Fellows Symposium on Transplantation Medicine

Sunday, September 25
8:00 am - 8:30 am

Primary Mechanisms of Self-tolerance

Maria-Luisa Alegre, MD, PhD

September 23-25, 2011
Hilton DFW Lakes Executive Conference Center
Grapevine, Texas
www.a-s-t.org
Primary Mechanisms of Self-Tolerance

Marisa Alegre, MD, PhD

How Is Tolerance to Self-Antigens Normally Maintained?

Central Tolerance
- Ignorance, Anergy, Clonal deletion, Immune deviation, Suppression

Peripheral Tolerance

Thymic Selection of T Cells

- Very weak or no interaction (TCR that does not recognize self-MHC) => death by neglect
- Intermediate interaction => positive selection (survival) => export to the periphery of self-restricted, self-tolerant T cells
- Strong interaction => positive selection of Tregs
- Very strong interaction => death by negative selection

The Transcription Factor AIRE Allows Expression of Self-Antigens in the Thymus to Favor Negative Selection of Autoreactive T Cells

AIRE Knockout Mice Develop Autoimmunity
- Aire mutant people develop APS (autoimmune polyendocrinopathy syndrome)
- Aire (TF) is predominantly expressed in thymic medullary epithelial cells
- AIRE promotes ectopic expression of peripheral antigens

The University of Chicago

American Society of Transplantation Fellows Symposium

September 23-25, 2011
Grapevine, TX

AIRE Knockout Mice Develop Autoimmunity

Scienc 298:1395

Maria-Luisa Alegre, MD, PhD
www.a-s-t.org
Peripheral Expression of Aire and CD8 Tolerance

Peripheral antigen display by lymph node stroma promotes T cell tolerance to intestinal self

Negative Regulators of T cell Activation Terminate an Immune Response and Prevent Autoimmunity

Resting T Cell

Activated T Cell

Negative Selection

Anergy (tolerance)

Apoptosis

Peripheral Mechanisms to Prevent Autoimmunity: Lack of Costimulatory Ligands on Most Tissue Cells => Anergy

Peripheral Mechanisms to Prevent Autoimmunity: T Cell-Intrinsic Negative Regulators of T Cell Proliferation

Memory and exhausted CD8+ T cells express many inhibitory receptors

Maria-Luisa Alegre, MD, PhD

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Increased Viral Control after Simultaneous Blockade of LAG-3 and PDL-1

Different Types of Regulatory T Cells
- Treg CD4+/FoxP3+
- NKT cells
- TR1 cells (in humans)
- Th3 cells (generated after antigen encounter in a tolerogenic form; i.e., soluble, oral...)
- Occasional report of CD8+ cells, or CD4+/CD25- cells with suppressor activity

Foxp3 Encodes the Transcription Factor Scurfin
- Scurfin is a forkhead/winged/helix transcription factor
- The scurfy mouse strain has a Foxp3 mutation leading to deletion of forkhead domain => lethal CD4-dependent lymphoproliferation; same in Foxp3-deficient mice; lack CD4+/CD25+
- Foxp3 is selectively expressed in CD4+/CD25+ T cells (among T cells)
- Transfer of CD4+/CD25+ T cells to neonatal Foxp3-deficient mice corrects disease
- Ectopic expression (transgenic or retroviral) of Foxp3 confers suppressive function to CD4+/CD25- T cells

Two Types of FoxP3+ Tregs: Natural and Induced

FoxP3-Induced Surface Molecules and Cytokines Defining Tregs

Suppression of the Immune Response by Tregs
Suppressive Cytokines Produced by Tregs

Bettini and Vignali, Curr Opin Immunol, 21:612, 2009

T Cell Tolerance to Viral and Tumor Antigens


Mechanisms of T Cell Tolerance by Anti-CD154 in Transplantation

Stimulants: B6 (syngeneic) Balb/c (allogeneic) C3H (3rd party)

Anti-CD154 mAb Induces “Robust” Donor-Specific Transplantation Tolerance

Anti-CD154 (1mg d0, 7, 14) + DST

Redundancy and specificity of Tregs inhibitory cytokine function.
B Cell Tolerance

Clonal anergy- usually in periphery - impaired survival

a. Anergic B cells downregulate BCR and CXCR5 and are excluded from follicles, but this does not dictate survival

b. Increased dependence of anergic cells on TNF family survival factor BAFF. (BAFF signals contribute to phosphorylation and degradation of the pro-apoptotic protein Bim).

Marcus Clark, PhD, University of Chicago

Tracking the Fate of Alloreactive B Cells during Allograft Tolerance

Day 0:
B6 Heart Transplant into 3-83i Recipients
DST + Anti-CD154

(Li et al. PNAS 2007, JI 2008)

Tolerance Induces Deletion of 3-83 B Cells

(Analysis at 4 wks post-Tx)

Potential Role for Regulatory B Cells
<table>
<thead>
<tr>
<th>Summary</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Lymphocytes adhere to rules of tolerance centrally (thymus and BM) and in the periphery</td>
</tr>
<tr>
<td>• T cells can undergo anergy, deletion, iTreg differentiation, exhaustion</td>
</tr>
<tr>
<td>• B cells can undergo anergy, follicular exclusion, deletion and present antigen in a way that can inactivate T cells</td>
</tr>
<tr>
<td>• Tolerogenic DCs can promote iTreg differentiation or induce anergy of T cells.</td>
</tr>
</tbody>
</table>
Fellows Symposium on Transplantation Medicine

Sunday, September 25
8:30 am - 9:00 am

How is Tolerance Induced in the Graft?

Ronald G. Gill, PhD

September 23-25, 2011
Hilton DFW Lakes Executive Conference Center
Grapevine, Texas
www.a-s-t.org
**How is tolerance induced in the graft?**

Ronald G. Gill

University of Colorado, Denver
Colorado Center for Transplantation Care, Research, and Education

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**General Strategy for Facilitating Tolerance**

- Minimize function of graft destructive cells
- Facilitate activation of graft-‘protective’ cells

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**Allograft Tolerance:**

An antigen (donor)-specific response that results in preservation of the graft.

- *Not* immune deficiency
- *Not* immunosuppression

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The ‘context’ of antigen presentation dictates the fate of the donor-reactive T cell.

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**‘Plasticity’:**

A hallmark feature of the immune system

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**Allograft**

- No Response
- Destructive Immunity
- Non-destructive Immunity

- Clonal Deletion/Inactivation
- Active Regulation

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Ronald G. Gill, PhD
www.a-s-t.org
Potential Routes of Tolerance:

- Clonal Deletion / Inactivation ('Recessive')
- Active inhibition / regulation ('Dominant')

Evidence of T cell depletion during tolerance

- Requirement for killing mechanisms (perforin)
- Inhibition of apoptosis inhibits tolerance
- Direct evidence of T cell elimination

From: AD Wells et al. Nat. Med. 5:1303, 1999

T cell over-expression of the anti-apoptotic molecule Bcl-xL inhibits allograft tolerance induction

Exposure to donor antigen + anti-CD154 results in transient depletion of donor-reactive CD8 T cells

Active Regulation of the Alloresponse

- Types of Regulatory T cells
- ‘Linked’ Suppression
- ‘Infectious’ Tolerance
Active Tolerance

- Requires Antigen Recognition
- Predominantly a CD4-dependent response
- Does not require the deletion of graft-destructive T cells

‘Linked’ Suppression:
The APC serves as the intermediary between T effector (Teff) and T regulatory (Treg) cells

Varied Phenotypes of ‘Regulatory’ T cells

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Types of regulatory T cell in the immune system</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell type</td>
<td>Application or system tested</td>
</tr>
<tr>
<td>Effector T cells</td>
<td>Mouse allograft, GVHD and transplantation</td>
</tr>
<tr>
<td>Treg cells</td>
<td>Human xenotransplantation</td>
</tr>
<tr>
<td>IL-10 T cells</td>
<td>Human chimerism</td>
</tr>
<tr>
<td>CD8+ T cells</td>
<td>Human transplantation</td>
</tr>
</tbody>
</table>


‘Recessive’ or ‘Dominant’ tolerance?

Re-emergence of the modern concept of ‘regulatory’ allograft tolerance:


‘Infectious’ Tolerance

Key Relationship Between ‘Linked’ recognition and ‘Infectious’ Tolerance


How do Regulatory T cells ‘Regulate’?

a. Graft rejection
b. Chronic graft vs host disease
c. Inflammation

From: Ronald G. Gill, PhD
www.a-s-t.org
Do you need to be donor hypo-responsive to maintain allograft tolerance?

Targeting of LFA-1 + CD154 results in ‘dominant’, transferable transplantation tolerance

‘Dominant’ Tolerance Despite Potent Anti-Donor CTL Reactivity

Tolerant spleen cells exhibit normal anti-donor proliferative responses in vitro.

‘Linked’ Suppression:
Accounts for the lack of allograft rejection despite the presence of donor-reactive T cells

Barriers to Consistent Induction of Tolerance

- Genetic variability
- Pre-existing memory cells (‘heterologous’ immunity)
- Concurrent inflammation / infection
- ‘Dual’ role of immune mediators in rejection and tolerance
Both elimination of effector cells and generation of regulation may occur in the generation of allograft tolerance


Current Conundrum:

Some cells types / molecules can participate both in graft rejection and tolerance

Question: Are the ‘pro-inflammatory’ contributions of IFNγ, perforin, and NK cells important for immunity or tolerance?

Proposition: YES

Opposing Roles for NK Cells in Transplantation

Promote Inflammation

Regulate Alloreactivity

Varied Contributions of NK Cells to Immunity

Endothelial Cell Injury
Enhance Reperfusion Injury
ADCC
DC licensing
Promote Th1 Immunity

NK Cells Required Cardiac Allograft Rejection in CD28−/− Mice

S. Maier et al. Nat. Med. 7:557, 2001
Tolerance to Islet Allografts Requires NK Cells

(Beilke et al. Nat. Med. 11:1059, 2005)

Perforin: Role in Immunity and Tolerance

Serial Peripheral Blood Perforin and Granzyme B Gene Expression Measurements for Prediction of Acute Rejection in Kidney Graft Recipients

Perforin-Deficient Mice (pfp−/−) are Resistant to Tolerance Induction

Anti-LFA-1 Fails to Induce Long-Term Islet Allograft Survival in C57Bl/6 IFNγ−Deficient Recipients

Perforin: How can the same molecule be required both for immunity and for tolerance?

T cells

NK cells

Immunity (redundant)

Regulation (non-redundant)
Summary

- Tolerance is an active response to the graft
- Involves both deletional and regulatory components
- Some immune pathways contribute to both rejection and tolerance
Fellows Symposium on Transplantation Medicine

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Clinical Strategies of Tolerance Induction

Roslyn B. Mannon, MD

September 23-25, 2011
Hilton DFW Lakes Executive Conference Center
Grapevine, Texas
www.a-s-t.org
Clinical Strategies to Tolerance Induction
Roslyn B. Mannon, MD, FASN
AST Fellows Symposium 2011

The Contributions of Animal Models in Transplantation

- 600 BC Sushruta, autologous skin graft to face
- 1000 AD, Cosmos and Damian, leg transplant
- 1800’s, Hunter successfully replaced a premolar (in man)
- 1900, Landsteiner and Miller discover ABO, R&H blood groups
- 1902, Alexis Carrel developed the vascular anastomosis in a dog (forerunner).
- 1905, Carrel and Guthrie, heart transplant to neck of dog.
- 1906, Jabouley transplanted kidneys from sheep, goats, monkeys into man.
- 1908, Carrel transplanted kidneys between cats.
- 1920, Williamson accurately predicted tissue type in dogs as a forerunner of typing in man.

More Animal Contributions

- 1930, George Snell identified MHC antigens in the mouse (H-2 system).
- 1943, Peter Medawar evaluated responses between homografts and autografts in rabbits.
- 1945, Medawar proposed that rejection was an immunologic response in the recipient against the donor tissue.
- 1954, Merrill and Murray performed first kidney transplant in identical twins (largest of the large animal models).
- 1955, Welch performed first liver transplant in dogs.
- 1959, Merrill and Murray successful kidney transplant in man.
- 1960’s, Sir Roy Calne used azathioprine in human recipient of kidney transplant.

The Legacy of Sir Peter Medawar

- Experimental design
  - Crude cell/tissue mixture from an allogeneic adult mouse injected into 6 fetuses of a CBA female produced 5 pups
  - Pups skin-grafted at 8 weeks
  - Experiments repeated in neonatal mice
- Results
  - Of 5 skin grafts
    - 2 promptly destroy (likely acute rejection)
    - 1 with prolonged evolution (likely chronic rejection)
    - 2 accepted at 77 and 101 days
    - Implantation of LHI fragments from donor antigen-immunized mice lead to prompt rejection of these two surviving grafts
    - Inoculation of neonatal mice with various tissues of different strains was largely unsuccessful
  - Only 9 of 96 skin grafts experienced prolonged survival

Medawar Conclusions

- Medawar and colleagues
  - Effect of treatment was a continuum ranging from no effect on survival to indefinite survival
  - Regimen ineffective in neonatal (older) mice
  - Therapy consistently overcome by memory
- Misconceptions
  - A brief, limited intervention in most individuals can consistently produce tolerance to alloantigens
**Definition of Tolerance**

- Experimental definitions of tolerance are less applicable to clinical transplantation
  - Long-term allograft acceptance without ongoing immunosuppression in an immunocompetent recipient
  - Acceptance of subsequent donor but not 3rd party organs/tissues
- A working definition for functional or operational tolerance in humans would be continued stable allograft function in the absence of immunosuppression
  - Critical role of histology

**Mechanisms of Tolerance**

- Clonal exhaustion
- Anergy
- Deletion
  - Specific deletion of donor specific cells [bone marrow infusion]
  - Non specific deletion of donor reactive cells [rATG, Campath]
- Regulation
- Ignorance
  - Absence of secondary lymphoid organs
  - Immune privilege

**Limits to Animal Models—Ken Newell says,**

- Mice aren’t men
  - There is an increasing realization that the guiding principles gleaned from rodent transplant models may not translate to man
- Livers aren’t kidneys
  - Organ specific diversity in immunogenicity implies differences in immune response mechanisms and therefore tolerance.

**Man versus Mouse T Cell Repertoire**

<table>
<thead>
<tr>
<th></th>
<th>Man</th>
<th>Mouse</th>
</tr>
</thead>
<tbody>
<tr>
<td>T Cell Number</td>
<td>$10^8$</td>
<td>$2 \times 10^8$</td>
</tr>
<tr>
<td># Unique T Cell Clones</td>
<td>$2 \times 10^7$</td>
<td>$2 \times 10^6$</td>
</tr>
<tr>
<td>Clone Size</td>
<td>$1-4 \times 10^3$</td>
<td>$100-200$</td>
</tr>
<tr>
<td># Alloreactive T Cells</td>
<td>$10^{10}$</td>
<td>$1-2 \times 10^9$</td>
</tr>
<tr>
<td># Alloreactive T Cell Clones</td>
<td>$2 \times 10^5$</td>
<td>$1-2 \times 10^4$</td>
</tr>
</tbody>
</table>

**Heirarchy of Immunogenicity**

<table>
<thead>
<tr>
<th>Immunogenicity</th>
<th>Liver</th>
<th>Kidney</th>
<th>Heart</th>
<th>Islets</th>
<th>Pancreas</th>
<th>Intestine</th>
<th>Lung</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ease of Tolerance</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Costimulatory Blockade**
**Costimulatory Paradigm**

- **Positive**
  - Signal 1
  - APC
  - Signal 2
  - T Cell
  - Proliferation
  - Cytokine Production
  - Prevention of Anergy
  - T<sub>H</sub> differentiation

- **Negative**
  - Signal 1
  - APC
  - Signal 2
  - T Cell
  - Inhibition of proliferation
  - Inhibition of Cytokine Production
  - Apoptosis
  - Induction of Tregs?

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**Blockade of CD28/B7-1 Prolongs Cardiac Allograft Survival**

- Norway (RT1<sup>n</sup>) → Lewis (RT1<sup>l</sup>)
- 0.5 mg/d CTLA4Ig x 7d
- 0.05 mg/d CTLA4Ig x 7d
- None
- Isotype Control

Turka et al. PNAS 1992; 89: 11102

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**Growing Family of “Co-Stimulation”**

Li et al. Transplant Rev 2009; 229: 228-271

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**Combined Costimulatory Blockade Leads to Long Term Graft Survival of Heart and Skin Grafts**

- Norway → Lewis
- 0.5 mg/d CTLA4Ig x 7d
- 0.05 mg/d CTLA4Ig x 7d

Larsen et al. Nature 1996; 381: 434

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**LEA29Y (Belatacept) Rhesus Renal Allograft Model**

- 4-fold increase in avidity to CD80 and 2-fold to CD80.

- CTLA4Ig
- LEA29Y
- MMF/CsA
- Basiliximab

Larsen et al. AJT 2005; 5: 443

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**Belatacept (Nulojix®) in the Clinic**

- Not for tolerance induction
- FDA approved for prophylaxis against acute rejection in kidney recipients

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Roslyn B. Mannon, MD
www.a-s-t.org
**Regulation**

**Tolerance Promoted by Tregs**

Kingsley and Wood. Transplant International 2007; 20: 4

**Ex-Vivo Expanded Tregs Ameliorate Allograft Rejection In Vivo**

Nadig and Wood Nat Med 2010; 16:809

**Regulatory T Cells After Depletional Induction Therapy with Campath-1H**

JASN 2007; 18:1007

**Possible Strategies**

- Ex vivo Treg expansion followed by infusion at time of transplant
- Post transplant therapy to enhance Treg function and/or number

**Hematopoietic Chimerism**
Chimerism and Tolerance

- Ray Owen - Freemartin cattle, *Science* 1945
- Sir Peter Medawar - neonatal tolerance, *Nature* 1953
- Main and Prehn - acquired tolerance in adults induced by lethal irradiation plus full BMT = fully allogeneic chimeras *J Nat Can Inst* 1955
- Ildstad and Sachs - mixed allogeneic chimerism *Nature* 1984

Non-Myeloablative Strategies

- Anti-T cell mAbs, low does TBI (3 Gy), thymic irradiation (7 Gy) [Sharabi *J Exp Med* 1989]
- Low dose BM (20 million/mouse), busulfan, costim blockade [Adams *J Immunol* 2001]

New Strategy: Mixed Chimerism in Humans


Barriers to Tolerance

- Memory
- Homeostatic proliferation
- Heterologous Immunity

Memory Cells Resist Tolerance

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>1, 11, 14, 18, 12</th>
<th>29</th>
<th>40</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>Normal</td>
<td>12</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td>2</td>
<td>B7-A2DT + CD40L</td>
<td>14</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td>3</td>
<td>B7-A2DT + 7G3.4</td>
<td>14</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td>4</td>
<td>B7-A2DT + 7G3.4</td>
<td>14</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td>5</td>
<td>B7-A2DT + 7G3.4</td>
<td>14</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td>6</td>
<td>B7-A2DT + 7G3.4</td>
<td>14</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td>7</td>
<td>B7-A2DT + 7G3.4</td>
<td>14</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td>8</td>
<td>B7-A2DT + 7G3.4</td>
<td>14</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td>9</td>
<td>B7-A2DT + 7G3.4</td>
<td>14</td>
<td>12</td>
<td>12</td>
</tr>
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<td>10</td>
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<td>12</td>
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<td>B7-A2DT + 7G3.4</td>
<td>14</td>
<td>12</td>
<td>12</td>
</tr>
</tbody>
</table>

Valiyanikabil et al. *AJT* 2002: 2: 501
Memory Phenotype Cells Predominate Following Alemtuzumab Depletion

Weeks Post-Tx

Rejection

Memory Phenotype Cells Predominate Following Alemtuzumab Depletion

Homeostatic Proliferation Impairs Tolerance

Preferential Memory Cell Homeostatic Proliferation Following Subtotal Lymphocyte Depletion

Virally Induced Alloreactive Memory Cells Prevent Tolerance Induction

Summary/Conclusion

- Tolerance to an organ may engage a number of different mechanisms.
- A number of preclinical models have been utilized; the results in mice do not translate into NHP models, and also not to Man.
- However, these models provide valuable input into the mechanisms of human disease and have demonstrated the critical complicating factors between preclinical models and man.
Fellows Symposium on Transplantation Medicine

Sunday, September 25
10:00 am - 11:00 am

Literature in Transplantation:
Key Papers of 2010 - 2011
Combined Session

There are no advance slides for this session.

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Fellows Symposium on Transplantation Medicine

Sunday, September 25
11:00 am - Noon

Career Development in Basic and Translational Transplant Investigation

Facilitator: Robert L. Fairchild, Ph.D
Panel: Maria-Luisa Alegre, MD, PhD, Mark L. Barr, MD, William M. Baldwin, MD, PhD, Ronald G. Gill, PhD, Roslyn B. Mannon, MD and Kenneth A. Newell, MD, PhD

There are no advance slides for this session.

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