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

ACTIVATION (immunity)            SUPPRESSION (tolerance)

Autoimmunity                     Immunodeficiency

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

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

Costimulation - SIGNAL 2

Anergy as a result of insufficient costimulation

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

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

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?

Tregs preferentially suppress autoreactive cells

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**

- CTLA4 polymorphism affects Treg numbers
- 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%)

[Links to relevant studies and literature cited]
Levels of FOXP3 mRNA in Urinary Cells correlate with Reversal of an Episode of Acute Rejection of kidney allografts

Relative Risk of Graft Failure after an Episode of Acute Rejection

Cytokines involved in tolerance

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

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

Anti-CD3 may induce Tr1 cells

Treg and the gut

- The GI tract is the main interface where the body encounters exogenous antigens including commensal 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

Induction of Tr1 in vivo

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 autoimmune 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 (nTregs) and IL-10 producing T regulatory cells (Tr1).

3. Development of nTregs occurs in the thymus. Failure of these cells to develop results in autoimmune diseases affecting predominantly endocrine organs and the bowel. Development of nTregs is dependent on CD25 and possibly CTLA4 while survival of nTregs is dependent on CD86 and IL-2. Fasl, 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 CTLA4 and Fasl, on the surface of the activated T cells themselves.

6. Autoimmune diseases that are due to failure of T cell regulation include IPEX (Fasp3 deficiency), APECED (AIRE deficiency), and autoimmune proliferative syndrome (Fas deficiency). Genetic polymorphisms of many genes influence the onset or severity of autoimmunity.