# Regulatory T Cells & Tolerance

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## Regulatory T Cells and Maintenance of Tolerance

- Tolerance: *Lack* of an adaptive immune response to Ag despite exposure of lymphocytes to that Ag
  - Where is tolerance important?
    - Discrimination of non-self from self
      - e.g., virally infected from normal cell
    - Discrimination of harmless from dangerous
      - e.g., egg white from E. coli proteins in spoiled mayonnaise
  - Ideal: Focus response on dangerous non-self
  - Failure: autoimmunity, allergy, transplant rejection

# Autoimmunity:

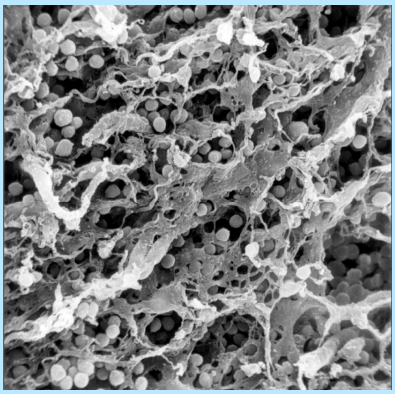
Distinguishing native tissue from foreign pathogen

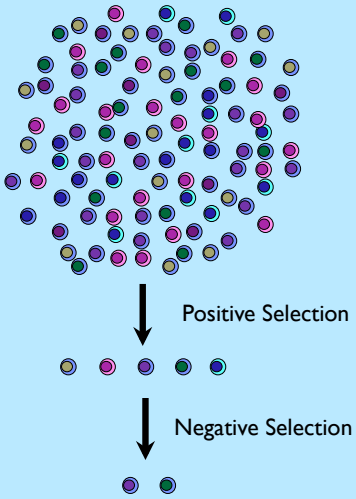
- Innate System - inherent in the receptors
  - Directed at microbial molecules (PAMP's)
- Adaptive System - not inherent in the receptors
  - Able to bind anything - protein, carbohydrate, lipid
  - Need safeguards to ensure non-reactivity with native (self) molecules - that is, to maintain *tolerance*

# 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)

## Affinity and Avidity

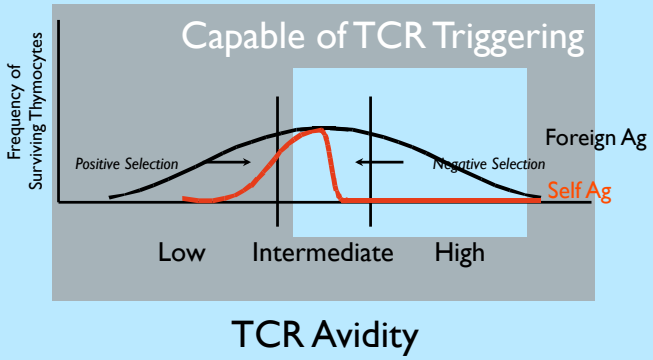
- "Affinity"– binding strength of one receptor to one ligand
- "Avidity"– aggregate binding strength of multiple receptors on one surface to ligands on another


- TCR avidity contributes to T cell receptor triggering:
  - High avidity - triggered at low antigen concentrations

## 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)

## Thymocyte Selection By TCR Avidity



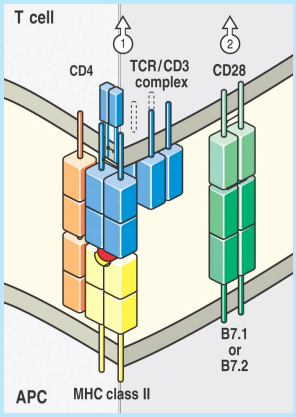
## Thymic Self-Representation

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

## Peripheral Tolerance:

Mechanisms intrinsic to the responder T cell

- Successful T cell activation requires two signals:




Signal 1 - TCR crosslinking
- Antigen-specific

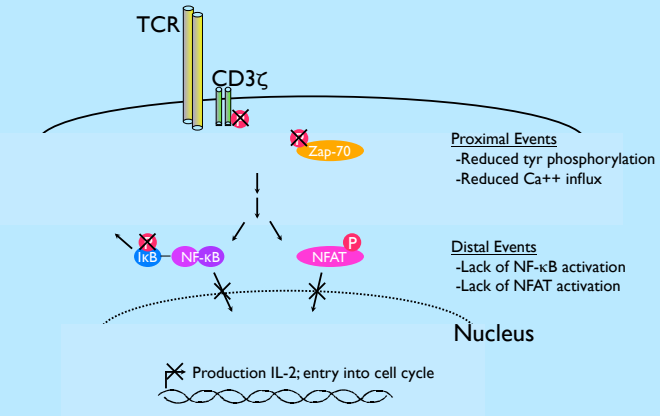
Signal 2 -
Provided by APC, induced to mature by "danger" signals (eg., TLR signals)

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

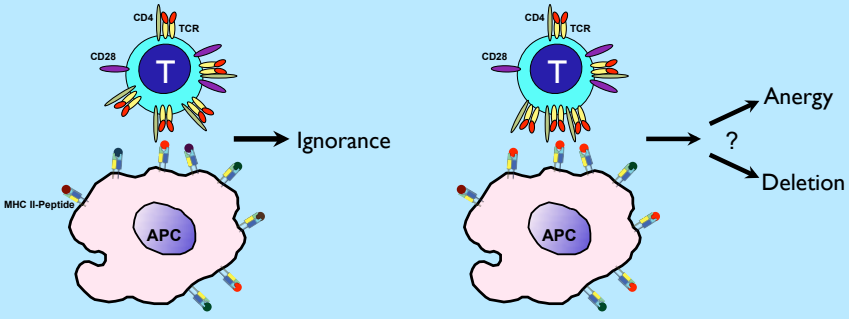



After Davidson, A. "Regulatory T Cells and Maintenance of Tolerance" (2006) P&S Pathophysiology Course: Immunology

# Other Mechanisms of Peripheral Tolerance Intrinsic to the Responder T cell

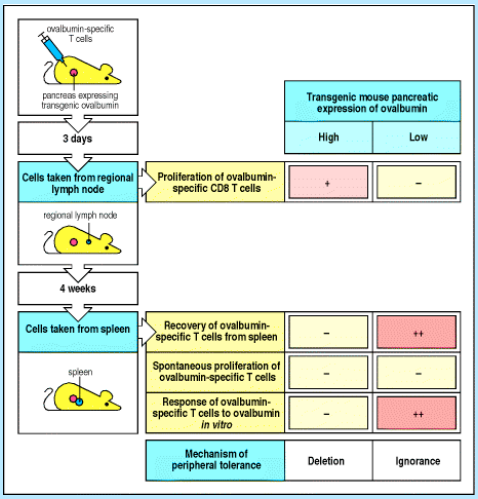
- Ignorance: Low TCR avidity for Ag/MHC and/or low antigen abundance result in failure to produce "signal 1"
  - Example: anatomically "privileged" Ags: CNS, eye (sympathetic ophthalmia)

- Clonal Deletion: High antigen density - persistent TCR triggering in the absence of costimulation



# Peripheral Tolerance:

Mechanisms intrinsic to the responder T cell

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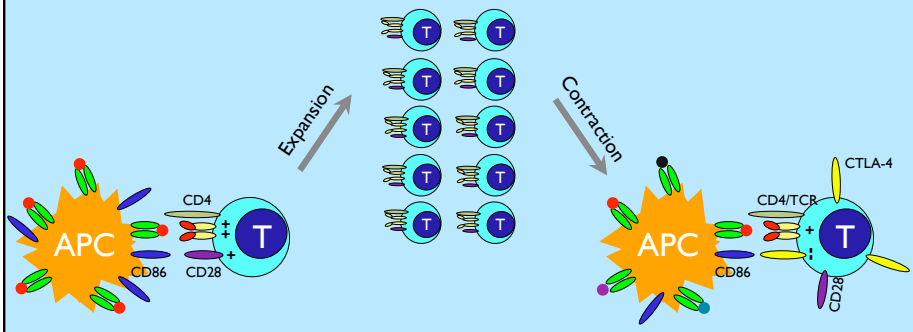
# Activation-Induced Cell Death (AICD)

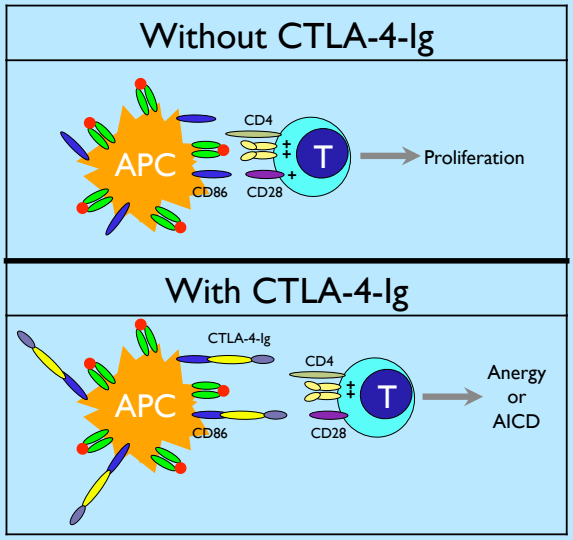
- Elimination of clonally expanded effector T cells
  - Terminal phase of an antigen response ⇨ homeostasis
  - With persistent or repeated TCR triggering ⇨ tolerance
- Cell death is apoptotic - largely fas mediated
  - fas is upregulated following T cell activation
  - fas- or fasL-deficiency→lymphoproliferation/autoimmunity
  - anti-fas or anti-fasL antibodies inhibit AICD (animal model)
- Inappropriate AICD has been proposed to account for pathological T cell loss in HIV, EBV, Varicella

# Clonal Expansion/Contraction

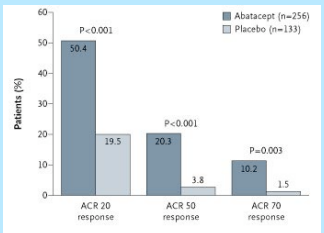
- CTLA-4: Natural brake on T cell response
  - Expressed on activated T cells
  - Binds CD80/CD86 with higher avidity than CD28
  - Delivers an inhibitory signal to responder T cell

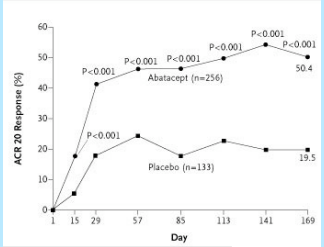



# CTLA-4-Ig: Abatacept (Bristol-Myers-Squibb)




Effectiveness in Rheumatoid Arthritis



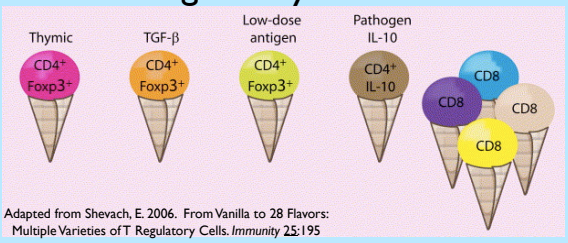




Genovese, MC, et al. 2005. New Engl. J Med. 353:1114.

# 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" - thymus-derived
  - "Adaptive" - conversion of naive CD4's in periphery
- Other $CD4^+$
  - $T_H3$
  - Tr1
- $CD8^+$ - Avidity model of peripheral $T_{reg}$'s

*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)

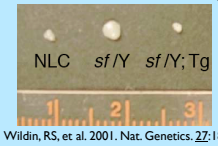
- 10% of circulating mouse $CD4^+$ cells bear CD25 (<1% of CD8's)
  - Upon triggering with CD3/CD28 crosslinking *in vitro:*
    - no proliferation
    - no secretion of IL-2, IL-4, or IFN-$\gamma$
    - contact-dependent inhibition of local "responder" T cells
  - Capable of self-renewal *in vivo*
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

## "Natural" $T_{reg}$'s Arise in the Thymus

- Proposed "third role" of the thymus (in addition to positive and negative selection)
  - Alternative to cell death for thymocytes with significant avidity for self
    - Thymocyte TCR triggering is required for $T_{reg}$ differentiation
    - CD28 costimulation is required for normal $T_{reg}$ levels
    - Third, $T_{reg}$-specific signal - postulated
  - $T_{reg}$ induction is presumably driven by self recognition, however the $T_{reg}$ repertoire is unknown

## 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

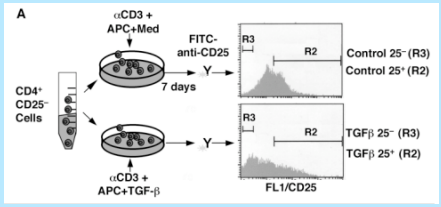
## $CD4^+/CD25^+$ "Lineage Marker" FoxP3?

- Possible "master controller" for $T_{reg}$ program
  - FoxP3 represses TCR-triggered IL-2 transcription, induces CTLA-4 and CD25 transcription
  - $FoxP3^+$ $T_{reg}$ function is stable - transferrable by adoptive transfer from one individual to another (in mice)
  - Mouse: $FoxP3^+$ T cells are >90% $CD4^+/CD25^+$ and functionally suppressive

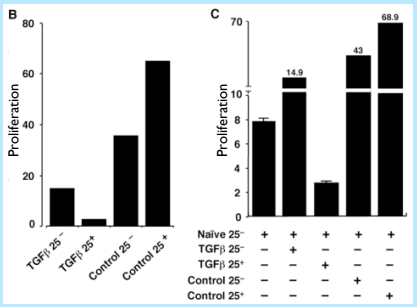
## "Adaptive" $T_{reg}$'s Arise in the Periphery

- Naive $CD4^+$ T cells ⇨ $CD4^+CD25^+FoxP3^+$ under the following circumstances:
  - TCR X-linking in presence of TGF-β *(in vitro)*
  - Slow infusion low dose soluble antigen *(in vivo)*
- Adaptive $T_{reg}$ function
  - Mouse - potent suppressors of responder T cells (indistinguishable from natural $T_{reg}$'s)
  - Human - despite FoxP3 expression, suppression less consistently demonstrated; role unclear

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




Chen, W. et al. J. Exp. Med. 2003.198:1875.



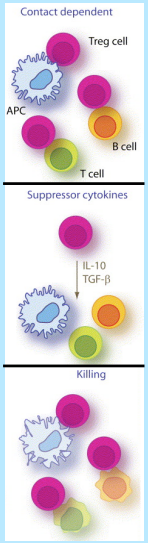



JEM

## Proposed Mechanisms of $CD4^+/CD25^+$ $T_{reg}$ Suppression

- CTLA-4 contact: "reverse signaling" through CD80/CD86
- Secreted or surface IL-10 and TGF-β
- "Soaking up" of local IL-2
- Killing via perforin/granzyme

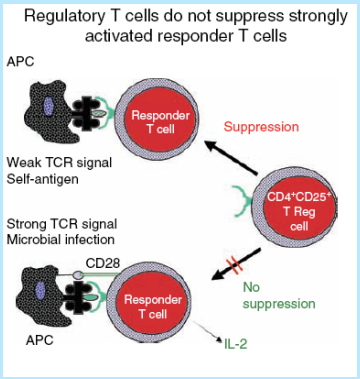
- Not clear if TCR triggering is required to activate suppressive function




Adapted from Shevach, E. 2006. *Immunity* 25:195

# $T_{reg}$ 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 $CD4^+$ T cells with regulatory effects (but no CD25) 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

## $CD4^+$ $T_{reg}$ as a Phenomenon

- Strengths
  - Clear suppression of effector T cells
  - Reproducible by multiple investigators
- Weaknesses
  - Mechanism of "call to action" undefined
    - How are inappropriate T cell responses recognized?
  - Mechanism of suppressive effect remains undefined
  - Many of the defining principles derived in mice
    - Human $\stackrel{?}{=}$ Mouse

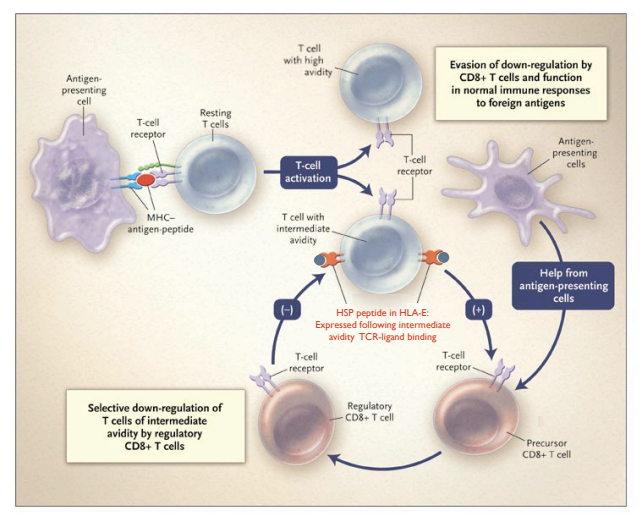
## Emerging Model of T Cell Regulation: Self vs. Non-self Discrimination

- Key Facts - we know that:
  - TCR avidities for foreign antigen range low to high
  - but avidities for self are restricted (thymic selection)
  - The T cell can "sense" avidity - basis of thymic selection
- Problem: Intermediate avidity self-reactive T cells in the periphery may encounter circumstances with high enough levels of self Ag presentation and costimulation to become activated ⇨ autoimmunity

## T Cells Self-Police

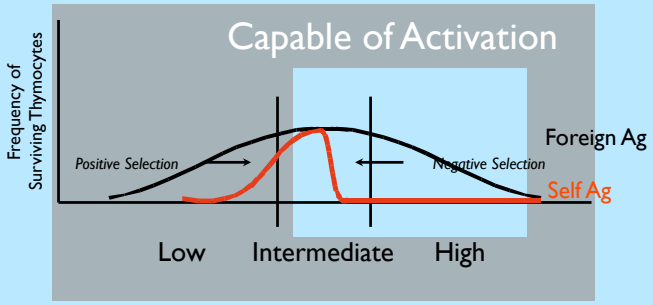
- CD4+ T cells triggered following intermediate avidity interaction with MHC/peptide "flag" themselves
  - Based only on avidity - completely independent of what the Ag actually is
  - One of these "flags" has been identified: peptide derived from HSP-60 displayed on the cell surface in the groove of an HLA-I like molecule called HLA-E
- $CD8^+$ $T_{regs}$ recognize $HSP\text{-}60^+$ $CD4^+$ effector cells and target them for suppression

## Avidity Model of T Cell Regulation




Jiang, H. & Chess, L. 2006. Regulation of Immune Responses by T Cells. *New England Journal of Medicine* 354:1166.

# Selection By TCR Avidity:
## Central & Peripheral Repertoire Molding




TCR Avidity

# Selection By TCR Avidity:
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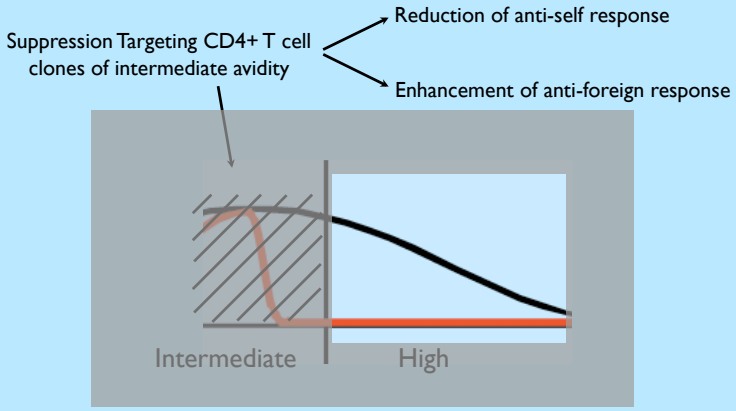

## Summary

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). 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 that appears to require 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-$\beta$.
5. Other $CD4^+$ regulatory cells include $T_H3$ cells (oral antigen; TGF-$\beta$-secreting); Tr1 cells (antigen stim. in presence of IL-10; IL-10-producing).
6. One population of regulatory T cells that expresses CD8 appears to target $CD4^+$ responder cells that have been activated by a TCR–antigen/MHCII interaction of *intermediate* avidity. This mechanism, so far demonstrated most definitively in mice, is the one mechanism to date that provides a rationale for self/non-self discrimination on a functional level.