T Cell Tolerance

Mark Anderson, MD, PhD
UCSF Diabetes Center
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Immunological tolerance

• Definition:

• Significance:
THE PHENOMENON OF TOLERANCE:

Seminal observations in 1945 by R.D. Owen that cattle dizygotic twins display red cell (chimerism/mosaicism) in adult life.

Owen interpreted that placenta of cattle dizygotic twins undergo anastomosis early in fetal life permitted blood cells and their precursors to move from one twin to the other.
Tolerance is acquired.

eg. Dizygotic twins in cattle:

 Shared placental circulation in utero, resulting in interchange of hemopoietic stem cells

Adults accept grafts or transfusions from twin, despite the fact that the donor cells bear genetically dissimilar erythrocyte and MHC alloantigens

Fig. 2: Dizygotic twins in cattle.
OBITUARY

Ray D. Owen
1915–2014

Michael P Cancro

NATURE IMMUNOLOGY VOLUME 15 NUMBER 12 DECEMBER 2014
THE PHENOMENON OF TOLERANCE:

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In 1953, BILLINGHAM, BRENT, and MEDAWAR demonstrated that immunological tolerance could be acquired by introduction of donor cells during fetal development - tolerance permanent and Ag specific
THE TECHNIQUE OF FREE SKIN GRAFTING IN MAMMALS

BY R. E. BILLINGHAM* AND P. B. MEDAWAR

From the Department of Zoology, University of Birmingham

(Received 2 March 1951)

(With Plates 5–7 and One Text-figure)

J. Exp. Biol. 28, 385-402
Type C: reciprocal interchange of grafts between animals grouped in pairs. Such a test may be used (except with cows; see below) to distinguish monozygotic from dizygotic twins or to decide whether a breeding pair are sufficiently alike to be chosen as the parents of the succeeding generation of an inbred strain. For this second purpose the test is far from exhaustive, for if a group of potential parents contains $p$ individuals of one sex and $q (> p)$ of the other, only $p$ pairings of the $pq$ that are possible can be set up and tested by graft interchange.

Type D: parallel recipients. Grafts are transplanted from a chosen donor to two (or more) recipients. A test making use of transplanted tumours is essentially of this sort. The homograft that survives longest is borne by the individual having most antigens in common with the antigens of the donor.

Type E: parallel donors. Grafts are transplanted from two (or more) donors to a single recipient. In general, the homografts will survive for different lengths of time, and the homograft that survives longest comes from the donor that has the fewest (or the weakest) of the antigens that are not also possessed by the recipient. Anderson et al. (1951) point out that this is the only transplantation method that could make it possible to distinguish between monozygotic and dizygotic twins in animals such as the cow, to which tests of Type C are inapplicable. The sensitivity of the test can obviously be increased by immunizing the recipient to skin from one of the donors beforehand.
'ACTIVELY ACQUIRED TOLERANCE' OF FOREIGN CELLS

By Dr. R. E. BILLINGHAM*, L. BRENT and Prof. P. B. MEDAWAR, F.R.S.

Department of Zoology, University College, University of London

Summary

(1) Mice and chickens never develop, or develop to only a limited degree, the power to react immunologically against foreign homologous tissue cells with which they have been inoculated in fœtal life. Animals so treated are tolerant not only of the foreign cells of the original inoculum, but also of skin grafts freshly transplanted in adult life from the original donor or from a donor of the same antigenic constitution.

(2) Acquired tolerance is immunologically specific: mice and chickens made tolerant of homografts from one donor retain the power to react against grafts transplanted from donors of different antigenic constitutions.

(3) Acquired tolerance is due to a specific failure of the host's immunological response. The antigenic properties of a homograft are not altered by residence in a tolerant host, and the host itself retains the power to give effect to a passively acquired immunity directed against a homograft which has until then been tolerated by it.

(4) The fertility of tolerant mice is unimpaired.

*Present address: University of Cambridge.
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(4) The fertility of tolerant mice is unimpaired.

Figure 1. A White A/Jax Mouse Carrying an Allogeneic Skin Graft (from a CBA Mouse) Five Months after Transplantation.

The A/Jax mouse had received 3 million CBA spleen cells when it was a 17-day-old fetus and had become fully tolerant of CBA histocompatibility antigens. The skin was transplanted five weeks after birth. At this age, normal A/Jax mice would be expected to reject such a graft within 10 days.
Central and peripheral tolerance to self antigens.

**Generative (primary) lymphoid organs**
- Newly emerged (immature) clones of lymphocytes
- Lymphoid precursor
- Self antigen present in generative lymphoid organ
- Immature lymphocytes with receptors for self antigens
- Maturation of clones not specific for self antigens present in generative organs
- Central tolerance: deletion of lymphocytes that recognize self antigens present in generative organs

**Peripheral (secondary) lymphoid tissues**
- Mature lymphocytes
- Foreign antigen
- Self antigen in peripheral tissues
- Peripheral tolerance: deletion or anergy of lymphocytes that recognize self antigens in peripheral tissues
- Immune response to foreign antigens

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TOLERANCE THRESHOLD

No positive selection

positive selection

negative selection

TCR – HLA / peptide Affinity
Consequences of self antigen recognition in thymus

From: Abbas & Lichtman, Cellular & Molecular Immunology, W. B. Saunders, 2003
APECED: an example of failed central (thymic) tolerance

APECED patients suffer a variety of autoimmune diseases and candidiasis

<table>
<thead>
<tr>
<th>Symptom</th>
<th>Frequency in Finnish patients (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Endocrine glands</strong></td>
<td></td>
</tr>
<tr>
<td>Hypoparathyroidism</td>
<td>85</td>
</tr>
<tr>
<td>Adrenal failure</td>
<td>72</td>
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<tr>
<td>Ovarian failure</td>
<td>60</td>
</tr>
<tr>
<td>Insulin-dependent diabetes mellitus</td>
<td>18</td>
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<tr>
<td>Testicular atrophy</td>
<td>14</td>
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<tr>
<td>Parietal cell atrophy</td>
<td>13</td>
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<tr>
<td>Hypothyroidism</td>
<td>6</td>
</tr>
<tr>
<td><strong>Other tissues</strong></td>
<td></td>
</tr>
<tr>
<td>Candidiasis</td>
<td>100</td>
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<tr>
<td>Dental enamel hypoplasia</td>
<td>77</td>
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<tr>
<td>Nail dystrophy</td>
<td>52</td>
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<tr>
<td>Tympanic membrane calcification</td>
<td>33</td>
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<tr>
<td>Alopecia</td>
<td>27</td>
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<tr>
<td>Keratopathy</td>
<td>22</td>
</tr>
<tr>
<td>Vitiligo</td>
<td>13</td>
</tr>
<tr>
<td>Hepatitis</td>
<td>13</td>
</tr>
<tr>
<td>Intestinal malabsorption</td>
<td>10</td>
</tr>
</tbody>
</table>

- APECED is caused by a single gene defect
- Defective gene is called **Aire (Autoimmune Regulator)**
- Aire regulates the expression of self-proteins in the thymus for negative selection of T cells

Figure 11-19 The Immune System, 2/e (© Garland Science 2005)
Real Time-PCR Analysis of Different Tissue Types
Aire is expressed in thymic medullary epithelial cells
Thymus Architecture
Organ-Specific Infiltrates

<table>
<thead>
<tr>
<th>KO</th>
<th>Salivary</th>
<th>Ovary</th>
<th>Retina</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><img src="image1.png" alt="Image" /></td>
<td><img src="image2.png" alt="Image" /></td>
<td><img src="image3.png" alt="Image" /></td>
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<tr>
<td>WT</td>
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<td><img src="image5.png" alt="Image" /></td>
<td><img src="image6.png" alt="Image" /></td>
</tr>
</tbody>
</table>

KO and WTrepresent different genotypes or conditions.
Thymic Transplant Experiment

**Thymic Donor**
- WT
- KO

**Recipient**
- Athymic Nude

**dGuo Rx'd**

**Infiltrates and AutoAb's?**
- No
- Yes
Model

Central tolerance ("negative selection")

- Induced by high concentration of antigen in generative lymphoid organs
  - Typically seen with self antigens that are present in many tissues, including thymus and bone marrow
  - Many self antigens are expressed in medullary epithelial cells in thymus (role of AIRE transcription factor)

- High-affinity ("strong") recognition of self antigens
  - Eliminates high-affinity self-reactive (potentially most dangerous) lymphocytes

- It is not known why some immature T cells die and others develop into regulatory cells upon recognition of self antigens in the thymus
Central Tolerance and Allogeneic Responses

- Mixed lymphocyte reactions between allogeneic cells (i.e. live MHCa + irradiated MHCb cells) give detectable proliferation of T cells after 4 to 7 days
Mixed lymphocyte reaction (MLR)

Mix MHC$^a$ T cells and irradiated MHC$^b$ non-T cells as antigen-presenting cells

Measure proliferation of T cells by incorporation of $^3$H-thymidine

Measure killing of $^{51}$Cr-labeled target cells to detect activated cytotoxic T cells

T-cell proliferation depends largely on differences in MHC class II alleles

Generation of cytotoxic T cells depends largely on differences in MHC class I alleles

Figure 13-42 Immunobiology, 6/e. (© Garland Science 2005)
Central Tolerance and Allogeneic Responses

• Mixed lymphocyte reactions between allogeneic cells (i.e. live MHCa + irradiated MHCb cells) give detectable proliferation of T cells after 4 to 7 days

• Whereas lymphocyte reactions with most foreign antigens (i.e. MHCa T cells + MHCa APC’s + antigen) give no detectable proliferation of T cells after 4 to 7 days

• Why?

• Answer=precursor frequencies. Alloreactive T cells are 1/50 to 1/1,000 cells whereas antigen-specific T cell precursor frequencies are much lower

• Why?
T cells are “tuned” to react to self-MHC + foreign peptide

Allo-MHC “mimics” self-MHC + foreign peptide for many T cells
The molecular explanation of alloreactivity

Foreign peptide:self MHC binding

- T cell
- TCR
- Self MHC class II

antigen-presenting cell

Peptide-dominant binding

- T cell
- TCR
- Nonself MHC class II

antigen-presenting cell

MHC-dominant binding

- T cell
- TCR
- Nonself MHC class II

antigen-presenting cell

Figure 5-18 Immunobiology, 6/e. (© Garland Science 2005)
Figure 12-19 The Immune System, 2/e (© Garland Science 2005)
When a kidney is transplanted, the recipient's T cells attack the transplant.

When bone marrow is transplanted, the T cells in the transplant attack the recipient's tissues.

Transplant rejection

Graft-versus-host disease

Figure 12-11 The Immune System, 2/e (© Garland Science 2005)
Peripheral Tolerance
Central and peripheral tolerance to self antigens.

**Central tolerance**: deletion of lymphocytes that recognize self antigens present in generative organs.

**Peripheral tolerance**: deletion or anergy of lymphocytes that recognize self antigens in peripheral tissues.

Generative (primary) lymphoid organs:
- Newly emerged (immature) clones of lymphocytes
- Immature lymphocytes with receptors for self antigens
- Maturation of clones not specific for self antigens present in generative organs
- Lymphoid precursor
- Self antigen present in generative lymphoid organ

Mature lymphocytes:
- Self antigen in peripheral tissues
- Immune response to foreign antigens

Peripheral (secondary) lymphoid tissues:
Peripheral Mechanisms of the Induction of Tolerance

- Normal
- Immunologic Ignorance
- Deletion
- Inhibition
- Suppression

Activated T cell

T cell

Apoptosis

No activation

Regulatory T cell

Antigen-presenting cell

CD80

MHC

Peptide

Anatomical barrier

CD4

CD28

CD3

Fas ligand

Fas

CD152

Interleukin-10 TGF-β
Antigen sequestration

Immunologically privileged sites

- Brain
- Eye
- Testis
- Uterus (fetus)
- Hamster cheek pouch

Figure 13-12 Immunobiology, 6/e. © Garland Science 2005
Antigen sequestration example: Sympathetic Ophthalmia

Trauma to one eye results in the release of sequestered intraocular protein antigens.

Released intraocular antigen is carried to lymph nodes and activates T cells.

Effector T cells return via bloodstream and encounter antigen in both eyes.
Peripheral tolerance

Normal T cell response

Activated T cells

Anergy

Off signals

Functional unresponsiveness

Deletion

Apoptosis (activation-induced cell death)

Suppression

Block in activation
Peripheral anergic tolerance: antigen without co-stimulation signal

Figure 6-19 The Immune System, 2/e (© Garland Science 2005)
The B7:CD28 families of costimulators and receptors

From: Abbas & Lichtman, Cellular & Molecular Immunology, W. B. Saunders, 2003
Experimental models of T cell anergy

• T cells from TCR transgenic mice transferred into normal mice and exposed to tolerogenic antigen (e.g. aqueous peptide) or to cognate antigen expressed in tissues (islet β cells, serum)

• Difficult to study anergy with primary T cells in vitro
Model for systemic tolerance and autoimmunity: T cell response to serum antigen

**DO.11 (TCR transgenic)**

Transfer T cells

**sOVA-Tg (antigen transgenic)**

Assay DO.11 cells: phenotype; functional responsiveness

**DO.11 TCR-Tg:** CD4 T-cells specific for ovalbumin

**sOVA-Tg:** secreted form of ovalbumin expressed as transgene under control of metallothionine promoter; transgenic mice have 10-20 ng/mL of serum OVA
T cell unresponsiveness is induced by encounter with systemic antigen

DO.11 T cells transferred into BALB/c control or sOva-Tg mice, immunized with antigen-pulsed DCs in vivo, T cells purified and assayed ex vivo -- T-cells that encounter systemic antigen become unresponsive
Lymphocyte activation and tolerance

Normal immune response:
- Lymphocyte + Immunogenic antigen → Proliferation and differentiation

Tolerance:
- Lymphocyte + Tolerogenic antigen → Anergy (functional unresponsiveness)
- Anergy + Immunogenic antigen → No response

Deletion (cell death):
- Lymphocyte + Immunogenic antigen → Death
## Protein antigens immunogenicity vs. tolerogenicity

<table>
<thead>
<tr>
<th>Factor</th>
<th>Factors that favor stimulation of immune responses</th>
<th>Factors that favor tolerance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amount</td>
<td>Optimal doses that vary for different antigens</td>
<td>High doses</td>
</tr>
<tr>
<td>Persistence</td>
<td>Short-lived (eliminated by immune response)</td>
<td>Prolonged (repeated T cell stimulation induces apoptosis)</td>
</tr>
<tr>
<td>Portal of entry; location</td>
<td>Subcutaneous, intradermal; absence from generative organs</td>
<td>Intravenous, oral; presence in generative organs</td>
</tr>
<tr>
<td>Presence of adjuvants</td>
<td>Antigens with adjuvants: stimulate helper T cells</td>
<td>Antigens without adjuvants: nonimmunogenic or tolerogenic</td>
</tr>
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<td>Properties of antigenpresenting cells</td>
<td>High levels of costimulators</td>
<td>Low levels of costimulators and cytokines</td>
</tr>
</tbody>
</table>
Tolerance induced by aqueous protein antigen

T cells from TCR transgenic specific for peptide of OVA transferred into normal mice, exposed to antigen in different forms
T cell anergy

- Multiple mechanisms demonstrated in different experimental systems
- The contribution of peripheral T cell anergy to tolerance to any natural self antigen is essentially unknown
“Activation-induced cell death”: death of mature T cells upon recognition of self antigens

From Abbas and Lichtman. Basic Immunology 2nd ed, 2006
Healthy female - at 18 months developed cervical adenopathy. Biopsy showed ‘reactive hyperplasia’. Pt developed anemia with hypersplenism, hematuria, proteinuria and renal insufficiency due to mesangial glomerulonephritis, then primary biliary infiltration. Evaluation at NIH: lymphadenopathy persists, ANA (+) 1:320, CD4-CD8- cells 25% of αβ T cells, increased B cells; Fas surface expression is normal. Heterozygous Fas splice mutation resulting in loss of exons 3, 4 (AA 52-96)
The death receptor (Fas) pathway

Fas-mediated activation-induced cell death

- Cross-linking of Fas by FasL
- Binding of adapter protein, FADD, to cross-linked Fas
- Binding and autocatalytic activation of caspase-8

Activation of effector caspases → Active caspase-8 → Apoptosis
Peripheral deletion of mature T cells by self antigen recognition: activation-induced cell death

- Death may be via mitochondrial pathway or death receptor (Fas) pathway
  - Fas is also involved in deletion of self-reactive B cells
- Not known which natural self antigens maintain tolerance by AICD
Protein antigens immunogenicity vs. tolerogenicity

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The B7:CD28 families of costimulators and receptors

From: Abbas & Lichtman, Cellular & Molecular Immunology, W. B. Saunders, 2003
Approaches to Tolerance Induction: 
**CTLA-4 Down-regulation**

CTLA-4 is a CD28-dependent activation molecule that down-regulates T cell function by inhibiting IL-2 production and cell cycle progression.
CTLA-4−/− T cells resist tolerance induction: proliferation of cells exposed to tolerogen
Peripheral Mechanisms of the Induction of Tolerance

- **Normal**: Activated T cell
- **Immunologic Ignorance**: T cell
- **Deletion**: Apoptosis
- **Inhibition**: No activation
- **Suppression**: No activation
Harnessing co-stimulation for tolerance in vivo
Tolerance Induction following Co-stimulation Blockade

**ISLETS**

**SKIN**
Dendritic cell subsets

- Immature dendritic cells capture antigens from sites of entry; mature DCs present antigen to and activate naïve T cells in lymphoid organs
- Some DCs may induce tolerance
- Problems:
  - Lack of definitive markers
  - Most studies based on culture derived DCs
Induction of tolerance by targeting antigen to dendritic cells *in vivo*

- anti-DC antibody
- antigen
- Injected into mice
- Dendritic cell
- endocytosis of antigen
- presentation of antigen
- Antigen-specific T cell
- Tolerance

Dendritic cells have not been activated by microbial products
Harnessing co-stimulation for tolerance in vivo (cont)
Beware!
The B7:CD28 families of costimulators and receptors

<table>
<thead>
<tr>
<th>Expression</th>
<th>APCs; inducible</th>
<th>APCs; inducible</th>
<th>APCs, B cells, other tissues; inducible</th>
<th>APCs, B cells, other tissues; inducible</th>
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</thead>
<tbody>
<tr>
<td>Name</td>
<td>B7-1 (CD80)</td>
<td>B7-2 (CD86)</td>
<td>ICOS-L</td>
<td>PD-L1</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>PD-L2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>B7-H3</td>
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</tbody>
</table>

Ligands on APCs and other cells

Receptors on T cells

<table>
<thead>
<tr>
<th>Name</th>
<th>CD28</th>
<th>CTLA-4</th>
<th>ICOS</th>
<th>PD-1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Expression</td>
<td>T cells; constitutive</td>
<td>T cells; inducible</td>
<td>T cells; inducible</td>
<td>T cells, B cells, Myleoid cells; inducible</td>
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<tr>
<td>Major function</td>
<td>Costimulation of naive T cells</td>
<td>Negative regulation of immune responses; self-tolerance</td>
<td>Costimulation of effector T cells</td>
<td>Negative regulation of T cells in peripheral tissues</td>
</tr>
</tbody>
</table>

From: Abbas & Lichtman, Cellular & Molecular Immunology, W. B. Saunders, 2003
PD-L1, PD-L2 and their receptor PD-1

- PD-L1 (programmed death 1 ligand), PD-L2 (programmed death 2 ligand) and do not bind to CD28, ICOS, or CTLA-4, but bind to PD-1 (programmed death 1 molecule).

- PD-1 shows 24% of sequence similarity to CD28 and CTLA-4.

- An ITIM motif (Immunoreceptor Tyrosine-based Inhibitory Motif) is present in the cytoplasmic tail of PD-1. The ITIM motif is known to be present in receptors that have an inhibitory function on lymphocyte responses.
What’s known about PD-L1:PD-1 pathway?

Loss of PD-L1:PD-1 signaling results in autoimmunity in many settings:

- BALB/c.PD1KO mice = autoimmune cardiomyopathy
- C57BL/6.PD1KO mice = lupus-like disease
- 129.PDL1KO mice = permits EAE (usually resistant)
- C57B/6.PDL1KO mice = exacerbated EAE

Mechanism-wise…

Blockade or deficiency of PDL1:PD-1 signaling frequently associated with:

- increased proliferation (seems most potent at suboptimal stimulation)
- reduced apoptosis
- increased IFN-γ (sometimes also IL-2)
- some think involved in Treg function
PD-L1 Blockade in NOD accelerates diabetes

A 10 wk old female NOD

B 4 wk old female NOD

C 1 wk old female NOD

Safety, Activity, and Immune Correlates of Anti–PD-1 Antibody in Cancer

Suzanne L. Topalian, M.D., F. Stephen Hodi, M.D., Julie R. Brahmer, M.D., Scott N. Gettinger, M.D., David C. Smith, M.D., David F. McDermott, M.D., John D. Powderly, M.D., Richard D. Carvajal, M.D., Jeffrey A. Sosman, M.D., Michael B. Atkins, M.D., Philip D. Leming, M.D., David R. Spigel, M.D., Scott J. Antonia, M.D., Ph.D., Leora Horn, M.D., Charles G. Drake, M.D., Ph.D., Drew M. Pardoll, M.D., Ph.D., Lieping Chen, M.D., Ph.D., William H. Sharfman, M.D., Robert A. Anders, M.D., Ph.D., Janis M. Taube, M.D., Tracee L. McMiller, M.S., Haiying Xu, B.A., Alan J. Korman, Ph.D., Maria Jure-Kunkel, Ph.D., Shruti Agrawal, Ph.D., Daniel McDonald, M.B.A., Georgia D. Kollias, Ph.D., Ashok Gupta, M.D., Ph.D., Jon M. Wigginton, M.D., and Mario Sznol, M.D.
Using PD-1 to break tolerance
Peripheral Mechanisms of the Induction of Tolerance

Normal:
- Antigen-presenting cell
- CD80
- MHC
- Peptide
- Anatomical barrier
- Activated T cell
- CD3
- CD4
- CD28

Immunologic Ignorance:
- Fas ligand
- Fas
- CD152

Deletion:
- Apoptosis

Inhibition:
- Interleukin-10
- TGF-β
- Regulatory T cell
- No Activation/Tolerance

Suppression:
- No activation
Regulatory T cells protect against autoimmunity

- Treg's co-express CD4 and CD25 and are reactive to self-antigens
- FoxP3 is a transcription factor expressed by Treg's
- FoxP3-deficient humans and mice develop severe autoimmune disease
Development of CD4+CD25+ cells in mice occurs mainly after day 3 of life

Autoimmune Disease as a Consequence of Developmental Abnormality of a T Cell Subpopulation

By Masanao Asano,*† Masaaki Toda,† Noriko Sakaguchi,* and Shimon Sakaguchi†

Thymectomy before day 3 causes autoimmunity but replacement of CD4+CD25+ cells prevents it.
CD4⁺ CD25⁺ regulatory T cells control tolerance in autoimmune diabetes

% Incidence of Diabetes

Weeks post transfer

0 3 6 9 12 15
CD4+CD25+ cells express FoxP3

Foxp3 programs the development and function of CD4+CD25+ regulatory T cells

Jason D. Fontenot, Marc A. Gavin & Alexander Y. Rudensky

FoxP3-deficient (Scurfy) mice get autoimmunity and are rescued by CD4+CD25+ cells from WT donors.
Regulatory T cells protect against autoimmunity

- Treg’s co-express CD4 and CD25 and are reactive to self-antigens
- FoxP3 is a transcription factor expressed by Treg’s
- FoxP3-deficient humans and mice develop severe autoimmune disease
Mechanisms of action of regulatory T cells

- Antigen recognition
- T cell proliferation and differentiation
- Effector functions of T cells

Apoptotic differentiation: Anti-inflammatory cytokines (IL-10, TGF-β)

From: Abbas & Lichtman, Cellular & Molecular Immunology, W. B. Saunders, 2003
Outstanding issues on Treg’s

- What is their exact mechanism of suppression? In vivo vs. in vitro observations

- How are they selected for? Are they preferentially self-reactive?
Harnessing Treg’s for tolerance
Reversal of diabetes in NOD mice with Treg’s

In Vitro–expanded Antigen-specific Regulatory T Cells Suppress Autoimmune Diabetes

Qizhi Tang,¹ Kammi J. Henriksen,¹ Mingying Bi,¹ Erik B. Finger,¹ Greg Szot,¹ Jianqin Ye,¹ Emma L. Masteller,¹ Hugh McDevitt,² Mark Bonyhadi,³ and Jeffrey A. Bluestone¹

¹UCSF Diabetes Center, Department of Medicine, University of California San Francisco, San Francisco, CA 94143
²Department of Microbiology and Immunology, Stanford University School of Medicine, Stanford, CA 94305
³Xcyte Therapies, Inc., Seattle, WA 98104

J. Exp. Med. © The Rockefeller University Press • 00 Volume 199, Number 11, June 7, 2004 1455–1465
**Human Treg Expansion In Vitro**

Treg Trial

• Phase 1 study with infusion of autologous Tregs expanded in vitro
  - First effort in autoimmunity
  - Prior trial with related cell product in GVHD (Brunstein et al, Blood 2010)

• Subjects > 18, within 2 yrs of dx and with measurable C-peptide

• Dose escalation
  - First and second cohorts have done well
  - Now completing third cohort
### Mechanisms of immune tolerance

<table>
<thead>
<tr>
<th>Type of tolerance</th>
<th>Mechanism</th>
<th>Site of action</th>
</tr>
</thead>
<tbody>
<tr>
<td>Central tolerance</td>
<td>Deletion Editing</td>
<td>Thymus Bone marrow</td>
</tr>
<tr>
<td>Antigen segregation</td>
<td>Physical barrier to self-antigen access to lymphoid system</td>
<td>Peripheral organs (eg, thyroid, pancreas)</td>
</tr>
<tr>
<td>Peripheral anergy</td>
<td>Cellular inactivation by weak signaling without co-stimulus</td>
<td>Secondary lymphoid tissue</td>
</tr>
<tr>
<td>Regulatory cells</td>
<td>Suppression by cytokines, intercellular signals</td>
<td>Secondary lymphoid tissue and sites of inflammation</td>
</tr>
<tr>
<td>Cytokine deviation</td>
<td>Differentiation to T_{H2} cells, limiting inflammatory cytokine secretion</td>
<td>Secondary lymphoid tissue and sites of inflammation</td>
</tr>
<tr>
<td>Clonal exhaustion</td>
<td>Apoptosis post-activation</td>
<td>Secondary lymphoid tissue and sites of inflammation</td>
</tr>
</tbody>
</table>
Peripheral Mechanisms of the Induction of Tolerance

1. Normal
   - Antigen-presenting cell
   - CD80
   - MHC
   - Peptide
   - Anatomical barrier
   - T-cell receptor
   - CD28
   - CD4
   - Activated T cell

2. Immunologic Ignorance
   - CD80
   - MHC
   - Peptide
   - Fas ligand
   - Fas
   - T cell

3. Deletion
   - CD152
   - CD80
   - MHC
   - Peptide
   - Apoptosis

4. Inhibition
   - Interleukin-10
   - TGF-β
   - Regulatory T cell
   - No activation

5. Suppression
   - Interleukin-10
   - TGF-β
   - Regulatory T cell
   - No activation
Immunological tolerance

• Definition:
  - specific unresponsiveness to an antigen that is induced by exposure of lymphocytes to that antigen (tolerogen vs immunogen)

• Significance:
  - All individuals are tolerant of their own antigens (self-tolerance)
  - Foreign antigens administered in particular ways induce tolerance
  - The induction of tolerance may be exploited to prevent graft rejection, to treat autoimmune and allergic diseases, and to allow gene therapy