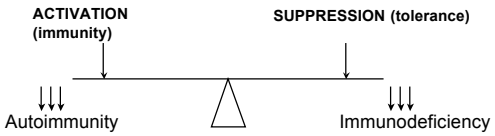
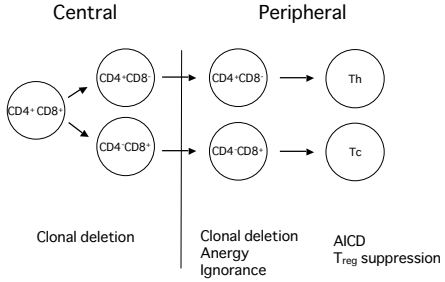
**Immunoregulation:** A balance between activation and suppression that achieves an efficient immune response without damaging the host.




**Significance:** The induction of tolerance may be exploited to prevent graft rejection, and to treat autoimmune diseases, allergies and malignancies.

## Mechanisms of Immune Tolerance




## Central tolerance

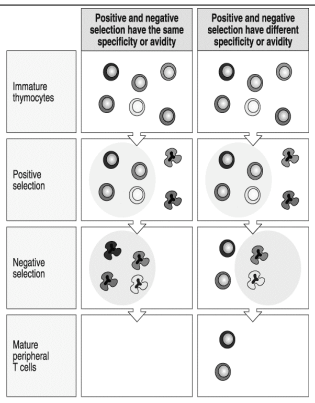



Fig 7.35 © 2001 Garland Science

Failure of negative selection in the thymus results in autoimmunity

- APECED, or multiple polyendocrinopathy Type I is due to mutation in a gene called AIRE
- AIRE controls expression of important self-antigens on thymic medullary epithelial cells
- In the absence of AIRE, T cells recognizing these self-antigens fail to undergo negative selection

## Regulation of the T cell response

## TCR engagement - SIGNAL 1

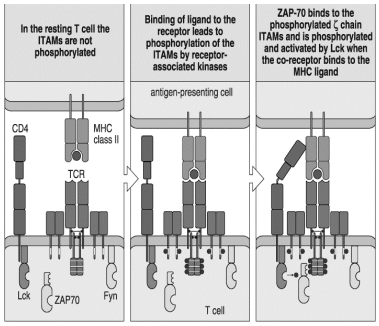
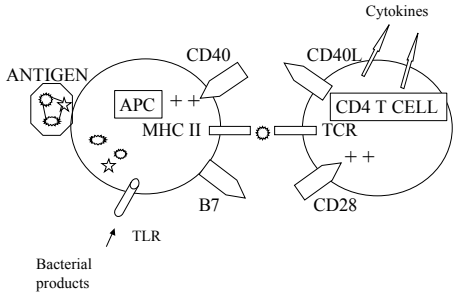



Fig 6.11 © 2001 Garland Science

## Costimulation - SIGNAL 2




## Anergy as a result of insufficient costimulation

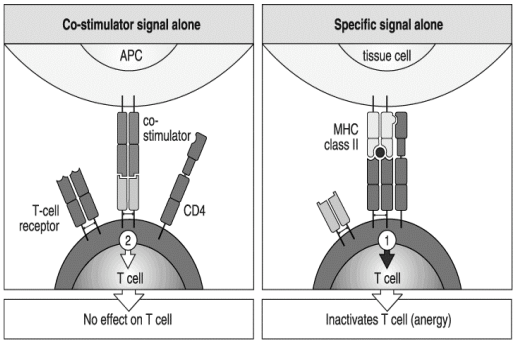
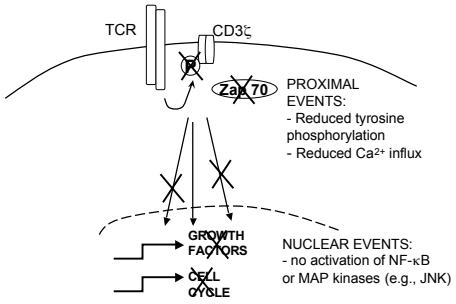
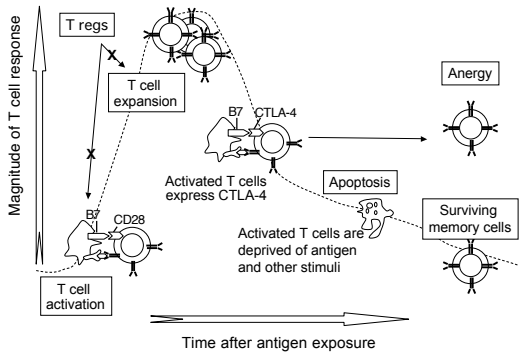



Fig 8.21 © 2001 Garland Science

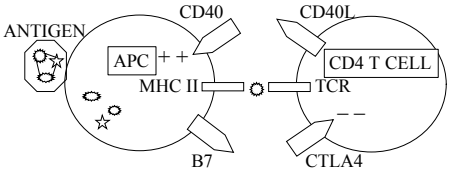
## Molecular basis of anergy in T lymphocytes



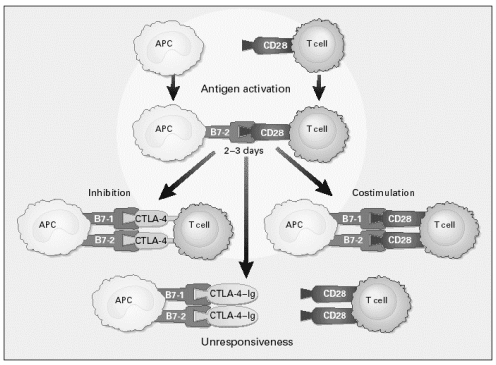

## Regulation of T cell homeostasis during immune responses



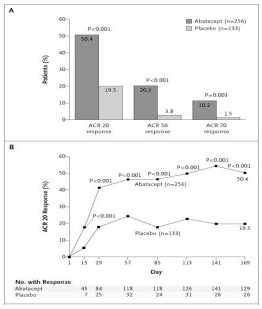

## CTLA4 engagement inhibits activated T cells by competing for B7 (CD80/CD86)



CTLA4Ig is the first biologic to inhibit costimulation




Abatacept (CTLA4-Ig) abrogates signs and symptoms of Rheumatoid Arthritis

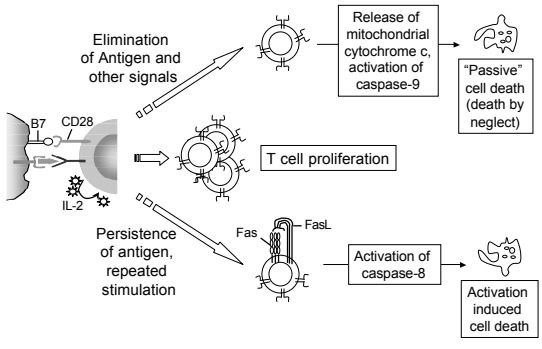



Genovese MC et al Volume 353:1114-1123

The NEW ENGLAND JOURNAL of MEDICINE

Regulation of activated T cells at the end of an immune response can occur by clonal deletion

Activation induced cell death (AICD)




Fas deficiency causes autoimmune lymphoproliferative syndrome

- Genetic disease with incomplete penetrance
- Splenomegaly and lymphadenopathy
- Autoimmune hemolytic anemia and thrombocytopenia
- Other autoantibodies
- Inflammatory autoimmune disease uncommon
- Increased circulating double negative T cells
- May develop lymphomas

# Regulatory T cells

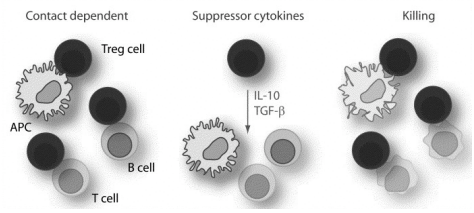
## Types of regulatory T cells

- Naturally arising CD4+ CD25+ (nTreg)
- Peripheral CD4+ CD25+ Tregs (aTreg)
- IL-10 secreting (Tr1)
- TGF-β secreting (Th3)
- Qa-restricted CD8 (Qa Treg)
- CD8+ CD28-
- γδ T cells
- NK T cells
- Others

## How are Tregs induced?

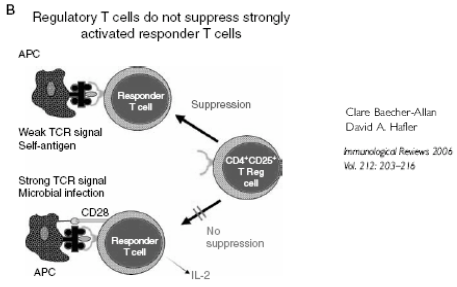
- Some arise naturally as a distinct population (e.g., nTreg and NK T cells)
- Others are induced as a result of antigen exposure in a permissive cytokine environment (e.g., aTreg and Tr1 cells)
- Multiple subsets reflect the importance of maintaining immune homeostasis and self-tolerance under many different circumstances

## How do regulatory T cells work?




Shevach E Immunity 25, 195–201, August 2006

## Tregs preferentially suppress autoreactive cells




Clare Baecher-Allan
David A. Hafler
*Immunological Reviews* 2006
Vol. 212: 203–216

## CD4+ CD25+ regulatory T cells

## Natural Tregs

- Arise in the thymus upon ?medium avidity prolonged antigen/MHC exposure
- Require CD40 and CD28 for development and CD28 and IL-2 for survival (perhaps TGF-β as well)
- Function in suppressing inflammatory responses in the periphery in a cell contact-dependent manner
  - Effect depends on the ratio of effectors:suppressors
- A similar subset arises in the periphery from naïve precursors after exposure to antigen (adaptive Treg)

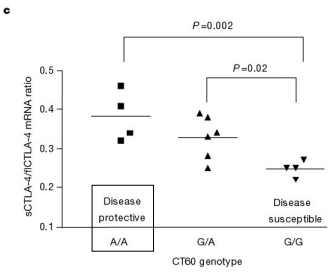
## Autoimmune diseases arise in the setting of deficient nTregs

- Neonatal thymectomy results in multiple endocrinopathy in rodents
- In this situation Treg depletion is transient but sufficient to induce autoimmunity
- Transfer of CD4+ CD25- cells to SCID results in autoimmune disease especially bowel disease. Reversed by CD4+ CD25+ cells
- IPEX in humans
  - Immune dysregulation
  - Polyendocrinopathy
  - Enteropathy
  - X-linked
  - Due to deficiency of Foxp3 gene

## CTLA4 and immune tolerance

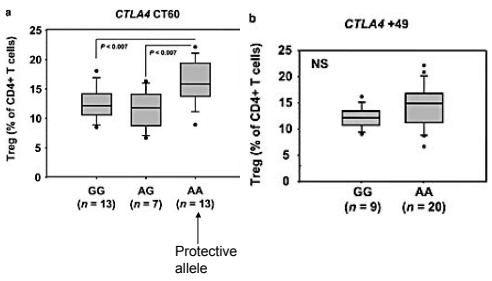
- Natural Tregs constitutively express CTLA4
- CTLA4 deficiency results in fatal autoimmune proliferative disease
- Blocking anti-CTLA4 antibodies induce autoimmunity
- CTLA4 polymorphism is associated with various autoimmune diseases (e.g., diabetes, thyroid diseases and Addison's disease)

## CTLA4 polymorphism associated with diabetes regulates the amount of soluble CTLA4




Ueda et al Nature. 2003 May 29;423(6939):506-11

## CTLA4 polymorphism affects Treg numbers



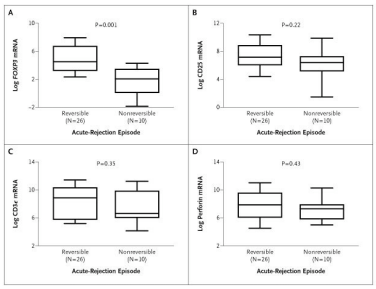

Atabani et al., Eur J Immunol. 2005 Jul;35(7):2157-62

## Ipilimumab (blocking anti-CTLA4) induces autoimmunity

- 137 patients with melanoma, 61 with renal cell carcinoma
- 41 developed enterocolitis
- 13 developed hypophysitis, 8, dermatitis, 4 arthritis, 2 uveitis, 1 hepatitis, 1 nephritis, 1 aseptic meningitis
- Clinical tumor response was associated with enterocolitis (35% vs 2-11%)

## Levels of FOXP3 mRNA in Urinary Cells correlate with Reversal of an Episode of Acute Rejection of kidney allografts

Foxp3 CD25

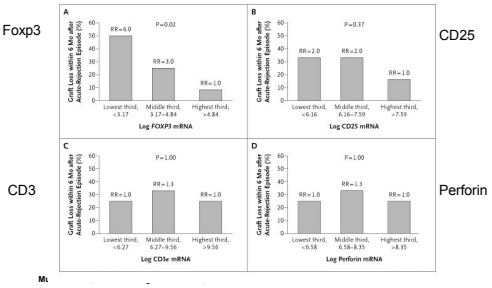



CD3 Perforin

Muthukumar et al., *New Engl. J. Med.* 353:2342-2351, 2005

## Relative Risk of Graft Failure after an Episode of Acute Rejection

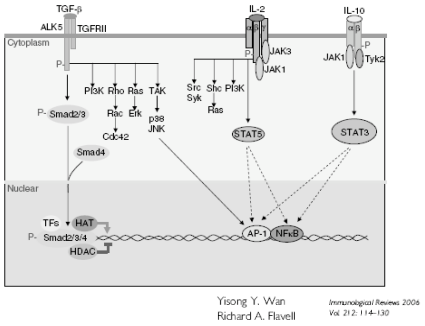
Foxp3 CD25




CD3 Perforin

Muthukumar et al., *New Engl. J. Med.* 353:2342-2351, 2005

## Cytokines involved in tolerance




Yisong Y. Wan
Richard A. Flavell

*Immunological Reviews* 2006
Vol 212: 114-130

## Cytokines in immune tolerance – IL-2

- IL-2 deficiency results in fatal lymphoproliferation and inflammation with reduction or CD4+ CD25+ Tregs
- IL-2 neutralization can augment autoimmunity
- An allelic variant of CD25 is associated with diabetes in humans

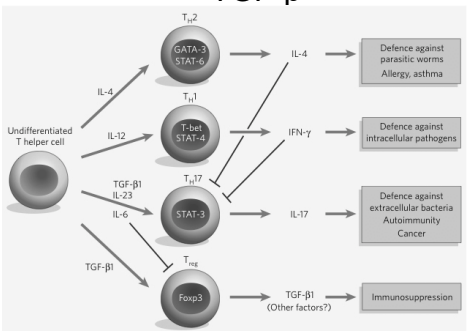
## PTPN22 and autoimmunity

- PTPN22 is a tyrosine phosphatase
- The susceptible variant is a gain of function mutant
- Causes less TCR signaling and less IL-2 production by T cells
- Susceptibility to multiple endocrine autoimmune diseases and rheumatoid arthritis (but not lupus)

## Cytokines in immune tolerance – TGF-β

- TGF-β1 is the form expressed in the immune system
- Expressed as a latent form bound to inhibitors
- Promotes the generation of aTregs together with IL-2 and in the absence of IL-6
- TGF-β deficiency results in fatal autoimmune proliferative disease

## Induction of peripheral Tregs by TGF-β




## IL-10 producing regulatory T cells

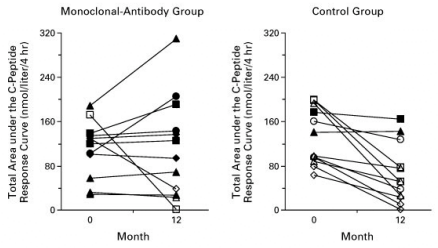
## Tr1 cells

- Induced in the periphery by antigen exposure of naïve T cells in the presence of IL-10
- Cytokine profile is
  - high IL-10, TGF-β, IL5
  - low IFN-γ, IL-2
  - no IL-4
- Can be CD4 or CD8
- Proliferate poorly
- Migrate to inflamed tissues
- Immune suppression through cytokines, not contact
- IL-10 polymorphisms that alter transcription are associated with autoimmunity

## Role of Tr1 cells *in vivo*

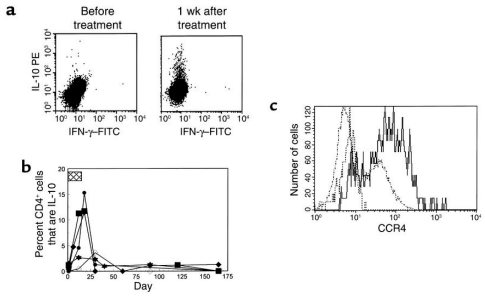
- Regulate diabetes and mucosal tolerance in rodents
- Because they migrate to inflamed sites they can modulate responses to infectious agents, allergens and transplant antigens
- Can be induced *in vivo* by IL-10 in combination with the immune suppressant, rapamycin

## Prevention of diabetes by anti-CD3




Herold K et al N Engl J Med. 2002 May 30;346(22):1692-8

## Anti-CD3 may induce Tr1 cells




Herold K et al J Clin Invest. 2003 Feb;111(3):409-18

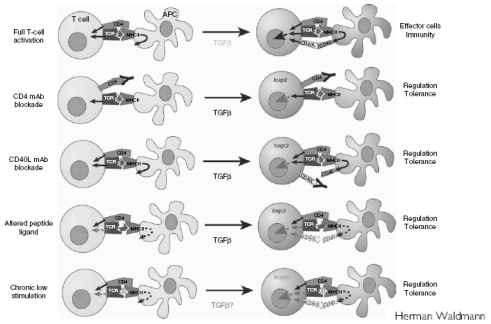
## Treg and the gut

- The GI tract is the main interface where the body encounters exogenous antigens including commensual organisms and dietary antigens
- Loss of tolerance leads to autoimmune bowel disease (e.g., celiac disease or colitis)
- Colitis does not occur in germ free animals
- Colitis is a prominent feature of diseases that involve loss of Tregs or Treg producing cytokines

## Can regulatory T cells be harnessed for therapeutic purposes?

## Expansion of Tregs for therapeutic purposes

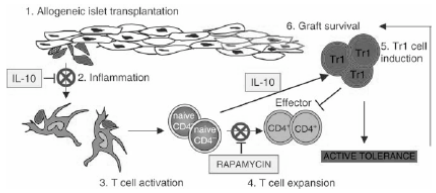
- nTregs can be expanded in vitro in mice with polyclonal activation and IL-2
  - Expansion in vitro in the presence of rapamycin may prevent co-expansion of activated cells.
- Tr1 cells
  - Can be expanded using immature DC or other tolerogenic IL-10 producing subsets of DC
  - Can be expanded *in vivo* with IL-10 and rapamycin
  - ? Induced by anti-CD3 monoclonal antibodies




Herman Waldmann
Elizabeth Adams
Paul J. Fairchild
Stephen Cobbold

*Immunological Reviews 2006*
*Vol. 212: 301–313*

## Induction of Tr1 *in vivo*




Maria Grazia Roncarolo
Silvia Gregori
Manuela Battaglia
Rosa Bacchetta
Katharina Fleischhauer
Megan K. Levings

*Immunological Reviews 2006*
*Vol. 212: 28–50*

## Foxp3 therapy

- Foxp3 transduced cells can protect from autoimmunity but only if there are sufficient antigen specific cells
- Has been more successful for transplant than for organ specific autoimmunity

## What signals favor Treg development?

- What determines thymic deletion vs. Treg development?
- May be a function of the type of APC
- Costimulatory molecules and cytokines required for Treg development are also needed for activation of effector cells
- Solving these puzzles will help lead the way to therapeutic interventions

## Summary

1. A fraction of naïve T cells that are released into the periphery contain potentially pathogenic autoreactive specificities. Regulation of these T cells is required to avoid pathogenic autoimmunity.

2. Multiple subsets of autoreactive T cells have been described. Of these, the two most well characterized are natural T regulatory cells (nTreg) and IL-10-producing T regulatory cells (Tr1).

3. Development of nTregs occurs in the thymus. Failure of these cells to develop results in autoimmunity diseases affecting predominantly endocrine organs and the bowel. Development of nTregs is dependent on CD28 and possibly CTLA4 while survival of nTregs is dependent on CD28 and IL-2. Foxp3, a master transcriptional regulator, is required for the function of nTregs. nTregs mediate suppression via contact-dependent mechanisms.

4. Development of Tr1 cells is dependent upon exposure to antigen in the presence of IL-10. Tr1 cells produce IL-10 and mediate both antigen-specific suppression and "bystander" suppression.

5. Functional downregulation of activated T cells in the periphery involves multiple mechanisms. These include regulatory T cells as well as expression of inhibitory molecules, such as CTL4 and Fas, on the surface of the activated T cells themselves.

6. Autoimmune diseases that are due to failure of T cell regulation include IPEX (Foxp3 deficiency), APECED (AIRE deficiency), and autoimmune proliferative syndrome (Fas deficiency). Genetic polymorphisms of many genes influence the onset or severity of autoimmunity.