Immune Regulation and Tolerance

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Regulation of Immune Responses

Innate Immunity:
NK cells
Mast cells
Dendritic cells

Cellular Immunity:
Central tolerance
Peripheral Tolerance
Clonal Downsizing
T regulatory cells

Humoral Immunity:
Antibody Regulation
B regulatory cells

Can these be manipulated to promote tolerance?
Innate Immunity: Mast cells

- Mast cells are required for skin graft tolerance in some animal models
- Mast cells secrete MCP6 (protease) that cleaves IL-6, reducing inflammation
- Tregs secrete IL-9, mast cell growth factor, that prevents mast cell degranulation


NK Cells

- Rejection promoting functions:
  - Killing of Tregs or immature DCs
  - Activation of recipient T effector cells
- Tolerance promoting functions:
  - Killing of donor DCs
  - Inhibition of T effector cells by IL-10 secretion or competition for IL-15
Dendritic Cell Regulation

• Most efficient & versatile APC.
• Widespread tissue distribution → continuous sampling of self and exogenous Ags.
• Only APC that can stimulate naïve T cells.
• DCs function in both central & peripheral tolerance.

Dendritic Cell Subtypes

• Heterogeneous population found in thymus, lymphoid tissue, circulation, peripheral tissues
• Multiple DC subsets; 2 major human types:
  – “conventional” myeloid DC – APC when activated by inflammation, TLR signals, and AG uptake, migrate to lymph nodes → induce adaptive immunity
  – plasmacytoid DC – recognize ssRNA, ssDNA through TLR7, TLR9; secrete IFNα in innate responses; poor immunostimulatory ability favors development of T regs
**Myeloid DC Maturation**

- **Immature DC**
  - High endocytosis, phagocytosis capacity
  - Low levels of MHC class II, CD40, CD80, CD86

- **Mature DC**
  - High levels of MHC class II, CD40, CD80, CD86
  - Strong co-stimulation of T cells
  - Highly migratory via lymphoid homing receptor, CCR7
  - Secrete IL-12 → Th1

**DC Maturation**

- **“Semi-mature” DC**
  - Generated in vitro in presence of TNFα, IL-10 + TGFβ, or dexamethasone, Vitamin D3
  - Intermediate levels of MHC class II, CD40, CD80, CD86
  - Express inhibitory molecules ILT3, PD ligand 1; PD-L1 helps induce T regs

ILT3 = immunoglobulin-like transcript; PD L1 = programmed death ligand 1
Tolerogenic Dendritic Cells

Central tolerance
- Intrathymic DC
  - Negative selection
  - Generation of naturally occurring CD4+CD25+ T regs

Peripheral tolerance
- In absence of inflammation, maintain peripheral tolerance to self-Ags
- Induce T cell depletion, anergy
- Express immunomodulatory molecules
- Secrete immunosuppressive factors

Generation of tolerogenic DC

- Regulation depends on state of DC maturation:
  - Immature DC are tolerogenic
  - Deliver only Signal 1
- “Alternatively activated” monocyte-derived DC → generated by exposure to vitamin D3 and dexamethasone
- RNAi → can generate Rel-B silenced tol DC (Rel B = transcription factor for NFκB)
Tolerogenic Mechanisms

Suppressive factors:
- Cytokines IL-10, TGFβ
- IDO – indolamine dioxygenase: IDO depletion of tryptophan inhibits T cell proliferation
- PD-L1 binds to PD-1 receptor on activated T cells
- Ho-1 – heme oxygenase-1 degrades heme and inhibits inflammation

*In vitro* generated Tol DC: treated with dexamethasone or Vitamin D3 + IL-10; ± donor Ag


Considerations for DC Therapy

**Infusion**
- **Time**: Pre-Tx, peri-Tx, or post-Tx
- **Dose**: cell number per recipient body weight
- **Route**: Intravenous or subcutaneous
- **Frequency**: Once or several infusions
- **Optimum combined immunosuppression**

**Culture and preparation**
- Flasks or bags
- mDC or pDC
- Treatment e.g., maturation resistance
- Verification of phenotype i.e., immature or semi-mature
- Pulsing with donor antigen

**Recovery**
- Donor or recipient
- Bone marrow or blood (apheresis)
- Freshly isolated or stored product

Central Tolerance: Thymic Selection (Education)
Steps in Thymic Education/Selection

- T cell precursors, CD4-CD8- (Double Negative) enter sub-capsular region, rearrange genes.
- DP (CD4+ CD8+) cells proliferate and undergo **Positive** selection in cortex.
- Transition to single + cells, CD4+ or CD8+.
- **Negative** selection at cortico-medullary junction – DC induce negative selection and induce CD4+Cd25+ FoxP3 T regs
- Mature, self-tolerant cells exit from medulla.

Self-Restriction & Self- Tolerance

Positive selection ensures that mature T cells are self-MHC restricted.
Negative selection deletes cells with TCR which would be auto-reactive.
>95%, of precursor T cells fail positive or negative selection → apoptosis.
Signals from TCR:MHC direct both:
  + signal → maturation
  - signal → apoptosis
Affinity Model for Thymic Selection

Selection- Regulatory Events

Key regulatory components:
1. RAG genes
2. bcl genes
   pro-apoptotic: Bax, Bad
   pro-survival: bcl-2, bcl-x
3. Caspases (cysteine proteases)

Positive Selection: RAG↓↓, bcl-2↑↑
Negative Selection: Bax ↑↑, Caspases ↑↑

Extrathymic Development

- Thymus involution and atrophy with age → decreased thymic education and selection.
- With older BMT patients, deficient education of stem cells → non-tolerant donor-derived T cells → GVHD.
- In adults, skin may be alternate, epithelial differentiation site.

B Cell Selection & Regulation

- Occurs in bone marrow for immature B cells (SIgM+).
- Exposure to membrane self antigens → apoptosis (≤75%).
- Exposure to soluble self antigens → anergy.
- Cells not deleted or inactivated → mature as IgM+, IgD+ B cells.
- After somatic hyper-mutation, self-reactive B cells are controlled by T cells (by lack of T_{m,2} cells with auto-antigenic specificity).
Peripheral Tolerance: Mechanisms

Homeostasis – Immune System

Migration from BM, Thymus

Receptor engagement

Naïve Cells

No receptor engagement

Apoptosis

Before Ag Exposure

Ag + 2nd Signals

Activation

Differentiation

Effectors

Clonal Downsizing

Survival Signals

Memories

Maintenance of memory cells

Feedback Mechanisms

Inhibition

Apoptosis

After Ag Exposure
Clonal Downsizing

• Lymphocyte proliferation must be controlled to maintain homeostasis
• Occurs through programmed self-destruction by apoptosis
  – Apoptosis - mediated by caspases; results in nuclear condensation, DNA cleavage, intact plasma membrane, apoptotic bodies
  – Pyroptosis – inherently inflammatory; requires caspase 1 (not involved in apoptosis); results in rapid plasma membrane rupture; triggered by infection, stroke, heart attack, cancer

Clonal Downsizing

Passive cell death
• Apoptosis following cytokine withdrawal resulting from AG clearance, decrease in activation signals
• Does not require death receptor

Activation Induced Cell Death
• Apoptosis stimulated through TNF-related receptors (eg. Fas, Fas L)
• Receptors are up-regulated after prolonged AG stimulation.
• KIR on T cells may also contribute to AICD
One Model for PCD

Following cessation of APC interaction with T cells, class II molecules re-distribute to non-lipid rafts → activate apoptosis signals. Decreased production of cytokines evokes PCD in T cells.


Activation Induced Cell Death

Calcineurin signal pathway induces Fas expression; after several days of activation, Fas:FasL interaction → T cell apoptosis. Clonal downsizing may be critical for tolerance.
Regulation via Negative Signals

ITIM receptors are found on many cell types:

- FcγRIIB: IgG receptor on T, B, & mast cells
- CTLA-4 (CD152): T cells
- KIR, NKG: NK cells
- KIR: Subset of T cells
- ILTs, LAIRS: Various cells

ITAM v.s. ITIM Receptor Regulation

Factors affecting ITAM:ITIM Balance:

- Ag/Aby concentration
- Duration of stimulation
- Level of receptor expression:

<table>
<thead>
<tr>
<th>Initial High [AG]</th>
<th>Prolonged AG</th>
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<tbody>
<tr>
<td>on    off</td>
<td>off on</td>
</tr>
<tr>
<td>TCR   CTLA-4</td>
<td>TCR       CTLA-4</td>
</tr>
<tr>
<td>↑      ↑</td>
<td></td>
</tr>
<tr>
<td>ZAP-70 SHP</td>
<td>ZAP-70     SHP</td>
</tr>
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</table>
T Subsets: KIR Regulation

Inhibition or Lack of Co-Stimulation

Soluble allo- MHC molecules – “oral tolerance”.

Belatacept: 2nd Generation CTLA4-Ig
2x \( \uparrow \) affinity for CD80 (B7.1)
4x \( \uparrow \) affinity for CD86 (B7.2)
Yin and Yang: Co-Stimulation

Positive Co-Stimulatory Pathways
CD28, CD40, CD70, ICOS, CD134

(+) Proliferation
Cytokine Synthesis
T helper differentiation

(-) Anergy
Apoptosis
Regulation

Negative Co-Stimulatory Pathways
CTLA-4, PD-1

T Regulatory Cells

- Provide suppression of immune responses
- Multiple types, defined by various markers and cytokines produced.
  - CD4+ T\textsubscript{reg} (natural/thymic, adaptive)
  - CD8+CD28- T\textsubscript{sup}
  - CD1 restricted CD8+ T\textsubscript{reg}
  - NKT\textsubscript{reg}
CD4+ T Regulatory Cells

- **CD4+CD25+FoxP3+ T regs** (natural, nTregs)
  - Naturally occurring; generated in thymus
  - 5-10% of CD4+ T cells
  - FoxP3+ expression (forkhead-winged transcription factor)

- **Adaptive CD4+CD25- T regs** (aTregs)
  - Develop in periphery from naïve CD4+CD25-T cells; acquire transient FoxP3 expression
  - Different subsets of adaptive T regs induced depending upon activation conditions
    - CD4+CD25+FoxP3+
    - Tr1
    - Th3 found in mucosal immune system

Development Pathways: T regs

Kand SM, Tang Q, Bluestone JA. AJT.2007;7:1457
Possible T reg Mechanisms for Suppression
Possible T reg Mechanisms for Suppression

- Anti-CD3, ATG – global depletion of T cells may favor enrichment of TGFβ+ T regs
- Anti-CD52 (alemtuzumab) – after profound depletion, possible enrichment of T regs, but may also expand memory T cells
- mTOR inhibitors (rapamycin, everolimus); some evidence that rapamycin promotes selection and/or survival of T regs

**mTOR Pathways and FoxP3 Expression**

- Both nTreg and aTreg require stable FoxP2 expression
- Induction of FoxP3 requires SMAD3/4 activated by TGFβ signals
- Rapamycin inhibits mTORC1 and ± mTORC2

Chi H, Nat Rev Immunol, April 20, 2012

**Possible Approaches for Adoptive T reg Cell Therapy**

1. Isolation and *in vitro* expansion of nT regs
   - Markers for isolation: CD4+CD25hi +CD45RA+ (eliminates T memory)
2. Generation of aT regs by stimulation with donor APC in presence of TGFβ and IL-2
3. Generation of T regs by ectopic gene expression
   - Stimulate with donor APC, then transduce responding T cells with FoxP3

T memory cells: Barrier to Tolerance

- Heterogeneity within T cell memory → high frequencies of alloreactive T memory cells
  - Viral cross reactivity
  - Homeostatic proliferation after lymphocyte depletion

- Resistance of memory T cells to tolerance induction strategies
  - ↓ requirement for both TCR and Co-stimulation signals; lower activation threshold
  - ↑ levels of survival factors: Bcl-2
  - ↑ resistance to CD4+CD25+FoxP3 T regs


Down Regulation of Cellular Immune Responses

- Anergy
  - CD28
  - Ag Stimulation c/o 2nd signal → Functional Unresponsiveness

- Anergy
  - CTLA-4
  - CTLA-4: B7 interaction

- AICD/PCD
  - Fas: FasL interaction - AICD
  - Ag clearance-PCD

- Cell/Cytokine regulation
  - Cytokines
  - T & B Regulatory Cells
  - TGFβ, IL-10, IL-4, IL-13
  - Inhibition of proliferation & effector functions; Influence DC Maturation

- Apoptosis
  - B Regs
  - T Regs

Inhibition of effector functions; Influence DC Maturation
Regulation by Components of Humoral Immunity

Antibody and B cells

Antibody Regulation
Immune Complexes

Idiotypic Regulation

Aby 1 → Aby 2 → Aby 3 Etc…
Effector & Regulatory B Cells
Modulators of CD4+ T Cell Functions

- B cell depletion is effective treatment in some T cell mediated auto-immune disorders (MS, IDDM, RA, SLE)
- Effects of Rituximab (anti-CD20)
  - ↓ proportions of auto-reactive T cells, sparing other non-auto T cells
  - ↑ frequency of CD4+CD25+ FoxP3 T regs

Evidence for B regs in Transplantation

- CD20+ B cells found in renal biopsies classified as acute cellular rejection (Sarwal M, et al. Transplant. 2008;85:1705)
- Depletion of B cells from non-sensitized recipients may ↑ T cell rejection. (Clatworth, et al. NEJM 2009;360:2683.)
Functions of Effector & Regulatory B Cells

• Antigen Presentation & Co-simulation
  – B cells are very effective APC when antigen concentration is low
  – Co-stimulate via CD80, CD86

• Cytokine Production
  – Subsets, based on cytokine profiles, can augment T cell responses or down-regulate them

Effector / Regulatory B Cell Subsets

1. Be1 – derived from naïve B cells primed in presence of Th1 cytokines
   – Secrete IL-10, IL-6, TNFα, IFNγ
2. Be2 – derived when primed in presence of Th2 cytokines
   – Secrete IL-2, IL-4, IL-13
3. B reg (also called B10) – when primed by antigen, CD40L, and/or TLR
   – Secrete IL-10

Roles Of Effector / Regulatory B Cells


Regulatory Immune Cell Populations

<table>
<thead>
<tr>
<th>Cell Population</th>
<th>Surface Markers</th>
</tr>
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<tbody>
<tr>
<td>T regs</td>
<td>CD4+CD25hiCD127loFOXP3+</td>
</tr>
<tr>
<td>B regs</td>
<td>CD19+CD20+CD27-CD38hiIgDhiIgMhi</td>
</tr>
<tr>
<td>Transitional B cells</td>
<td><img src="image1.png" alt="Image" /></td>
</tr>
<tr>
<td>Regulatory Macrophages</td>
<td>No specific markers defined</td>
</tr>
<tr>
<td>Plasmacytoid DCs</td>
<td>CD4hiCD8-CD11cdisCD45RAhiMHC cl-IIlow</td>
</tr>
<tr>
<td>Myeloid derived suppressor cell</td>
<td>CD11b+CD33+CD34+HLA-DRlow</td>
</tr>
<tr>
<td>Granulocyte-like MDSCs</td>
<td>CD15+CD33+HLA-DRlow</td>
</tr>
<tr>
<td>Monocyte-like MDSCx</td>
<td>CD14+CD33+HLA-DRlow</td>
</tr>
<tr>
<td>Mesenchymal stromal cells</td>
<td><img src="image2.png" alt="Image" /></td>
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Neuroendocrine Regulation

Possible Links

• CNS control of hormones:
  – Corticosteroids, growth hormone, adrenaline, thyroxine, prolactin
• Lymphocyte receptors for hormones:
  – Steroids, adrenalin, noradrenaline, enkephalins, endorphins
• Immunosuppressive effects:
  – Corticosteroids, endorphins, enkephalins
• IL-1, IL-6 stimulation of adrenal corticosteroid production
Sternberg EM. Nat Rev Immunol. 2006;3:18