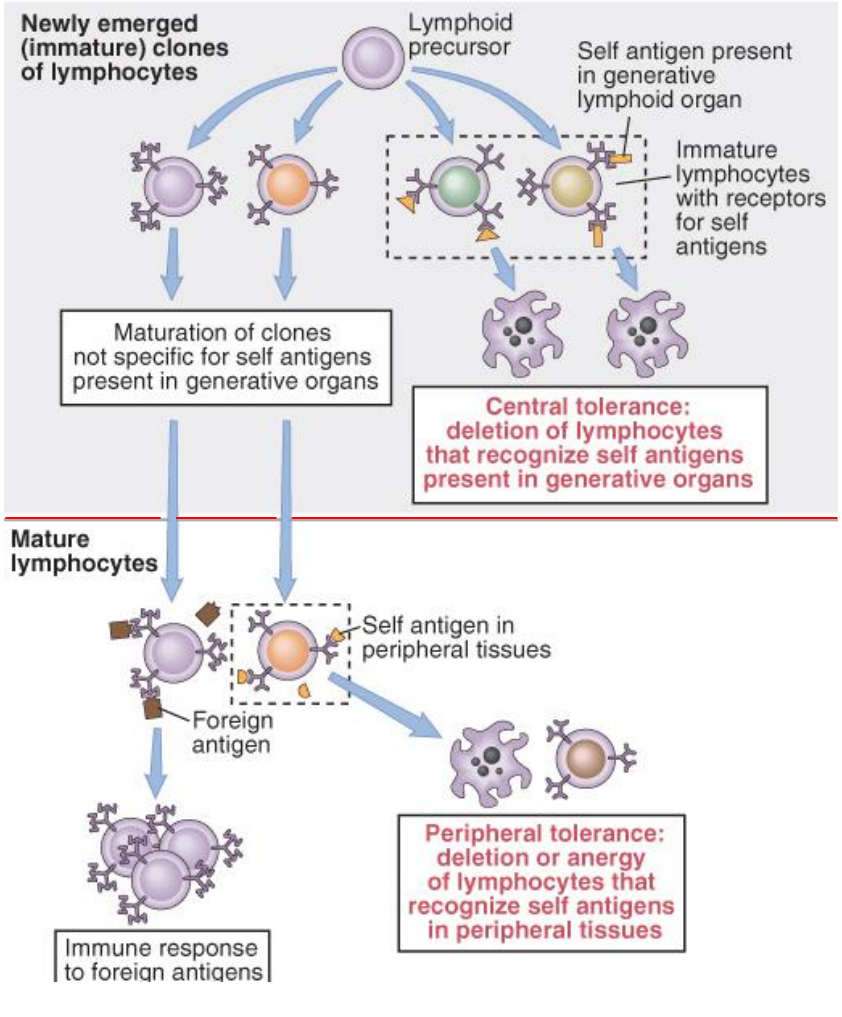
Central

- Thymic deletion
- Expression of Aire

Peripheral

-Anergy
-Deletion
-Ignorance
-Regulation





Mechanisms of central tolerance

What is central T cell tolerance?

- Deletion of T cells that recognise self-antigen in the thymus

How is it achieved?

- Thymic selection/deletion
- Aire

T cell repertoire selection

- Each T cell creates its own TCR by unique gene rearrangements
- Development of TCR repertoire is independent of antigen.
- Therefore some TCR will be:
  - strongly self-MHC/p reactive (high avidity)
  - unable to bind to self MHC/p (very low/no avidity)

So:

- How is MHC restriction guaranteed?
- How is autoimmunity controlled?

T cell repertoire selection

- Thymocytes undergo three selection processes in the thymus
  - β-selection
  - Positive selection
  - Negative selection

T cell repertoire selection

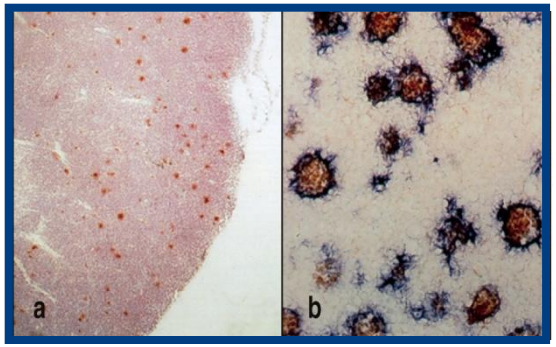
- Thymocytes undergo three selection processes in the thymus
  - β-selection
    - »Pre-TCR check point
    - »Is the β chain functional?
  - Positive selection
    - »self restriction
  - Negative selection
    - »Eliminates “danger”

β-Repertoire selection

Pre TCR checkpoint (β selection):

Is the new β chain functional?

YES NO ➔

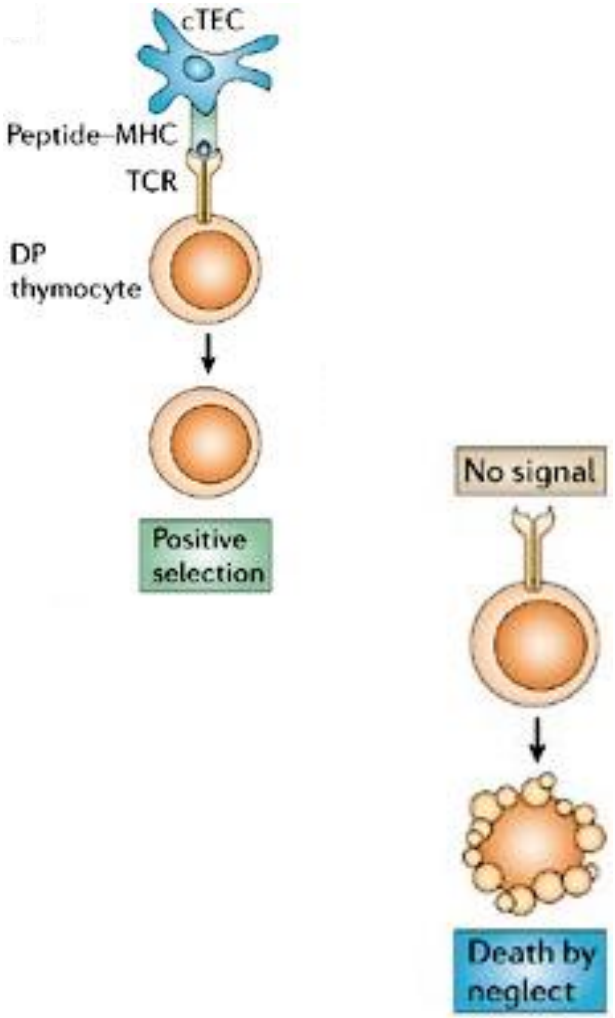


- cell survival
- cell division
- TcRα gene rearrangement
- differentiation to $CD4^+8^+TCR\alpha\beta^+$

Death by apoptosis

Positive Selection

- Selection for thymocytes bearing receptors capable of binding self-MHC molecules which results in Self MHC-restriction
- Selected for “usefulness”
- Cells that fail positive selection are eliminated within the thymus, they die by neglect



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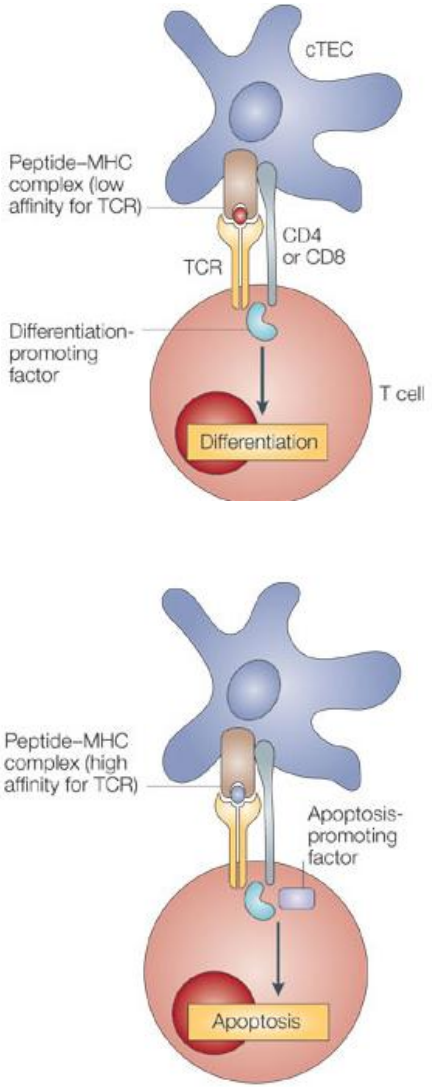
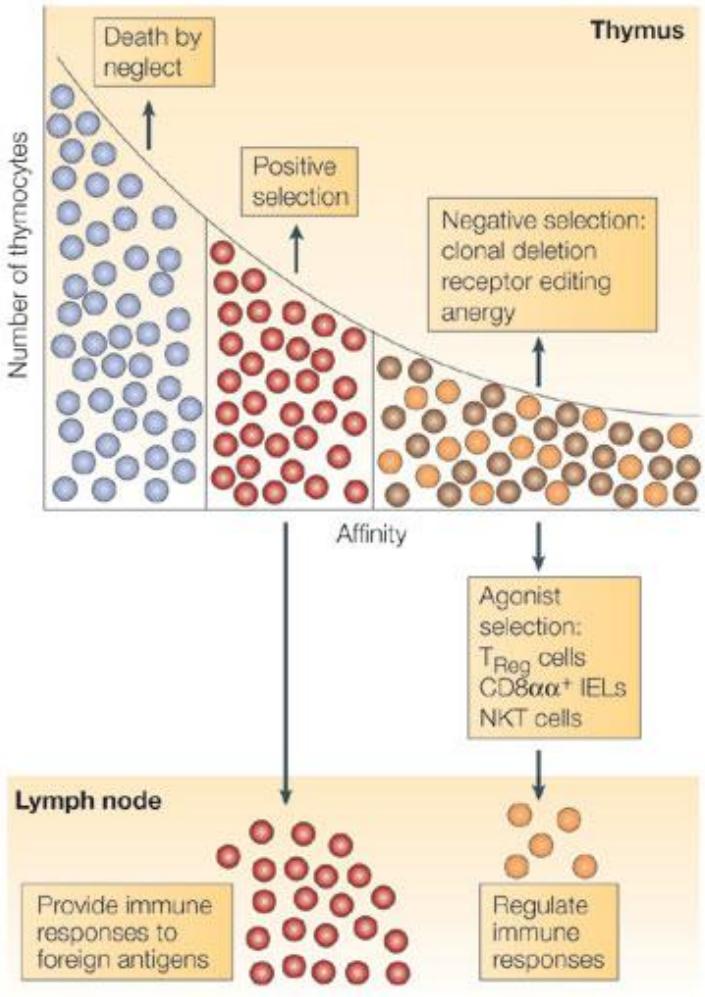
Negative Selection

- Selection that eliminates thymocytes bearing high-affinity receptors for self-MHC molecules alone or self-antigen presented by self MHC
- Selects against “danger” ie: autoreactivity
- Results in Self tolerance
- Results in Central tolerance



Repertoire Selection- summary



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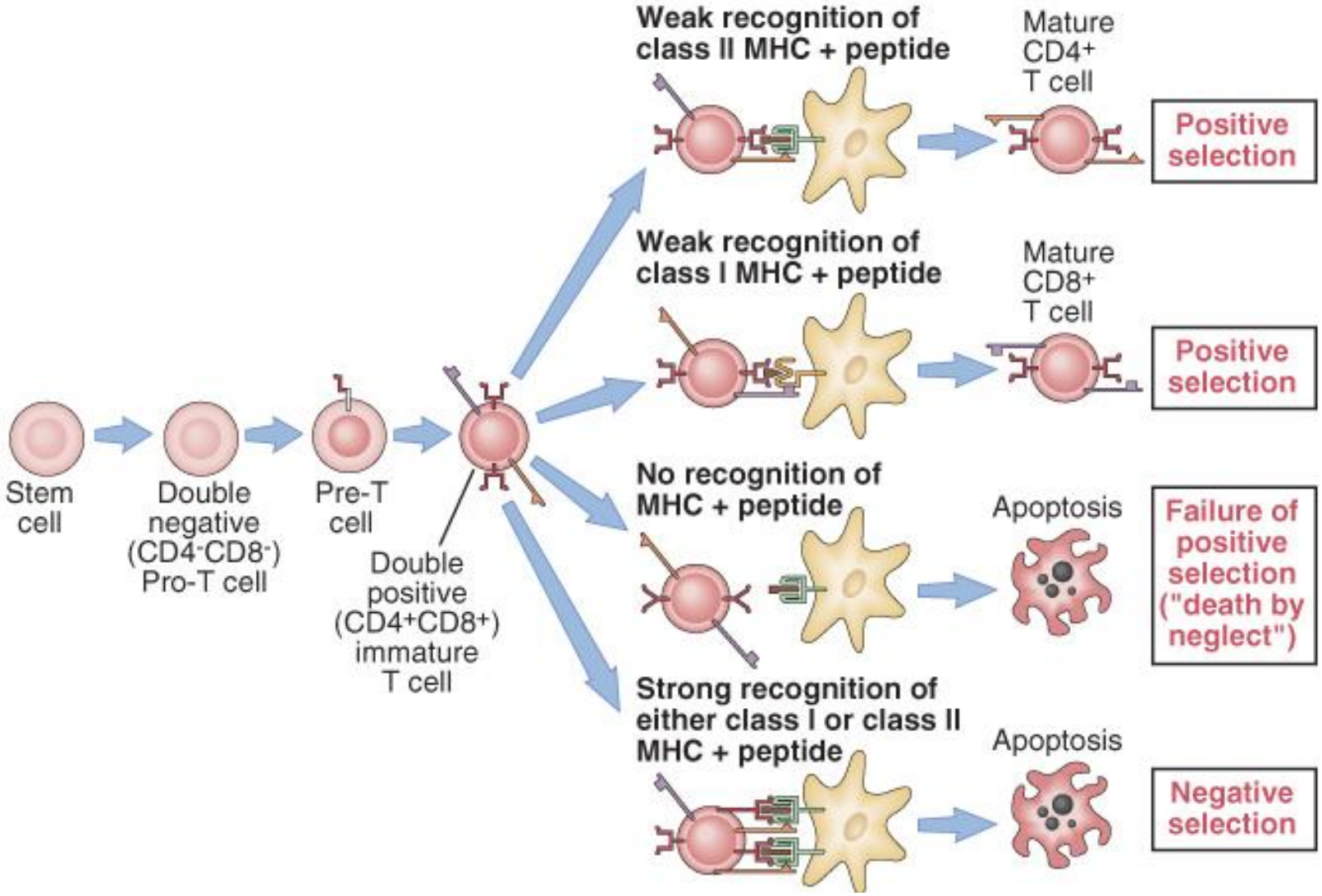
Only 5% of thymocytes survive selection



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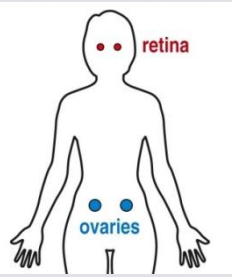
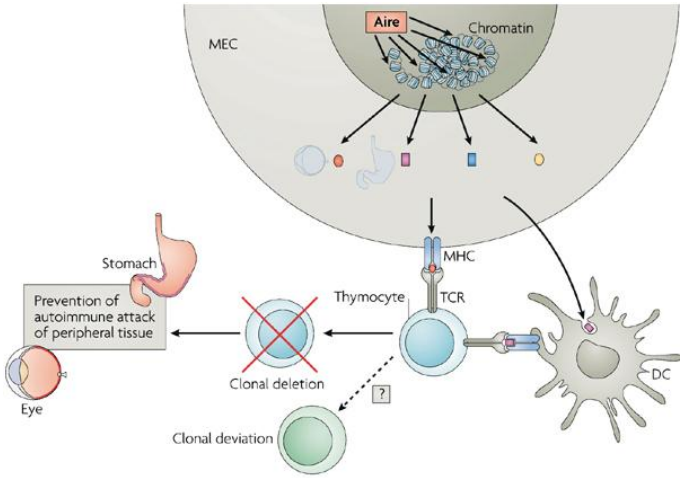
AIRE



- *Aire*: Autoimmune regulatory gene
  - Aire$^{-/-}$ mice

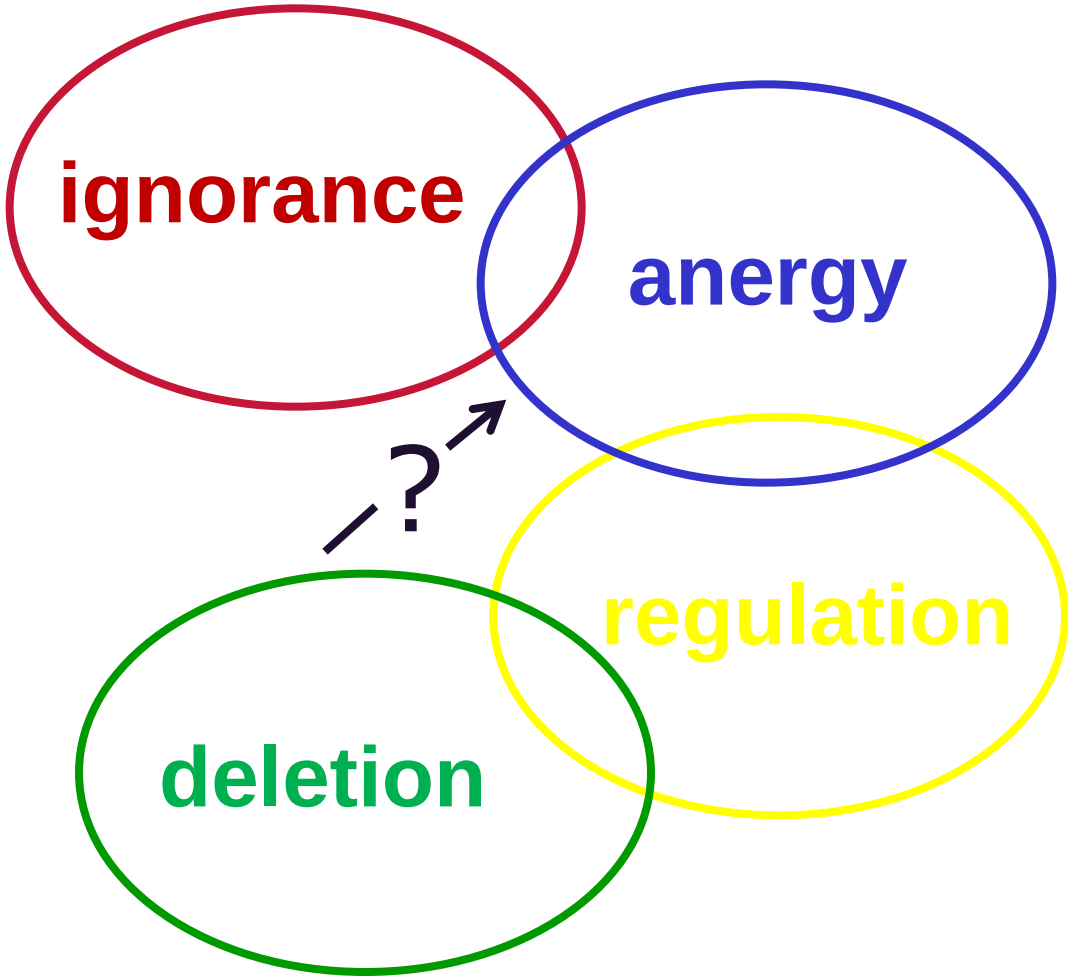
- Regulates the presentation of peripheral self-antigen in the thymus
  - tissue-specific antigens
  - developmental antigens



- APECED (autoimmune polyendochrinopathy, candidiasis-ectodermal dystophy) ; APS-1

Perspective: A decade of Aire. *Nature Reviews Immunology* 7, 645-650 (August 2007)

Mechanisms of peripheral tolerance



Deletion

T cells specific for self-antigen are deleted

- Chronic/persistant stimulation with self-antigen
- High/excessive dose of self-antigen
- Mechanisms:
  - Active: Fas-FasL interaction
  - Passive: cytokine (IL-2) deprivation

Anergy

"a state of long-lasting, partial or total unresponsiveness induced by partial activation"

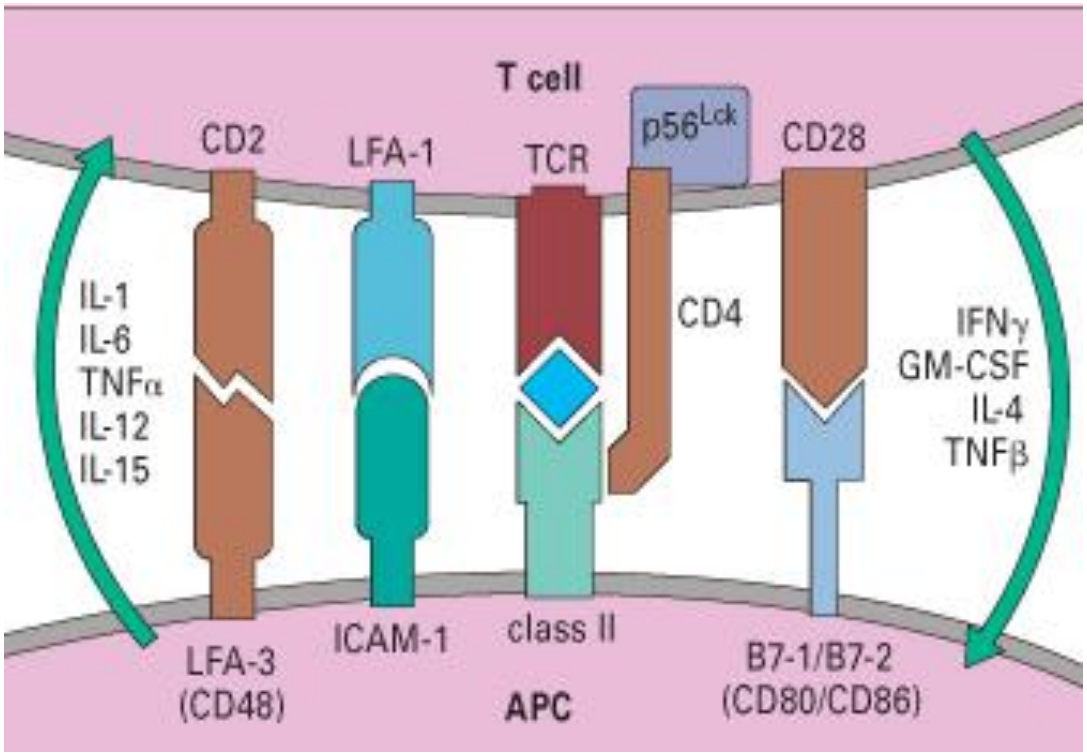
Induced by:

- Ag recognition (signal 1) in the absence of costimulation (signal 2)
  - Eg: CD80/86
- Ligation of co-inhibitors
  - Eg: CTLA-4
- Full signalling but without division

- *In vitro*: well defined (T cell clones)
- *In vivo*: difficult to distinguish from deletion/exhaustion

T cell: APC interaction

Molecular interactions during T cell activation
1: Cognate TCR:MHC/peptide (signal 1)
2: Costimulation (signal 2)
3: Adhesion
4: IL-2



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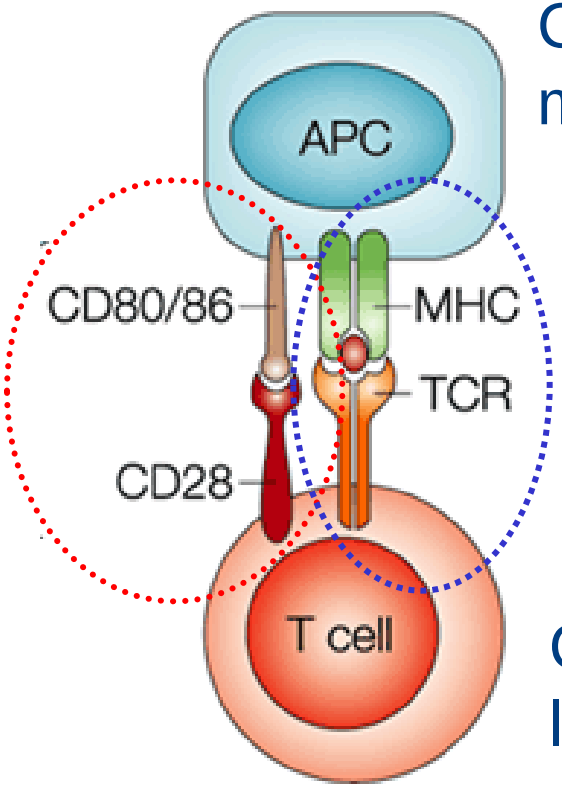
Signal 1 and Signal 2

Signal 2
Costimulation



Costimulatory molecule

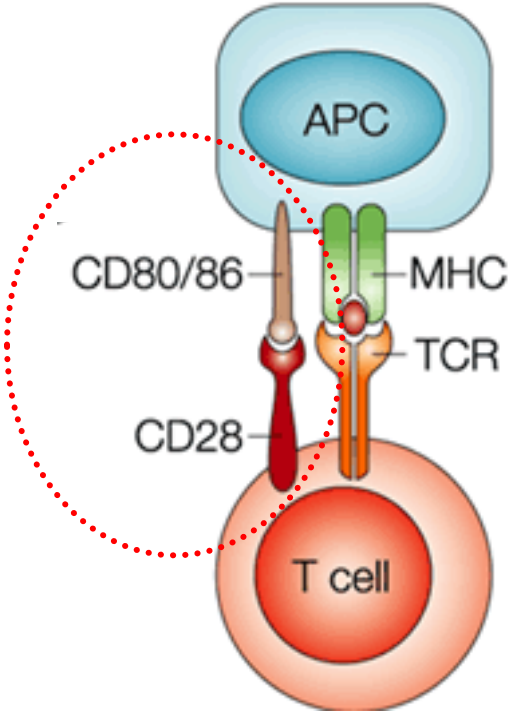
Signal 1

Costimulatory ligand

[1]

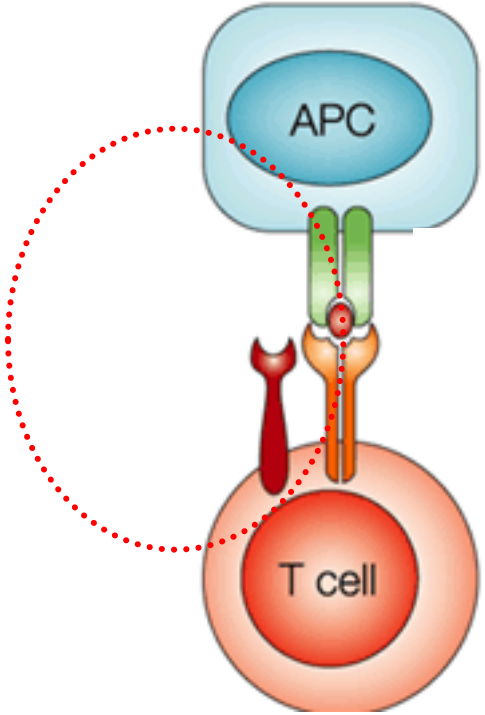
Recognition without costimulation

Signal 1 + Signal 2



Proliferation
Differentiation
Effector Function

No Signal 2



Cell dies or
ignores antigen

[1]

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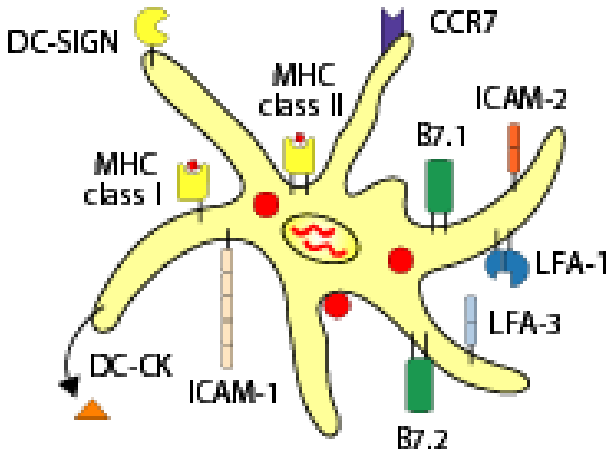
Absence of costimulation = Tolerance

The type of antigen presentation that occurs determines the outcome of activation or tolerance:

- Costimulation +ve cells:
  - mature DC
  - Macrophages
  - Activated B cells



- Costimulation –ve cells:
  - Fibrobalsts
  - Epithelial cells
  - Immature DC (low level expression NOT -ve)

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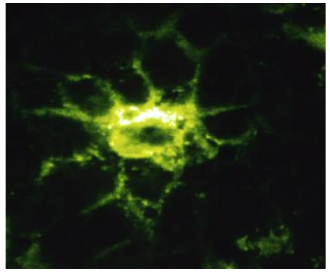
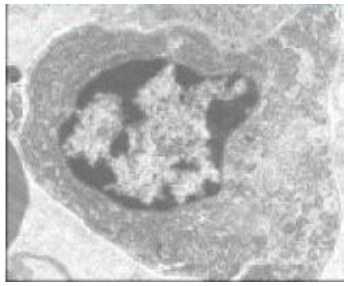
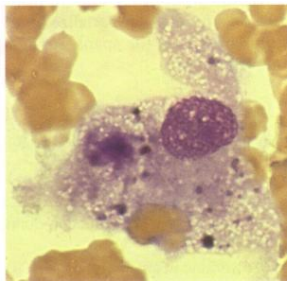
Conventional APC



DC



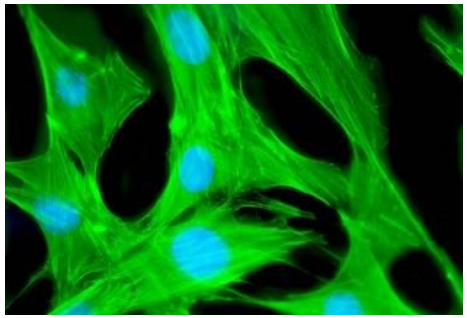
B cell



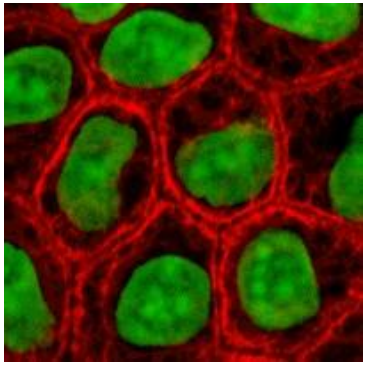
Macrophage

- Undergo maturation
- Constitutively express MHC class II molecules and CD80
- Induce T cell activation when mature
- Can induce T cell inactivation when immature

Non-conventional APC



Fibroblasts



Epithelial cells

- Fully differentiated
- MHC class II induced by inflammation
- Induce T cell in-activation
  - Role in peripheral tolerance to self antigen

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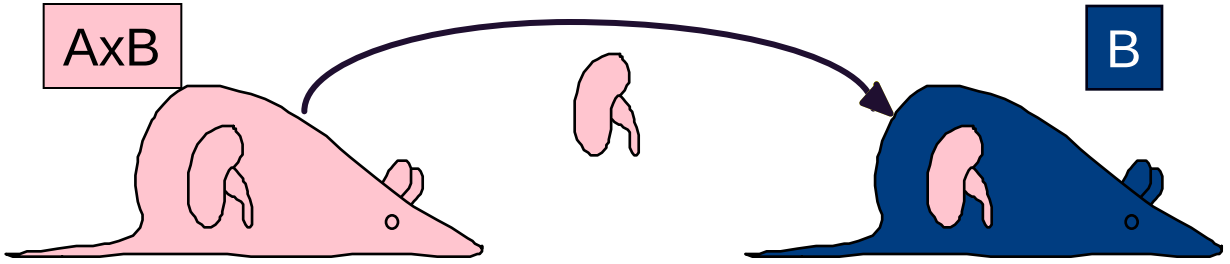
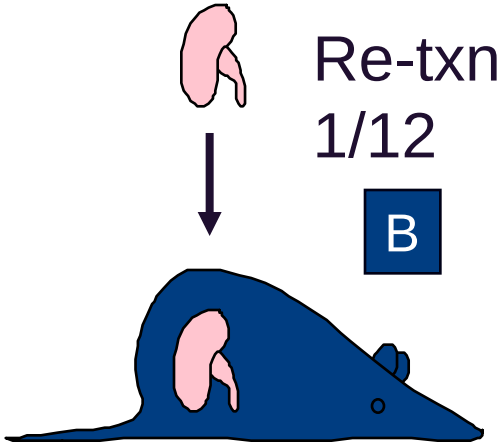
The rat model

Immunosuppression



The graft is not rejected:
the graft is fully antigenic
but is not immunogenic
(allo-MHC express by
epithelial cells)



no immunosuppression
no rejection

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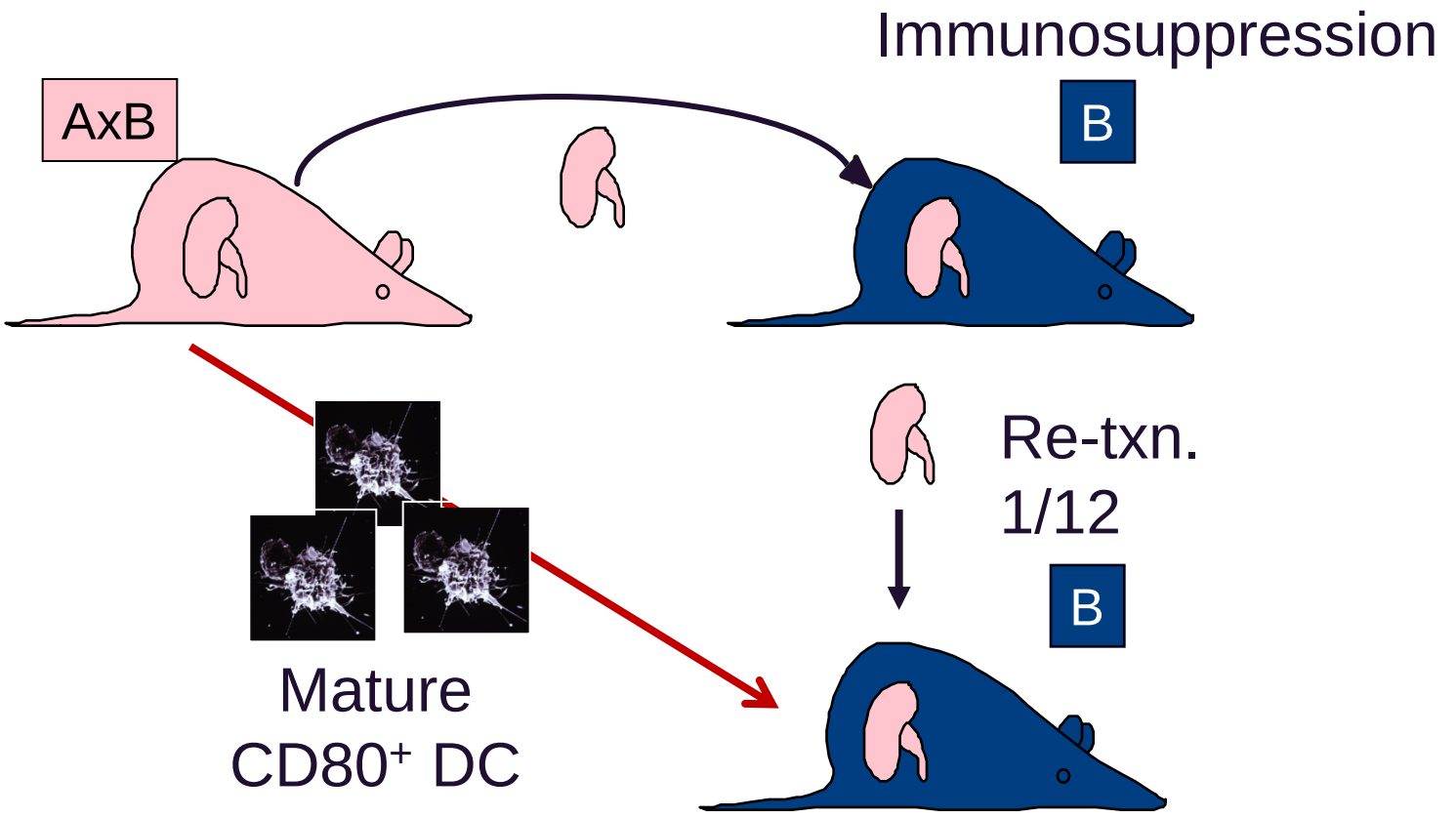
The rat model



The graft is immediately rejected!

Lechler & Batchelor 1982

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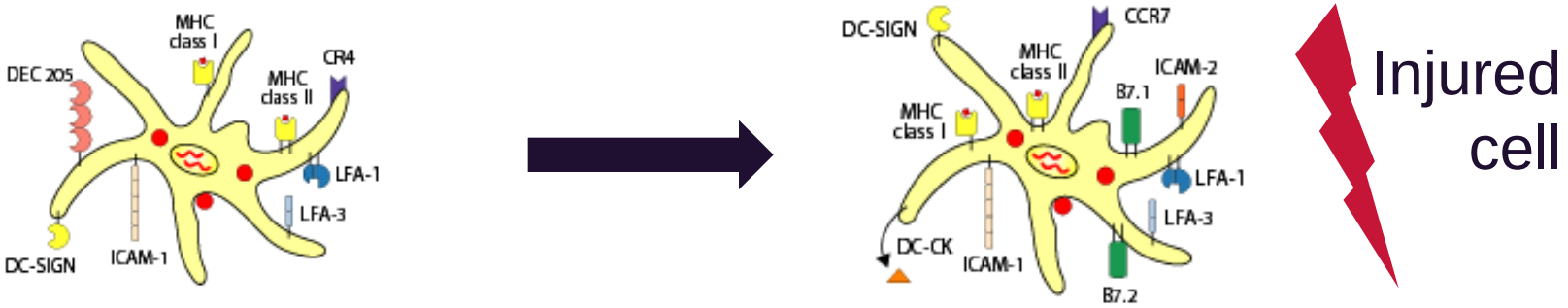
The “danger” hypothesis



Immature DC
Signal 1 only

Mature DC
Signals 1 & 2

- The immune system is activated when an antigen is associated with ‘danger/harm’
  - danger/harm results in DC maturation
- Activation state of DCs (immature vs mature) determines whether outcome is clonal expansion and effector function or abortive response

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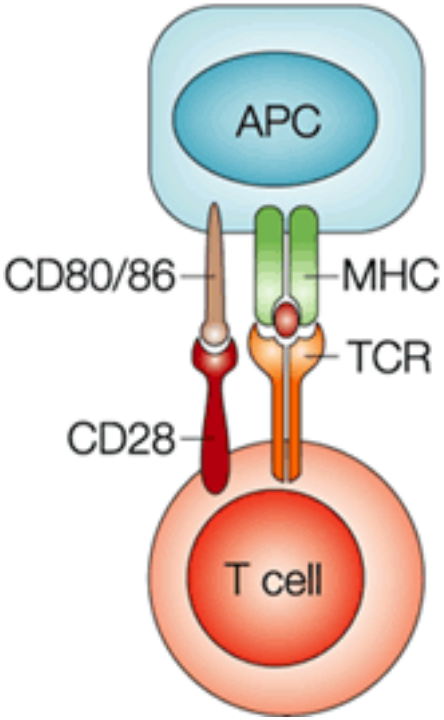
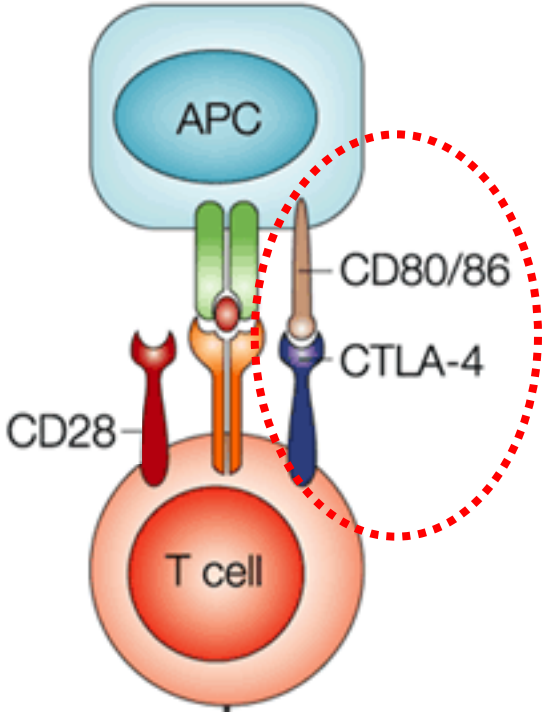
Coinhibition – negative costimulation

Signal 1 + Signal 2



Proliferation
Differentiation
Effector Function

Costimulatory molecule binds CTLA4

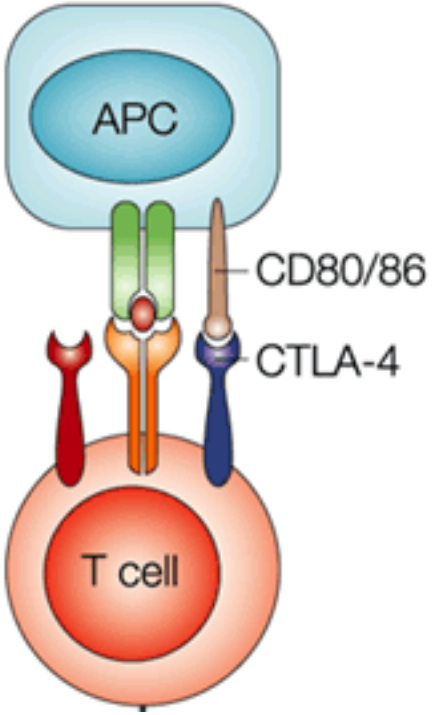


Cell cycle arrest

[1]

Coinhibition – “negative costimulation”

- CTLA4 is not constitutively expressed
  - Expressed only after T cell activation
- CTLA4 has a higher affinity for CD80 and CD86 than does CD28
- CTLA4 expression is transient
  - Rapidly internalised
- Co-inhibitory molecule
  - Tolerance induction
  - Limiting the immune response
- Multiple mechanisms (CS lecture)



Function of anergic T cells

- Anergic T cells can persist *in vivo* for a long period of time

- Anergic T cells can exert suppressor function *in vitro* and *in vivo*

- Overlap with regulatory T cells?

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Regulatory T cells

- First proposed in 1970 by Gershon.
- In 1970's and 1980's generation of suppressor hybridoma.
- Tolerance can be transferred with T cells in transplantation models.
- 1990's two important discoveries:
    - Regulatory cytokines (TGF-β and IL-10)
    - $CD4^+CD25^+$ T cells

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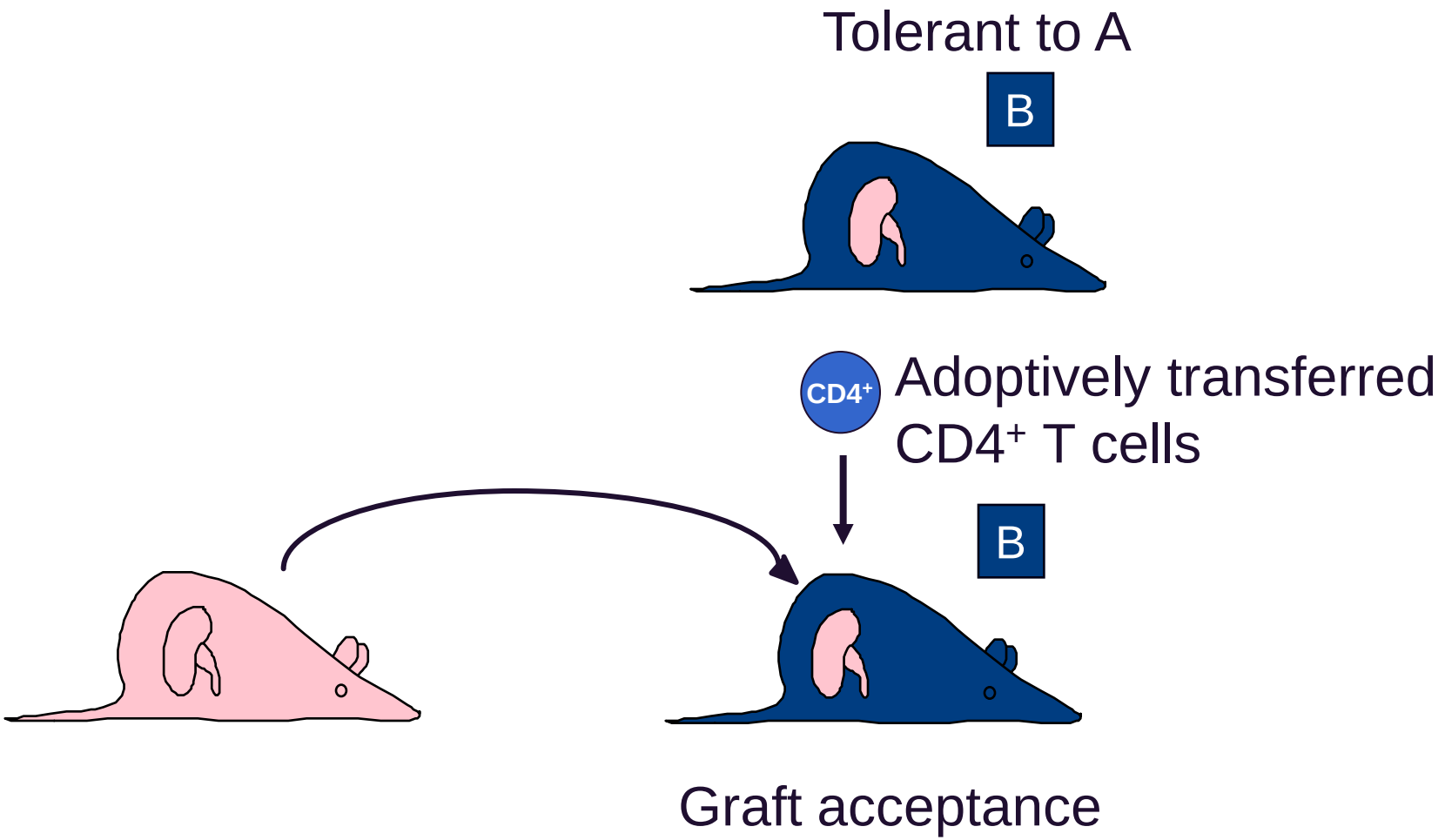
The rat model



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T*reg* families: induced and naturally occurring

Induced

in vitro and *in vivo*:

Anergic T cells

Tr1

Th3

$CD8^+CD28^-$

Naturally occurring:

$CD4^+CD25^+$

NK-T cells

$CD4^+CD25^-$

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T*reg* families: induced and naturally occurring

Induced

in vitro and *in vivo*:

Anergic T cells

Tr1

Th3

$CD8^+CD28^-$

Naturally occurring:

$CD4^+CD25^+$

NK-T cells

$CD4^+CD25^-$

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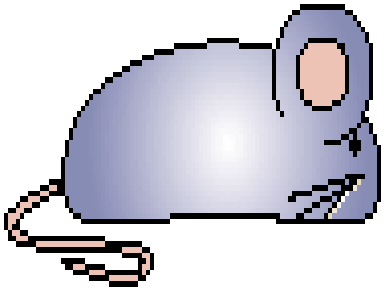
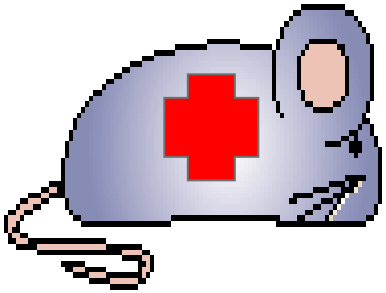
$CD4^+CD25^+$ T cells

Normal Mouse



Thymectomy 3-5 days after birth

Partially T cell depleted mouse



Mouse has severe polyautoimmune disease including: gastritis, thyroiditis, oophritis and diabetes

Sakakura T *et al* Science 1967 166:753-755

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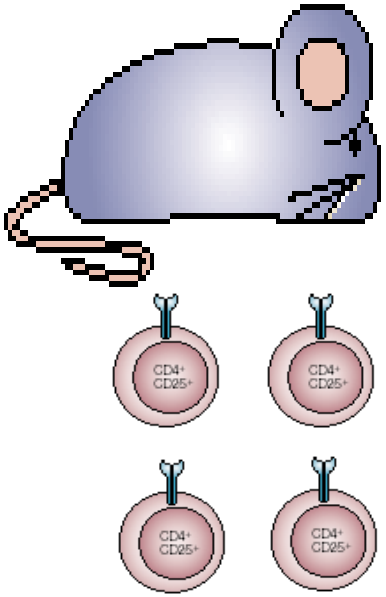
$CD4^+CD25^+$ T cells

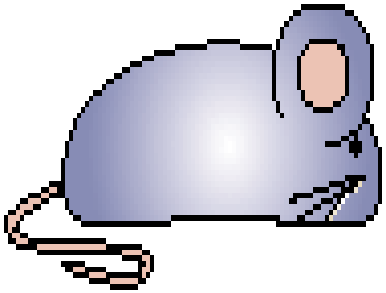
Normal mouse



CD4+CD25+ cells from normal mouse

Thymectomy 3-5 days after birth

Normal mouse



Adoptive transfer of CD4 T cells that express CD25, prevents the autoimmunity caused by day 3 thymectomy

Sakaguchi S et al 1995 J.Immunol 155:1151-1164

$CD4^+CD25^+$ T cells

- 5-10% of the $CD4^+$ T lymphocytes in healthy adult mice and humans.
- Generated in the thymus
- Constitutively express
  - CD25
  - CTLA-4
  - GITR
  - Foxp3
- Hyporesponsive/anergic and suppressive both *in vivo* and *in vitro*.

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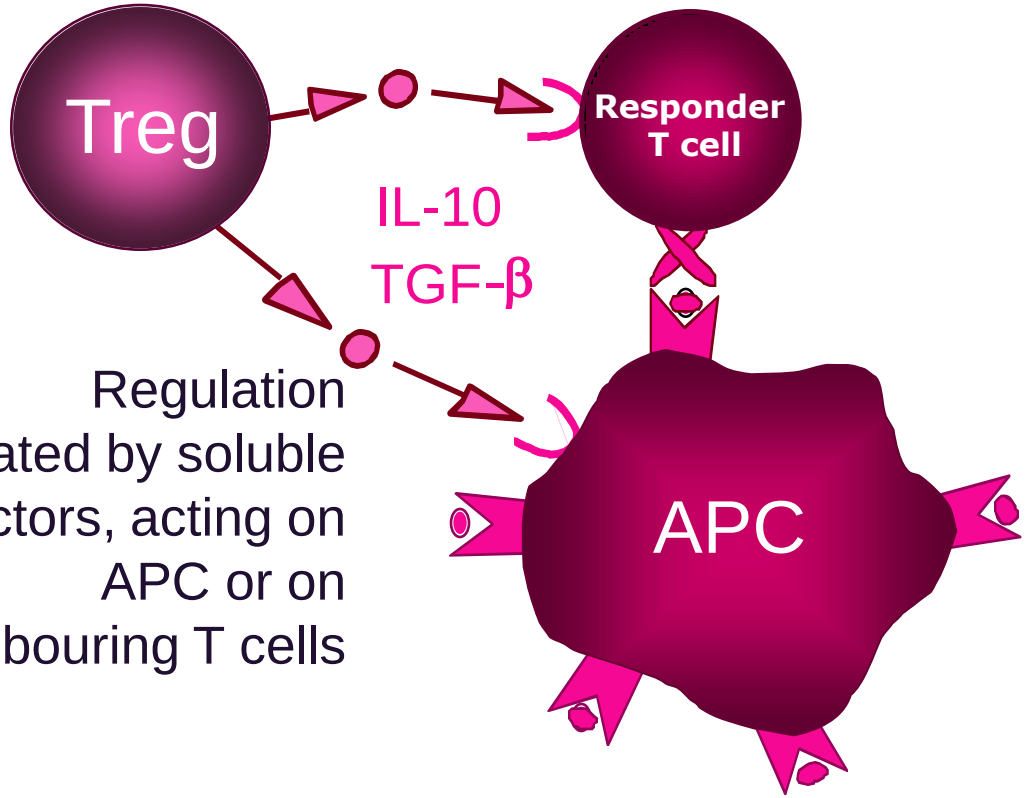
Foxp3

- Scurfy mice: natural mutants, develop multisystemic autoimmune disease.
  - Deletion of FoxP3
  - lack $CD4^+CD25^+$ T regulatory cells
- IPEX syndrome: X – linked (Immunodysregulation, Polyendocrinopathy, Enteropathy)
  - Mutations in Foxp3 identified in 13/14 patients
    - Truncation of FoxP3 protein
    - Commonly presents in infants with severe diarrhea and failure to thrive
    - Death by 12months if untreated

Sakaguchi *et. al*., 2003 and Wildin RS, J Med Genet, 2002 Clin.Exp.Imm 2004

The mechanisms of action of Treg….



Regulation mediated by soluble factors, acting on APC or on neighbouring T cells

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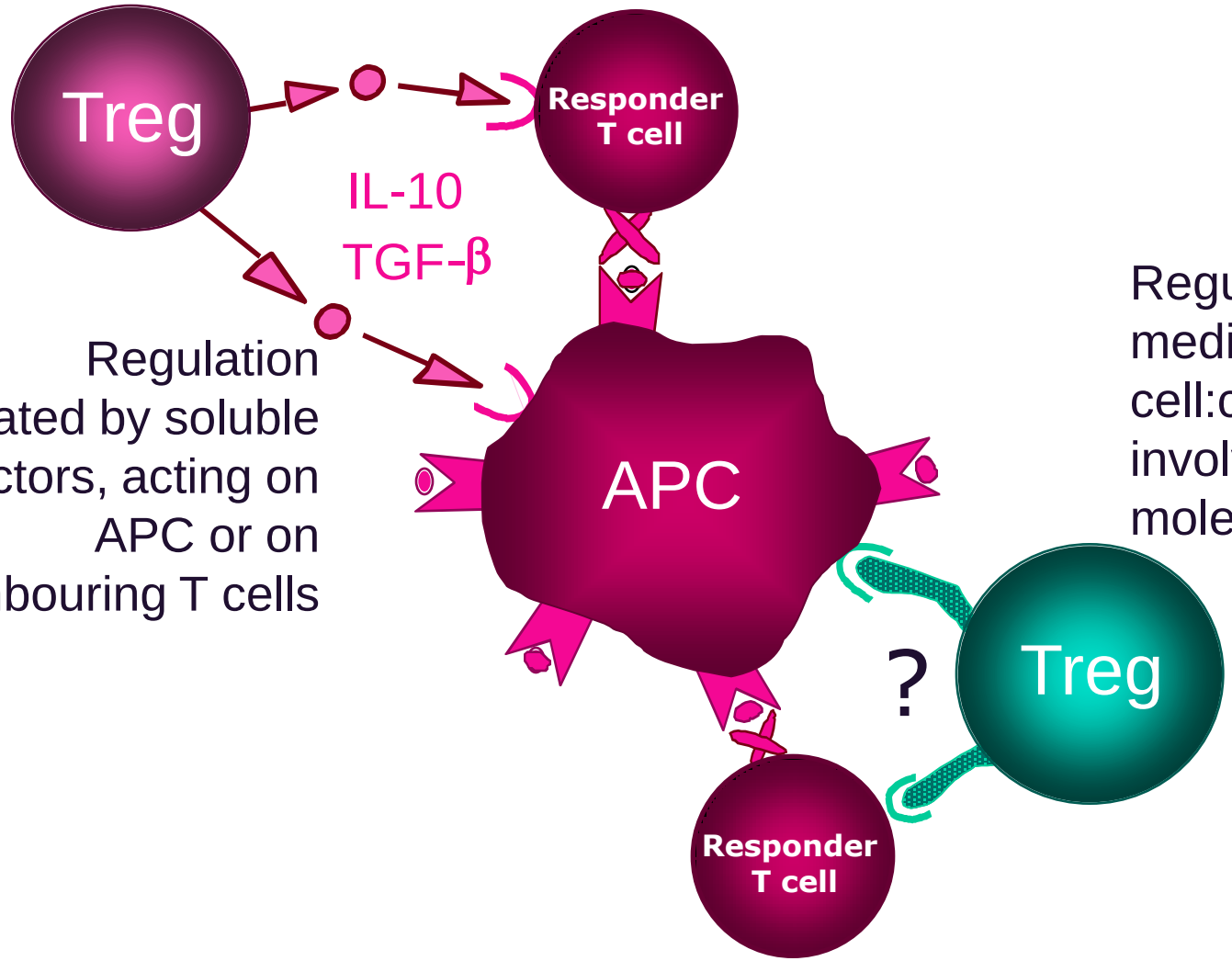
The mechanisms of action of Treg….



Regulation mediated by soluble factors, acting on APC or on neighbouring T cells

Regulation mediated by cell:cell contact, involving unknown molecules

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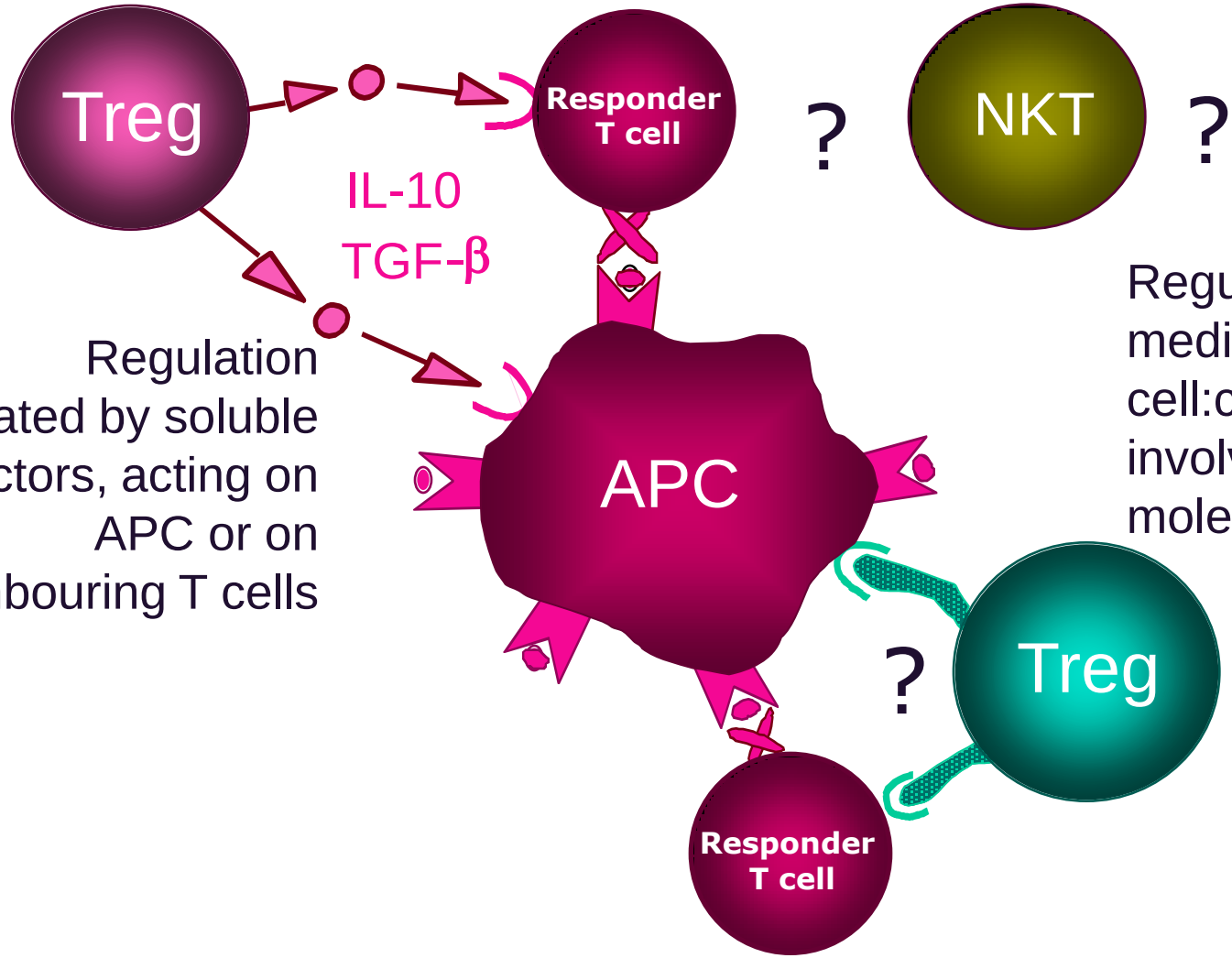
The mechanisms of action of Treg….



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$CD4^+CD25^+$ regulatory function in animal models

Autoimmunity

- Mice with EAE show significant improvement when infused with $CD4^+CD25^+$ T cells from naïve mice
  - Zhang X *et al* 2004. Int Immunol. 16:249-56
- The onset of diabetes in NOD mice can be delayed by transfer of $CD4^+CD25^+$ T cells from syngeneic NOD
  - Salomon B *et al* 2000. Immunity. 12:431-440

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Are $CD4^+CD25^+$ important in human disease?

Goodpasture's Disease

- Loss of tolerance to type IV collagen (glomerular basement membrane and alveoli)
- Characterised by glomerulonephritis and occasionally pulmonary haemorrhage
- Symptoms rarely relapse+remit – patients enter complete remission and this coincides with the emergence of $CD4^+CD25^{hi}$ T cells specific for type IV collagen
  - Salama AD *et al* Kidney Int 2003 64:1685-1694.

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Are $CD4^+CD25^+$ important in human disease?

Systemic Lupus Erythematosus (SLE)

- SLE patients have fewer peripheral $CD4^+CD25^{hi}$ T cells
  - Bagavant H *et al* 2004 Scand. J of Immunol 60:52-63
- $CD4^+CD25^+$ T cells from MRL/Mp mice (polygenic model of SLE) are resistant to CD4+ $CD25^+$ T cell-mediated regulation
  - Monk *et al*. 2005. Arthritis & Rheum 52:1180

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Are $CD4^+CD25^+$ important in human disease?

Rheumatoid Arthritis

- Studies have shown no difference in the number of peripheral $CD4^+CD25^{hi}$ T cells
- The synovium of inflamed joints contained higher frequencies of $CD4^+CD25^{hi}$ T cells then peripheral blood and these were anergic and suppressive *in vitro*
  - Cao D *et al* 2003 Eur.J. Immunol 33:215-223

Imperial College London

$CD4^+CD25^+$ regulatory function in animal models

Transplantation Tolerance

- Rats can be made tolerant to cardiac allografts by transfer of $CD4^+CD25^+$ T cells from animals made tolerant to similar grafts with cyclosporin
  - Bach JF 2003 Nat Rev Immunol 3:189-198

Th3 cells – general features

- Th3 cells are central to mucosal immuno-regulation (GALT)
- Richman *et al* (1978) first demonstrated the induction of Ts cells by enteric antigen
- Many studies have characterised the cells responsible for GALT immunoregulation, including Th3 subset of $CD4^+$ T*regs*

Th3 cells – general features

- Mediate suppression by TGF-β
- Th3 cells provide help for IgA and suppress both Th1 and Th2 responses
- Differentiation from Th0 cells *in vitro* occurs in presence of TGF- β, IL-4, IL-10, anti-IL-12
- Th3 cells mediate ‘bystander suppression’
- “Eating” your way towards immunosuppression?

Tr1 cells – general features

- Tr1 cells specific for a variety of antigens arise *in vivo*; may also differentiate from naïve $CD4^+$ T cells in presence of IL-10 *in vitro*
- Minimal proliferation: autocrine suppression by IL-10
- Require activation for regulatory function
- Regulate both Th1 and Th2 responses

Tolerance - Therapeutic strategies

- Chimaerism:
  - donor bone-marrow transfer – *i.v.*
  - induction of central tolerance
- Targeting T cell surface molecules
  - Costimulators/coinhibitors
- TCR targeting
- Cell-based therapies

Targeting co-inhibitors

- CTLA4Ig (abatacept)
- LEA29Y (belatacept)
  - recombinant extracellular domain of CTLA4 with mutation that increase 10fold binding to CD80 – antagonist
- Prevents the priming of antidonor T- and B-cell responses and can prolong islet allograft survival indefinitely under cover of sirolimus/tacrolimus in nonhuman primates
- Clinical trials suggest effectiveness with low side-effect in RA and renal transplantation

Targeting CD154

- Humanized anti-CD154 mAb hu5C8/Antova (Biogen)
- used in renal transplantation
  - several autoimmune indications
- These trials were discontinued because of :
  - multiple thromboembolic events (binding to platelets)
  - the failure to prevent rejection in five of seven patients receiving renal transplants
- IDEC Pharmaceuticals is also developing an anti-CD154 mAb (IDEC 131) with a current focus on autoimmune diseases

TCR targeting

Anti-CD3 mAbs

- OKT3 was the first FDA approved monoclonal antibody for use in kidney transplantation.
  - Severe side effects (cytokine storm)
- Humanized Fc receptor-nonbinding anti-CD3 antibodies (teplizumab) do not elicit a very toxic cytokine syndrome
  - have entered the clinic in both autoimmune disease and transplantation settings. Indications: transplantation GVHD, autoimmiunity (diabetes, “Protégé” clinical trial phase II/III).
- Mechanism: generation of T regs

Cell-based therapies

- Adoptive transfer of regulatory T cells
  - Gagliani, N. *et al.* (2009). Autoimmune diabetic patients undergoing allogeneic islet transplantation: are we ready for a regulatory T-cell therapy?

    Immunol Lett. Dec 2;127(1):1-7.

- Negative vaccination (tolerogenic DCs)
  - Hill, M. and Cuturi, M.C. (2010). Negative vaccination by tolerogenic dendritic cells in organ transplantation. Curr Opin Organ Transplant. Sep 24 *E-pub ahead of print*.