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Immunological tolerance and immune regulation

Abul K. Abbas
UCSF

UCSF University of California San Francisco advancing health worldwide

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Lecture outline

- Central and peripheral tolerance
- Inhibitory receptors of T cells
- Regulatory T cells

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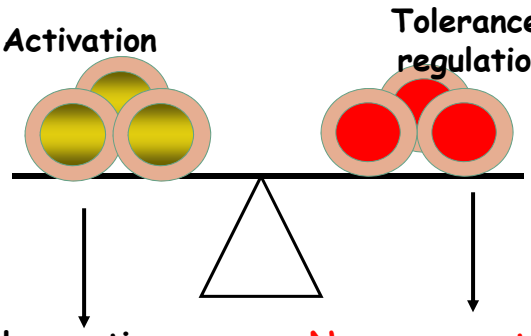
The immunological equilibrium: balancing lymphocyte activation and control



Normal: reactions against pathogens

No response to self and other harmless antigens

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Immunological tolerance

- Definition:
  - unresponsiveness to an antigen induced by exposure of lymphocytes to that antigen; antigen-specific (unlike "immunosuppression")
- Significance:
  - All individuals are tolerant of their own antigens (self-tolerance); breakdown of self-tolerance results in autoimmunity
  - Preventing immune responses to commensal microbes, fetal antigens
  - Therapeutic potential: Inducing tolerance may be exploited to treat autoimmune and allergic diseases

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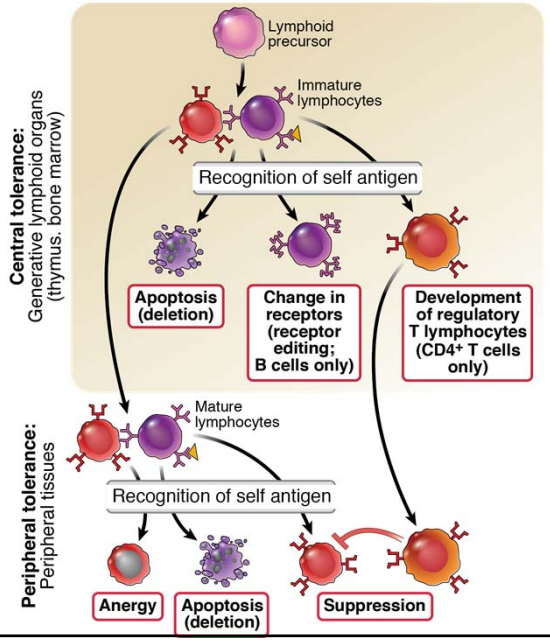
Central and peripheral tolerance to self



The principal fate of lymphocytes that recognize self antigens in the generative organs is death (deletion)

Some B cells may change their specificity (called "receptor editing")

Some T cells may differentiate into regulatory (suppressor) T lymphocytes

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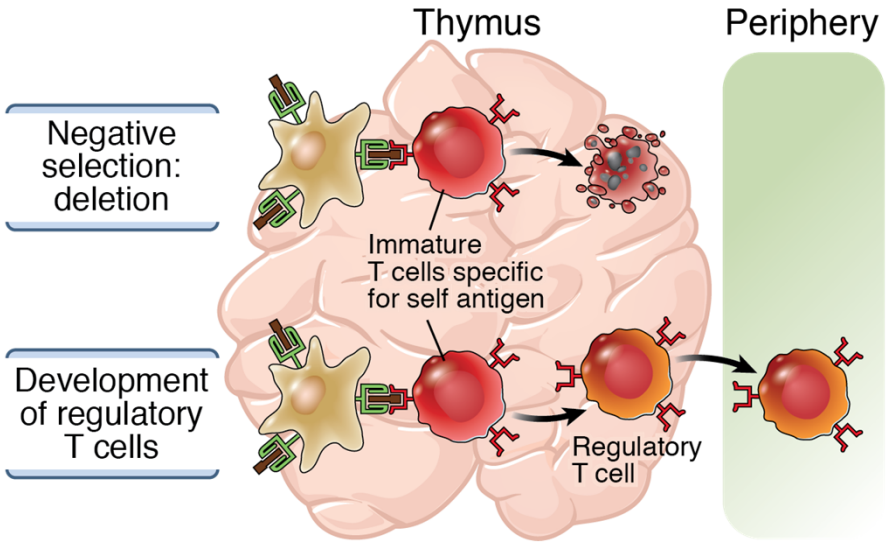
Consequences of self antigen recognition in thymus



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What self antigens are seen in the thymus?

- Ubiquitous cell-associated and circulating proteins
- The thymus has a special mechanism for displaying peripheral tissue antigens in thymic medullary epithelial cells, where they signal self-reactive thymocytes for death

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Consequences of AIRE mutation

- Human disease: autoimmune polyendocrinopathy with candidiasis and ectodermal dysplasia (APECED), also called autoimmune polyendocrine syndrome (APS-1)
  - Associated gene identified by positional cloning, named *AIRE* ("autoimmune regulator")
- Mouse knockout: autoantibodies against multiple endocrine organs, retina
  - Failure to express many self antigens in the thymus --> failure of negative selection

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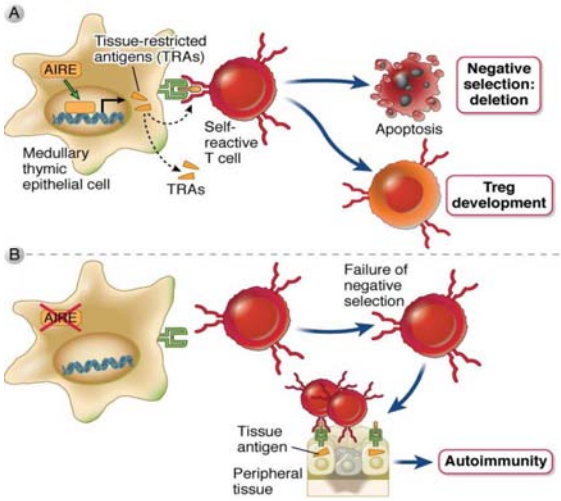
Deletion of self-reactive T cells in the thymus: how are self antigens expressed in the thymus?



AIRE (autoimmune regulator) is a regulator of gene transcription that stimulates thymic expression of many self antigens which are largely restricted to peripheral tissues

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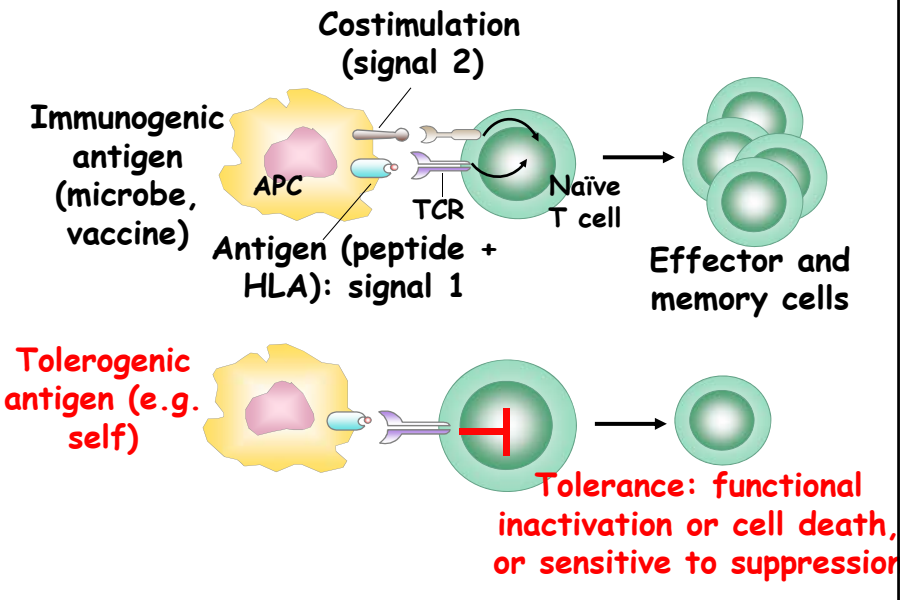
Peripheral tolerance



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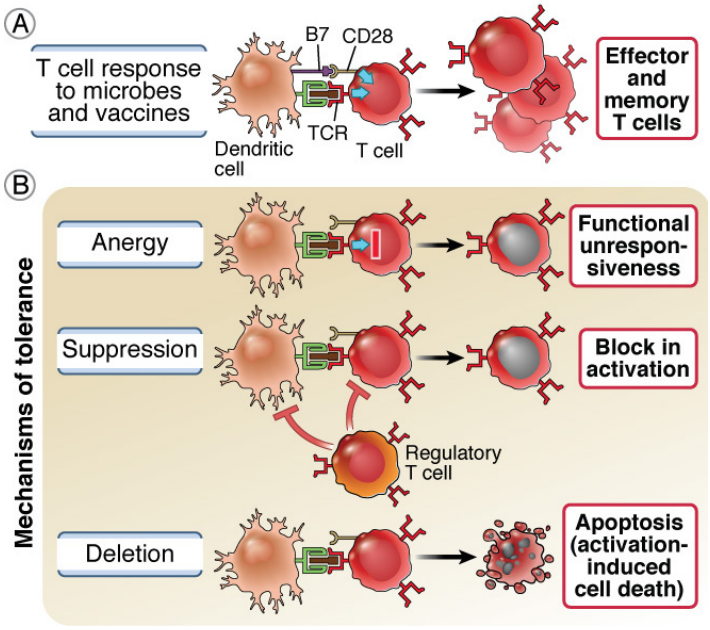
Peripheral T cell tolerance



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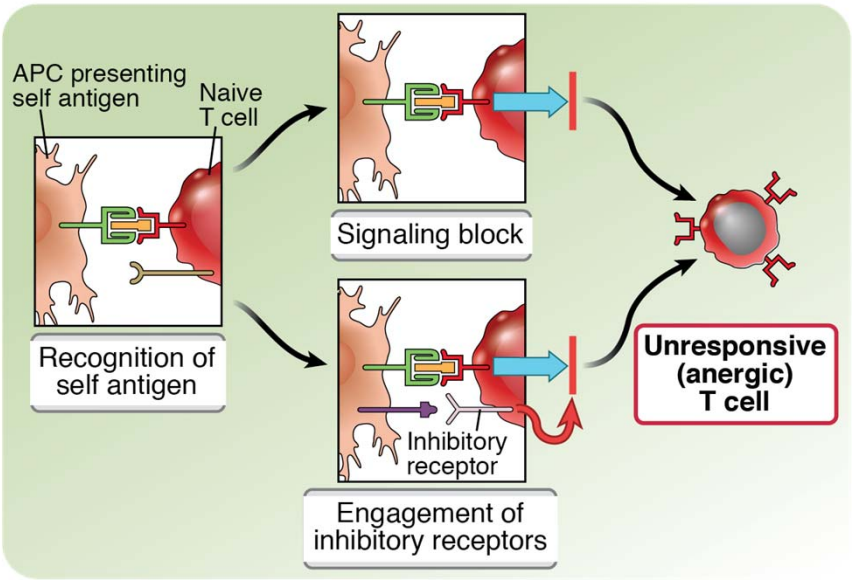
T cell anergy



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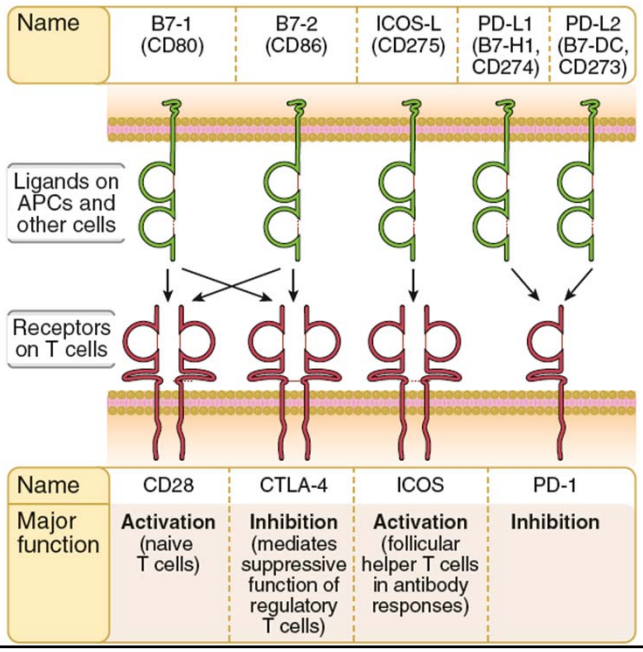
The B7:CD28 families



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Inhibitory receptors of T cells

- Prevent reactions against self antigens (their physiologic function)
  - Deletion or blockade of these receptors results in autoimmune diseases
- Suppress immune responses to some tumors, chronic infections (HCV, HIV)
  - Therapeutic application: checkpoint blockade for cancer immunotherapy

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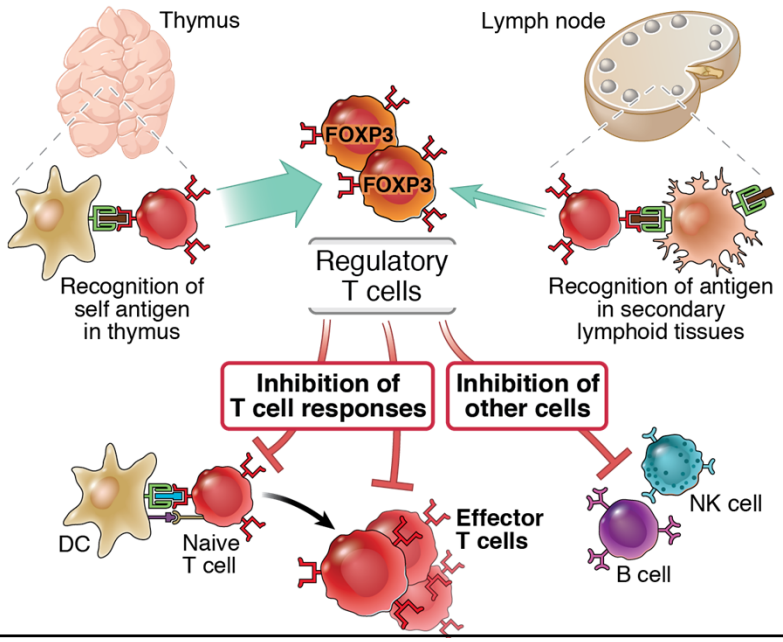
Regulatory T cells



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Properties of regulatory T cells

- **Phenotype:** CD4+, high IL-2 receptor (CD25), low IL-7 receptor, Foxp3 transcription factor; other markers
- **Essential features** of stable Tregs:
  - Foxp3 expression: requires demethylated non-coding CNS2 sequence in promoter
  - CD25 (IL-2Rα) expression: IL-2 is a necessary survival factor
  - CTLA-4 expression: required for suppressive function of most Tregs
  - (Inability to produce IL-2)

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The significance of Foxp3+ Tregs

- Genetic evidence: Foxp3 mutations --> autoimmune disease (IPEX); in mice, disease can be corrected by providing normal Foxp3+ cells
- Do defects in Foxp3+ Tregs or resistance to Treg-mediated suppression contribute to common autoimmune diseases?
  - Inconsistent and variable data

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Populations of Tregs

- Thymic, tTreg ("natural")
  - Induced by self antigen recognition during T cell maturation
- Peripheral, pTreg ("adaptive")
  - In response to antigen exposure in the periphery; contribution to preventing inflammatory disease?
- In vitro induced, iTreg (sometimes called Tr1)
  - Culture with TGFβ + IL-2; therapeutic options
- There may not be reliable markers for distinguishing these Tregs in a "bulk" population

Mechanisms of action of Foxp3+ Tregs

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- CTLA-4 on Tregs removes B7 on APCs, reduces CD28 engagement and T cell activation
  - Genetic deletion of CTLA-4 in Foxp3+ cells results in severe systemic autoimmunity and lymphoproliferation
  - Commonly used assay for Treg function in humans bypasses the need for B7 and cannot measure this activity

Mechanisms of action of Foxp3+ Tregs

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- CTLA-4 on Tregs removes B7 on APCs, reduces CD28 engagement and T cell activation

- Inhibitory cytokines produced by Tregs (IL-10, others?) suppress immune responses (DCs, Macs, T cells)
  - IL-10 deletion in Foxp3+ cells results in colitis
  - IL-10 is also produced by Foxp3- cells
- Consumption of IL-2
- Many others reported

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Role of Tregs in fetal tolerance

- In evolution, placentation developed at the same time as the ability to generate FoxP3+ peripheral Tregs
- Paternal antigens expressed in the fetus induce long-lived antigen-specific Tregs; elimination of these Tregs in mice results in fetal resorption

- Why does the placenta promote regulation?
- Role in humans? Are defects in Tregs the basis of recurrent fetal loss?
- Did peripheral Tregs evolve to protect the fetus? (And thymic Tregs for self antigens?)

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"Non-immune" functions of tissue Tregs

- Tregs in adipose tissue regulate lipid metabolism
- Tregs in muscle and other tissues produce growth factors that promote repair (trauma, infections, degenerative diseases)
- Tregs in skin stimulate cycling and differentiation of hair follicle stem cells

- Do Tregs adapt to their environment, or do distinct subsets exist that populate different tissues?

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Regulatory T cells

- Explosion of information about the generation, properties, functions and significance of these cells
- Will cellular therapy with ex vivo expanded Treg become a reality?
- Therapeutic goal: induction or activation of Treg in immune diseases

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The therapeutic potential of regulatory T lymphocytes

- Cell transfer of autologous Tregs to suppress immune responses
  - Grow up patient's Tregs ex vivo
  - Ongoing clinical trials in graft rejection, T1D show it is safe
  - Very little efficacy data
  - Technically difficult, individualized

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The therapeutic potential of regulatory T lymphocytes

- Cell transfer of autologous Tregs to suppress immune responses

- **Administer antigen or cytokine in ways that preferentially induce Tregs?**
  - **Antigen without adjuvant?**
  - **IL-2**

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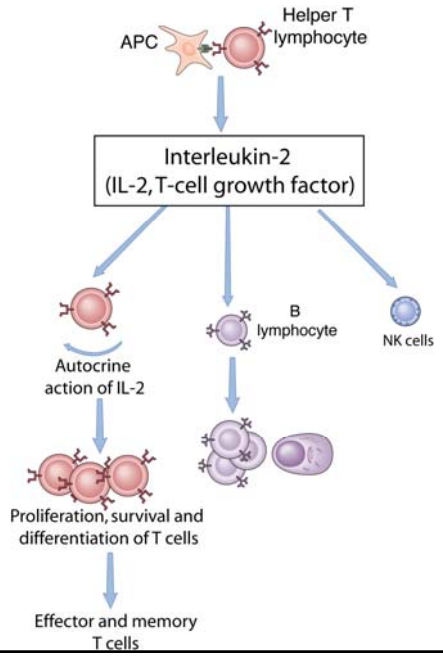
Functions of Interleukin-2: the dogma



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The unexpected biology of IL-2

- Interleukin-2 is the prototypic T cell growth factor (TCGF), required for initiating clonal expansion of T cells in response to antigen
- Prediction: what will be the consequence of eliminating IL-2 or the IL-2 receptor?

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The unexpected biology of IL-2

- Interleukin-2 is the prototypic T cell growth factor (TCGF), required for initiating clonal expansion of T cells in response to antigen
- BUT: knockout of IL-2 or the α or β chain of the IL-2R results not in immune deficiency but in systemic autoimmunity and lymphoproliferation

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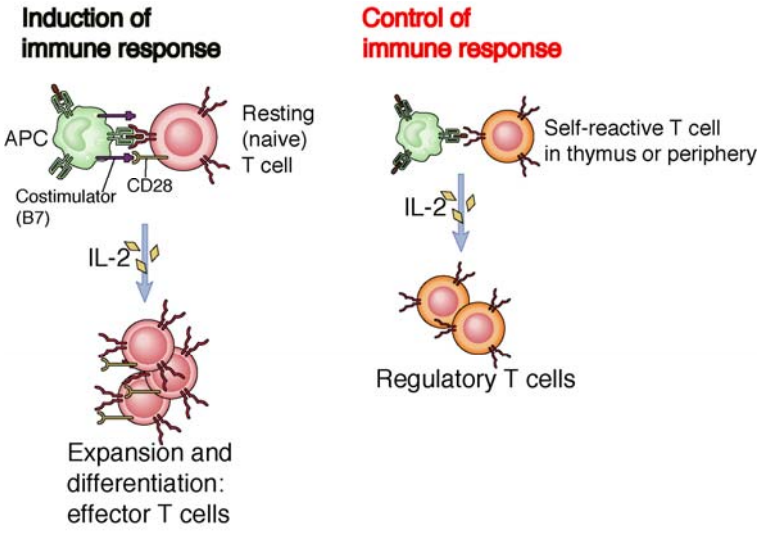
Dual roles of IL-2 in T cell responses



Surprising conclusion from knockout mice: the non-redundant function of IL-2 is in controlling immune responses

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The role of IL-2 in the lives of regulatory T cells

- IL-2 is a survival factor for maintaining Tregs in the periphery
  - Source of IL-2 is T cells that respond to antigens (microbes, other environmental antigens?)
- IL-2 is also required for the functional competence of Tregs
  - Promotes FoxP3+ CTLA-4+ T cells

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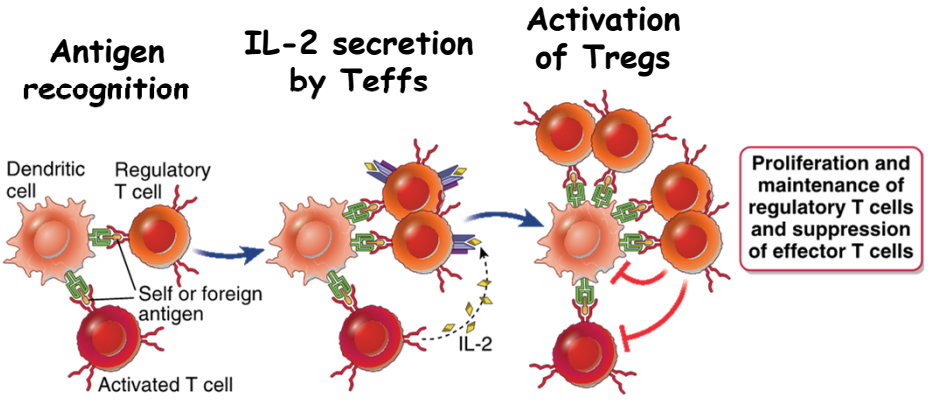
IL-2 dependent activation of regulatory T cells suppresses effector responses



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Therapeutic potential of IL-2: a revision

- IL-2 was originally used to boost immune responses in cancer, HIV infection (enhancing effector and memory T cells)
  - IL-2 treatment can increase number and functional activity if Tregs
- Use of IL-2 to boost Tregs: design IL-2 to bind to high-affinity CD25
  - Low-dose IL-2
  - Mutant IL-2 that binds preferentially to CD25

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Role of dendritic cells in self-tolerance

- Mature DCs (activated during innate immune responses to PAMPs and DAMPs) express costimulators, secrete cytokines, and initiate immune responses
- Immature ("resting") DCs live in tissues, display self antigens and maintain tolerance (by inducing anergy, deletion, and/or Tregs)

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The potential of "tolerogenic antigen-presenting cells"

- Exploiting antigen-pulsed DCs to induce tolerance
  - Phase 1 trial of DCs pulsed with citrullinated peptides in RA published in 2015
  - Maintaining DCs in tolerogenic state?
- Can DCs be modified to make them tolerogenic?
  - Expression of costimulator antagonists, immunosuppressive cytokines, other inhibitors: being tried in animal models of graft rejection

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Strategies for inducing tolerance

- Administration of antigen in tolerogenic form
  - Repeated doses of peptides without adjuvants
  - Understanding mechanism requires assays for antigen-specific lymphocytes
- Engaging inhibitory receptors
  - Risky, difficult to make antibodies that activate and not block receptors
- Treg targeted therapies:
  - Treg cell transfer
  - IL-2

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Regulating immune responses: where are we?

- Elucidating the mechanisms of immune regulation is one of the dominant themes of modern Immunology; obvious relevance to immune-mediated inflammatory diseases, therapeutics, vaccines
- Already leading to new therapeutic strategies
- Continuing challenge is to determine which mechanisms of tolerance fail in different autoimmune diseases