Regulatory T Cells and Maintenance of Tolerance

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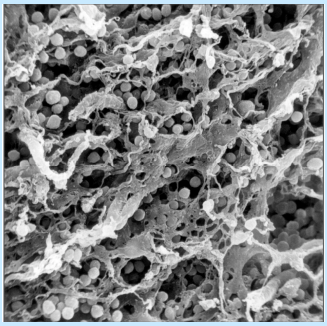
What constitutes immune "tolerance"?

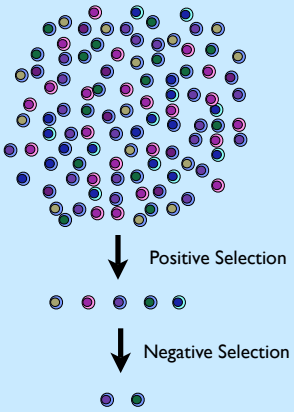
- Discrimination between:
  - non-self and self
    - e.g., virally infected from normal
  - harmless and dangerous
    - e.g., egg white from E. coli proteins in spoiled mayonnaise
  - Attack focused on dangerous non-self
  - Important components contributed by both innate and adaptive arms

Mechanisms Regulating Adaptive Response

Central	Peripheral
Selection During Development	Responder Cell Intrinsic ("Recessive")
B Cells - bone marrow	Ignorance
T cells - thymus	Activation Induced Cell Death
	Anergy
	Responder Cell Extrinsic ("Dominant")
	Regulatory (Suppressor) T Cells

Central Tolerance: Thymic Selection





Immunobiology, eds. Janeway, C., Travers, P., Walport, M., Shlomchik, M. (6th Ed., Garland Science, 2004)

Thymocyte Avidity Profile is "Molded"

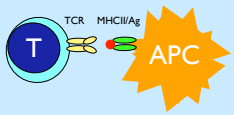
- T cells - mature from thymic precursors
- Specificities are "filtered" by positive and negative selection on self antigens
  - Anti-self - TCR avidities range from low to moderate
  - Anti-foreign - TCR avidities are "unfiltered" (range from undetectable to high)

Thymic Self-Representation

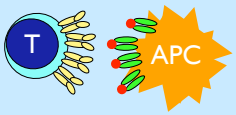
- AIRE: Autoimmune Regulator
  - Gene highly expressed in thymic epithelium
  - Encodes a transcriptional activator
  - Induces expression of "ectopic" self proteins
    - Pancreas-, retina- and ovary-specific
- APECED
  - Clinical condition from mutation in AIRE
  - Autoimmune attack on multiple endocrine structures (thyroid, parathyroid, adrenals, β-islets, gonads), vitiligo, alopecia

Affinity and Avidity

- "Affinity"– binding strength of one receptor to one ligand
- "Avidity"– aggregate binding strength of multiple receptors on one surface to ligands on another
- TCR avidity contributes to T cell activation:
  - High avidity - activated at low antigen concentrations
  - Moderate avidity - requires higher Ag concentrations
  - Low Avidity - largely "ignorant" (not activated)

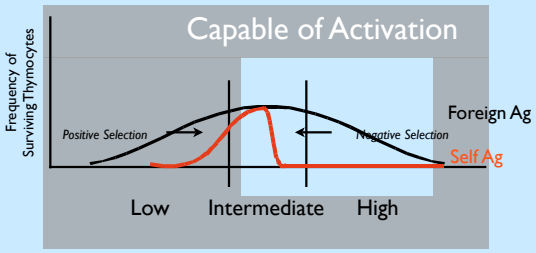


Affinity



Avidity

Thymocyte Selection By TCR Avidity

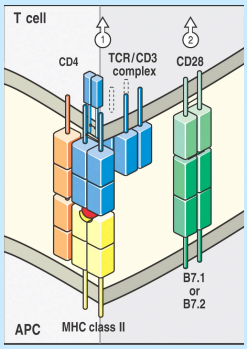


TCR Avidity

Peripheral Tolerance:

Mechanisms intrinsic to the responder T cell

- Successful T cell activation requires two signals:



Signal 1 - TCR crosslinking
- Antigen-specific

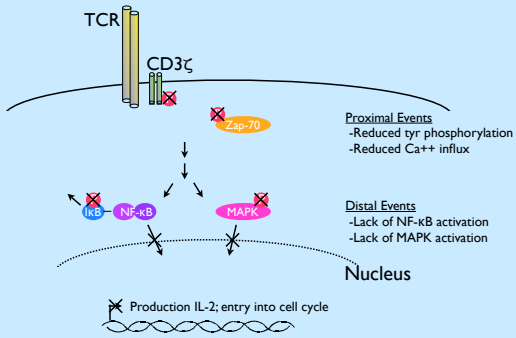
Signal 2 -
Provided by APC, induced to mature by "danger" signals (eg., TLR signals)

Signal 1 without Signal 2 can lead to anergy in the triggered T cell

Immunobiology, eds. Janeway, C., Travers, P., Walport, M., Shlomchik, M. (6th Ed., Garland Science, 2004)

T Cell Anergy

T cell unresponsiveness despite TCR engagement in the presence of costimulation

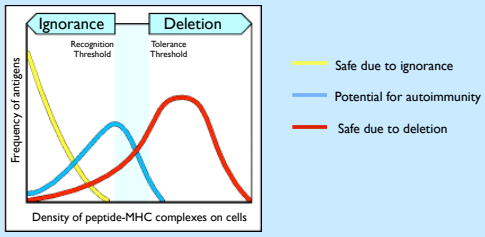


After Davidson, A. "Regulatory T Cells and Maintenance of Tolerance" (2006) P&S Pathophysiology Course: Immunology

Peripheral Tolerance:

Mechanisms intrinsic to the responder T cell

- Ignorance: Low TCR-Ag/MHC avidity and/or low antigen abundance result in failure to produce "signal 1"
  - Example: anatomically "privileged" Ags: CNS, eye (sympathetic ophthalmia)
- Clonal Deletion: High antigen density - persistent TCR triggering in the absence of costimulation → AICD



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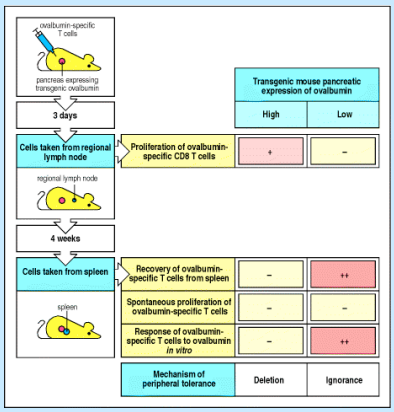
Peripheral Tolerance:

Mechanisms intrinsic to the responder T cell



Immunobiology, eds. Janeway, C., Travers, P., Walport, M., Shlomchik, M. (6th Ed., Garland Science, 2004)

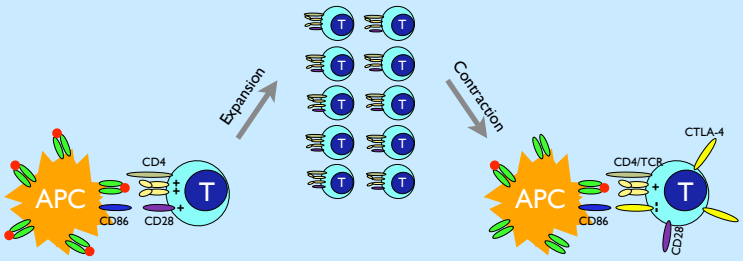
Peripheral Tolerance:

Mechanisms intrinsic to the responder T cell

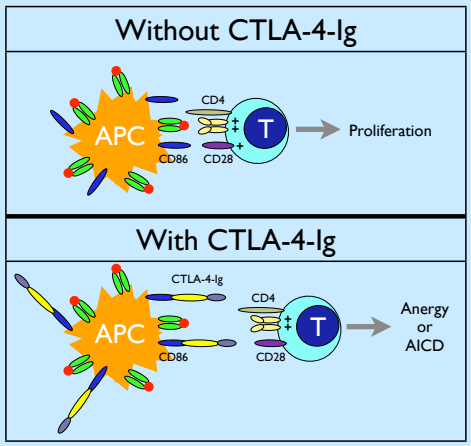
- Activation-induced cell death (AICD)
  - Elimination of clonally expanded effector T cells in the "terminal phase" of an antigen response
  - Persistent or repeated TCR triggering
  - Cell death is apoptotic - largely fas mediated
    - fas is upregulated following T cell activation
    - anti-fas or anti-fasL antibodies inhibit AICD
    - fas- or fasL-deficiency→lymphoproliferation/autoimmunity
  - Inappropriate AICD may result in pathological T cell loss in HIV, EBV, VZV

Clonal Expansion/Contraction

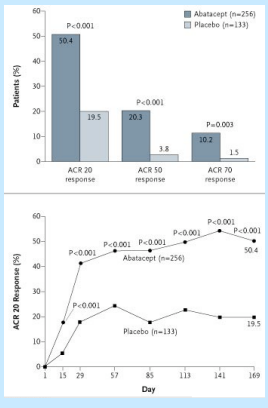
- CTLA-4: Natural break on T cell response
  - Expressed on activated T cells
  - Binds CD80/CD86 with higher avidity than CD28
  - Delivers an inhibitory signal to responder T cell



CTLA-4-Ig: Abatacept (Bristol-Myers-Squibb)



Effectiveness in Rheumatoid Arthritis

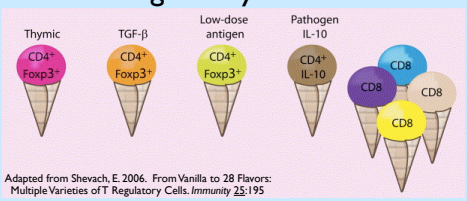


Genovese, MC, et al. 2005. New Engl. J Med. 353:1114.

Peripheral Tolerance:

Mechanisms extrinsic to the responder T cell

Regulatory T Cells



Adapted from Shevach, E. 2006. From Vanilla to 28 Flavors: Multiple Varieties of T Regulatory Cells. *Immunity* 25:195

- Concept of "dominant tolerance" has existed for 40 years
- Since 1995, multiple investigators have identified distinct sets of T_{reg}'s
- The relationship between these varieties *in vivo* remains unclear
- All T_{reg}'s are defined functionally: when mixed with "responder" T cells, T_{reg}'s inhibit the proliferative and/or cytokine response to antigen

Regulatory T Cells

- $CD4^+/CD25^+$ T_{reg}'s*
  - "Natural" or "thymic" - develop in the thymus
  - "Adaptive" or "induced - conversion of naive CD4's in periphery
- Other $CD4^+$
  - T_H3
  - Tr1
- $CD8^+$ - also described but less well established

*CD25 = IL-2Rα chain, required for the high-affinity IL-2R

$CD4^+/CD25^+$ T_{reg}'s

- 1969 - Early thymectomy (day 3-4) → autoimmune organ attack
  - Prevented if mice received splenic $CD4^+$ T cells from a normal adult
- 1995 - Identification by Sakaguchi
  - Transfer T cells into athymic mice→normal immune function
  - Transfer CD25-depleted T cells into athymics→autoimmunity
    - thyroiditis, insulitis, gastritis, adrenalitis, arthritis, etc.
    - short time window to rescue by transfer of $CD25^+$ fraction

$CD4^+/CD25^+$ T_{reg}'s (cont'd)

- 10% of circulating $CD4^+$ cells bear CD25 (<1% of CD8's)
  - Upon triggering with CD3/CD28 crosslinking *in vitro* →
    - no proliferation
    - no secretion of IL-2, IL-4, or IFN-γ
    - contact-dependent inhibition of local "responder" T cells
  - Capable of self-renewal *in vivo*
  - Constitutively express CTLA-4
  - Dependent on IL-2 for maintenance of regulatory phenotype or for survival
    - IL-2-deficiency and CD25-deficiency are both associated with autoimmunity

T_{reg} Function (*In Vitro* Studies)

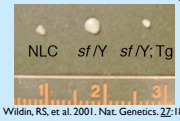
- Require activation via TCR to implement suppression
- Once activated, suppression of other cells is not MHC restricted
- Activated T_{reg}'s mediate "bystander" suppression
- Optimal suppression requires cell contact
- Targets include $CD4^+$ and $CD8^+$ T cells, B cells, MΦ, NK cells, mast cells

The majority (>80%) of circulating T_{reg}'s are of thymic origin

- The nT_{reg} career choice occurs late in thymic development
  - CD4 single positive stage
  - After positive and negative selection, but before exit
- Requirements:
  - TCR triggering by self Ag
  - CD28 ligation by CD80/86
  - IL-2 stimulation (also IL-15)
- Decisive event: activation of FoxP3
  - T_{reg} transcriptional program regulator

FoxP3: Transcription Factor Implicated in Autoimmunity

- Two naturally occurring diseases characterized by lymphoproliferation and autoimmunity
  - Human - IPEX*
  - Mouse - Scurfy



Wildin, RS, et al. 2001. Nat. Genetics. 27:18.

- 2001 - common genetic defect identified: FoxP3
  - Transcription factor that binds DNA using a protein domain termed a "forkhead box" (Fox)
  - Highly conserved between human, mouse, and rat
  - FoxP3-deficient subjects have no $CD4^+/CD25^+$ T cells

*Immune Dysregulation Polyendocrinopathy Enteropathy X-linked

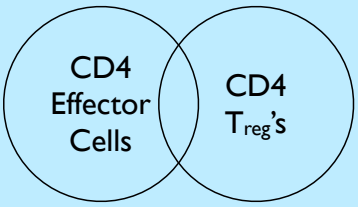
$CD4^+/CD25^+$ "Lineage Marker" FoxP3?

- Possible "master controller" for T_{reg} program
  - FoxP3 represses TCR-triggered IL-2 transcription, induces CTLA-4 and CD25 transcription
  - $FoxP3^+$ function is stable in nT_{reg}'s - transferrable by adoptive transfer
  - Mouse: $FoxP3^+$ T cells are >90% $CD4^+/CD25^+$ and functionally suppressive

What do nT_{reg}'s recognize?

- The natural target antigens of T_{reg}'s are unknown
- TCR receptor usage shows only ~20% overlap between nT_{reg}'s and effector $CD4^+$ T helpers

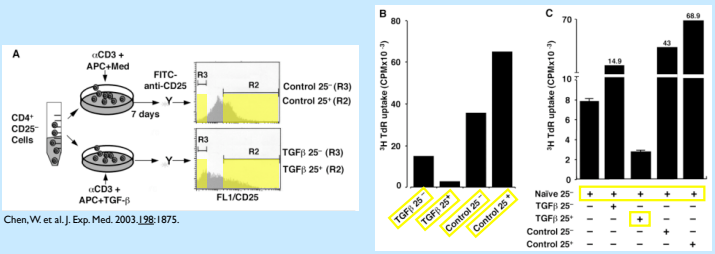
TCR Sequence Profiling



Inducible T_{reg}'s Arise in the Periphery

- Naive $CD4^+$ T cells → $CD4^+CD25^+FoxP3^+$ under the following circumstances:
  - TCR X-linking in presence of TGF-β *(in vitro)*
  - Suboptimal antigen presentation *(in vivo)*
    - Slow infusion low dose soluble antigen *(in vivo)*
    - Injection of antigen targeted to DC's
      - Fusion of Ag with anti-DC antibody
- Requirements
  - CTLA-4
  - IL-2
  - Relative lack of CD28 co-stimulation

TGF-β converts naive $CD4^+CD25^-$ T cells to $CD4^+CD25^+$ T_{reg} cells



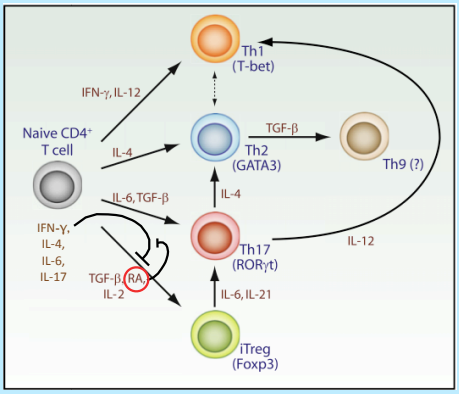
Chen, W. et al. J. Exp. Med. 2003;198:1875.

JEM

GI-Associated Lymphoid Tissue (GALT): A Tolerogenic Environment

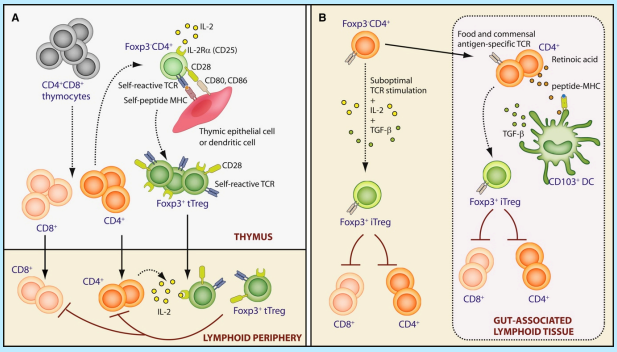
- Lamina Propria DC's balance T_{reg} vs. T_H17
  - Normally low levels of co-stimulation (CD80/86)
  - Secrete TGF-β and retinoic acid
    - RA reduces the sensitivity of naive $CD4^+$ T cells to inflammatory cytokines: IL-6, IFN-γ, IL-17
  - With tissue damage/pathogen invasion
    - Increase production of IL-6
    - Convert from T_{reg} generation to T_H17 generation

CD4+ T Cell Lineage Plasticity



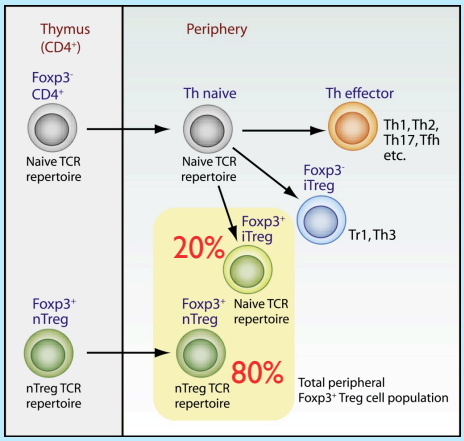
Zhou, et al. 2009. Immunity. 9:646.

Origins of T_{reg} Cells



Josefowicz. 2009. Immunity. 30:616.

Origins of T_{reg}'s (cont'd)



Lafaille. 2009. Immunity. 30:626

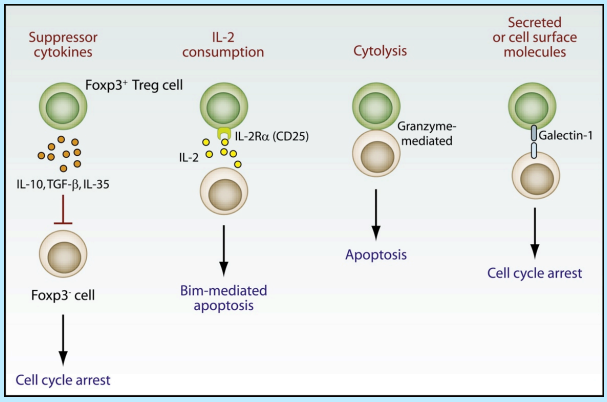
nT_{reg} - iT_{reg} Differences

Box 1. Differences between nTreg and iTreg Cells

		nTreg cell	iTreg cell
Generation:	Tissue	Thymus	GALT, spleen, lymph node, inflamed tissue
	Costimulation requirement	CD28	CTLA-4
	Cytokine requirement	TGF-β (?) IL-2 or IL-15	TGF-β IL-2
Specificity		Self (?)	Allergens, commensal microbiota, neoantigens (tumor), alloantigens, self (inflammation)

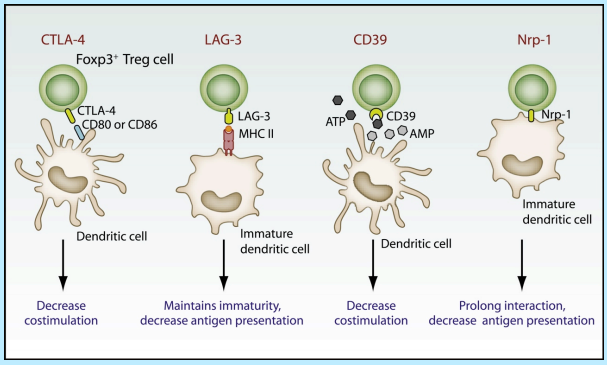
Lafaille. 2009. Immunity. 30:626

Proposed T_{reg} Suppressive Mechanisms: Direct



Shevach. 2009. Immunity. 30:636

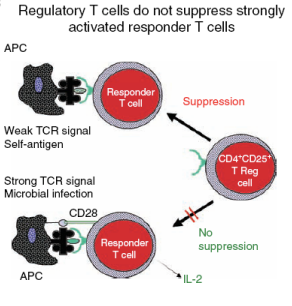
Proposed T_{reg} Suppressive Mechanisms: Indirect



Shevach. 2009. Immunity. 30:636

T_{reg} Mechanisms (cont'd)

Suppression is over-ridden by strong stimulus



Adapted from Baecher-Allan, C. 2006. Human Regulatory T Cells and Their Role in Autoimmune Disease. *Immunological Reviews* 212:203.

Other $CD4^+$ Regulatory T Cells

- Antigen-specific $CD4^+CD25^-$ T cells with regulatory effects can be generated under specific circumstances:
  - Oral administration of antigen → TGF-β-secreting $CD4^+$ T cells termed T_H3 cells*
  - Stimulation of $CD4^+$ T cells with antigen *in vitro* in the presence of IL-10 yields a regulatory T cell that secretes predominantly IL-10, and some TGF-β (termed Tr1 cells*)

*Nomenclature here is not important. Simply be aware that other $CD4^+$ regulatory T cells have been described, and appear to function via secretion of TGF-β and/or IL-10

Summary

1. Self and non-self are *relative* terms. For the lymphocyte, "self" is best defined as those antigens present during the cell's development (in bone marrow or thymus). By definition, thymocyte development can only be "molded" on self-antigens. Every mature T cell has a narrow range of TCR avidities for self but a wide range of avidities for foreign proteins.
2. T cells emerging from the thymus may have high enough avidity for self proteins as to permit activation under circumstances of high antigen concentration and co-stimulation. Peripheral tolerance mechanisms are therefore critical to dampening the self-reactivity of T cells.
3. Responder-intrinsic tolerance mechanisms include *ignorance* (low TCR avidity, low Ag conc.); *anergy* ("signal 1 w/o signal 2" → unresponsiveness); and *AICD* (T cell death due to increased sensitivity to apoptosis following activation; fas-dependent). CTLA-4 is critical to maintenance of T cell homeostasis (overall cell number), and may also function in AICD. CTLA-4-Ig harnesses this regulatory function for therapeutic use. Dysfunction in native fas or CTLA-4 lead to lymphoproliferation/autoimmunity.

Summary

4. Responder-extrinsic tolerance mechanisms are those mediated by committed regulatory cells. The most widely studied of these are $CD4^+/CD25^+$ T_{reg}'s, a lineage characterized by FoxP3 expression. These cells arise in both thymic development (natural) and in the periphery (adaptive) and require IL-2 for survival. The mechanism by which they target responder cells in unknown. Contact-dependent and -independent mechanisms of suppression have been implicated, involving CTLA-4 and TGF-β.
5. Other $CD4^+$ regulatory cells include T_H3 cells (oral antigen; TGF-β-secreting); Tr1 cells (antigen stim. in presence of IL-10; IL-10-producing).