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
Regulator Cell Extrinsic ("Dominant")
Regulatory (Suppressor) T Cells

Central Tolerance: Thymic Selection

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

- Capable of TCR Triggering
  - Frequency of T cell Phenotypes
    - Low
    - Intermediate
    - High
  - Positive Selection
  - Negative Selection

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

T Cell Anergy

- T cell unresponsiveness despite TCR engagement in the presence of costimulation

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, eyes (sympathetic ophthalmia)
- Clonal Deletion: High antigen density - persistent TCR triggering in the absence of costimulation
Peripheral Tolerance: Mechanisms intrinsic to the responder T cell

- **Activation-Induced Cell Death (AICD)**
  - Elimination of clonally expanded effector T cells
  - Terminal phase of an antigen response to homeostasis
  - With persistent or repeated TCR triggering to 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)**
  - Without CTLA-4-Ig
  - With CTLA-4-Ig

- **Peripheral Tolerance: Mechanisms extrinsic to the responder T cell**
  - Concept of “dominant tolerance” has existed for 40 years
  - Since 1995, multiple investigators have identified distinct sets of T<sub>reg</sub>'s
  - The relationship between these varieties in vivo remains unclear
  - All T<sub>reg</sub>'s are defined functionally: when mixed with “responder” T cells, T<sub>reg</sub>'s inhibit the proliferative and/or cytokine response to antigen

- **Regulatory T Cells**
  - CD4<sup>+</sup>/CD25<sup>+</sup> T<sub>reg</sub>’s*
    - “Natural” - thymus-derived
    - “Adaptive” - conversion of naive CD4<sup>+</sup> in periphery
  - Other CD4<sup>+</sup>
    - T<sub>17</sub>
    - T<sub>1</sub>
  - CD8<sup>+</sup> - Avidity model of peripheral T<sub>reg</sub>’s

*CD25 = IL-2R<sub>α</sub> chain, required for the high-affinity IL-2R
CD4+/CD25+ Treg'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

CD4+/CD25+ Treg's (cont’d)

- 10% of circulating mouse CD4+ cells bear CD25 (<1% of CD8s)
- 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 CD25 expression and regulatory phenotype
- IL-2-deficiency and CD25-deficiency are both associated with autoimmunity

“Natural” Treg’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 Treg differentiation
- CD28 costimulation is required for normal Treg levels
- Third Treg-specific signal - postulated
- Treg induction is presumably driven by self recognition, however the Treg repertoire is unknown

FoxP3: Transcription Factor Implicated in Autoimmunity

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

- 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 Treg program
- FoxP3 represses TCR-triggered IL-2 transcription, induces CTLA-4 and CD25 transcription
- FoxP3+ Treg 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” Treg’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 Treg function
  - Mouse - potent suppressors of responder T cells (indistinguishable from natural Treg’s)
  - Human - despite FoxP3 expression, suppression less consistently demonstrated; role unclear
TGF-β converts naive CD4+CD25− T cells to CD4+CD25+ Treg cells

Proposed Mechanisms of CD4+/CD25+ Treg 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

Treg Mechanisms (cont’d)

• Suppression is over-ridden by strong stimulus

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 Th3 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*)

CD4+ Treg 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 ≠ 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

* 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.
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+ Tregs recognize HSP-60+ CD4+ effector cells and target them for suppression

Avidity Model of T Cell Regulation

Selection By TCR Avidity:
Central & Peripheral Repertoire Molding

TCR Avidity

Capable of Activation

Foreign Ag

Low

Intermediate

Self Ag

High

Suppression Targeting CD4+ T cell clones of intermediate avidity

Reduction of anti-self response

Enhancement of anti-foreign response

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 leads to lymphoproliferation/autoimmunity.
4. Responder-extrinsic tolerance mechanisms are those mediated by committed regulatory cells. The most widely studied of these are CD4+CD25+ Tregs, 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 is unknown. Contact-dependent and -independent mechanisms of suppression have been implicated, involving CTLA-4 and TGF-β.
5. Other CD4+ regulatory cells include Th3 cells (oral antigen; TGF-β-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.