Tolerance: Why pregnancy is special

December 13, 2012

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Maternal - Fetal Medicine
David Geffen School of Medicine, UCLA
From the NCI website describing the immune system

“A child in the womb carries foreign antigens from the father as well as immunologically compatible self-antigens from the mother.

One might expect this condition to trigger a graft rejection, but it does not because the uterus is an "immunologically privileged" site where immune responses are somehow subdued.”
Medawar paradox

- Anatomic separation of mother and fetus
- Antigenic immaturity of the fetus
- Immunologic “inertness” (tolerance) of the mother
Current Theory of Maternal Tolerance of fetus

- Approach has been to treat the fetus as an allograft (transplant)
  - Considers immunoreactivity to the fetus
- Initial approach was to evaluate histology at the local interface.
- Application of what we know about transplant rejection
Current Theory of Maternal Tolerance of Fetal Allograft

- HLA - G
- Fas - Fas Ligand
- Uterine NK cells, Large Granular Lymphocytes
- Soluble factors
- Tryptophan Catabolism
HLA - G

- The maternal immune system encounters the fetus at the placental interface.
- The placental villi are bathed in maternal blood.
- The syncytiotrophoblasts is the predominate cell type at the interface.
  - Villous interface and in the decidua (replaces the endothelium of the spiral arteries)
HLA - G

• Is there something special about the syncytiotrophoblasts to make them less immunogenic?
  – No expression of MHC Class I or Class II.
  – Expression of antigenically restricted, non-classic MHC I: HLA-G
  – Unique to placenta
HLA - G

• Expression upregulated by inflammatory cytokines (TNF-\(\alpha\) and IFN-\(\gamma\))
• Inhibits proliferation of T cells
• Decreases the decidual production of IFN-\(\gamma\) and TNF-\(\alpha\).
• Possible induction of immune deviation
• Inhibits the cytotoxic activity of NK cells (and LGL)
Soluble Factors

- Inflammatory mediators suppress *in vitro* cultures of trophoblasts.
- TGF-β like molecules secreted by trophoblastic cells.
- IL-10 present in gestational tissues.
IL-10 and Pregnancy

Essential Role for IL-10 in Resistance to Lipopolysaccharide-Induced Preterm Labor in Mice

Sarah A. Robertson, Rebecca J. Skinner, and Alison S. Care


- C57/BL6 syngenic
- IL-10 -/- mice given varying doses of LPS on day 17 of pregnancy
- Did not attempt to identify the source of the cytokine changes.
  - Whole tissue analysis
Is the fetus an allograft?

• Women with renal transplants have a similar rate of serious rejection episodes (5%/year) regardless of pregnancy status.
  – Overall immunosuppressed?
• Women with autoimmune disease (except Lupus) experience an amelioration of their disease
Maternal immunologic awareness of the fetus

• Fetal cells are can be routinely detected in the mother
  – Cleared in large part
  – Microchimerism occasionally persists.

• T cells recognizing the fetus are reduced in number
  – Reversed post-partum
T Cell Awareness of Paternal Alloantigens During Pregnancy

Anna Tafuri, Judith Alferink, Peter Möller, Günter J. Hämmerling, Bernd Arnold*

Science (1995) 270:630

<table>
<thead>
<tr>
<th></th>
<th>Female</th>
<th>Male</th>
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<tbody>
<tr>
<td></td>
<td>(CBA H-2\textsuperscript{k})</td>
<td></td>
</tr>
<tr>
<td>Non-Preg (A)</td>
<td>DES-TCR</td>
<td>None</td>
</tr>
<tr>
<td></td>
<td>Specific for H-2K\textsuperscript{b}</td>
<td></td>
</tr>
<tr>
<td>Allogeneic* (B)</td>
<td>DES-TCR</td>
<td>H-2\textsuperscript{b}</td>
</tr>
<tr>
<td>Syngeneic (C)</td>
<td>DES-TCR</td>
<td>H-2\textsuperscript{k}</td>
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<tr>
<td>Allogeneic (D)</td>
<td>DES-TCR</td>
<td>H-2\textsuperscript{s}</td>
</tr>
</tbody>
</table>

![Graphical representation of T cell awareness](image)
• Pregnant DES - TCR mice grafted (days 3-5) with $K^b$ tumor cells
• Relative tolerance of tumor graft.
So, what is going on?
Peripheral Tolerance

- Self-reactive T cells are present in healthy individuals
- Uncontained immune responses, even to legitimate (non-self) targets, leads to normal tissue damage
- Robust mechanisms must exist to deal with over exuberant immune responses.
Peripheral Tolerance

• Major Mechanisms
  – Anergy/Co-stimulation
  – Nitric Oxide
  – Immune deviation
  – Immune regulatory cells
Regulatory T Cells

• Hypothesized for years
• Became a Taboo topic
• Finally, T regulatory cells isolated and characterized (1995)
  *Immunologic Self-Tolerance Maintained by Activated T Cells Expressing IL-2 Receptor α-Chains (CD25)*

  Breakdown of a Single Mechanism of Self-Tolerance Causes Various Autoimmune Diseases¹

  Shimon Sakaguchi,²* Noriko Sakaguchi,* Masanao Asano,¹ Misako Itoh,¹ and Masaaki Toda*  

• FoxP3 is the molecular hallmark
• Found to have roles in many aspects of autoimmunity
### Table 1. Induction of autoimmune disease in nu/nu mice by inoculating nu/+ T cell subpopulations

<table>
<thead>
<tr>
<th>Expt. Group</th>
<th>Treatment of Cells</th>
<th>Total No. of Mice</th>
<th>No. of Mice with Autoimmune Disease$^b$</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Gas</td>
</tr>
<tr>
<td>A</td>
<td>C</td>
<td>18</td>
<td>0</td>
</tr>
<tr>
<td>B</td>
<td>C</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>C</td>
<td>Anti-CD25 + C</td>
<td>12</td>
<td>(12)</td>
</tr>
<tr>
<td></td>
<td>(1 x 10$^4$)</td>
<td></td>
<td>(100)</td>
</tr>
<tr>
<td>D</td>
<td>Anti-CD25 + C</td>
<td>22</td>
<td>(22)</td>
</tr>
<tr>
<td></td>
<td>(5 x 10$^4$)</td>
<td></td>
<td>(100)</td>
</tr>
<tr>
<td>E</td>
<td>Anti-CD25 + C</td>
<td>10</td>
<td>(8)</td>
</tr>
<tr>
<td></td>
<td>(1 x 10$^4$)</td>
<td></td>
<td>(80.0)</td>
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<tr>
<td>F</td>
<td>Anti-CD25 + C</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Anti-CD4</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(5 x 10$^4$)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>G</td>
<td>Anti-CD25 + C</td>
<td>16</td>
<td>(14)</td>
</tr>
<tr>
<td></td>
<td>Anti-CD8</td>
<td></td>
<td>(87.5)</td>
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<tr>
<td>H</td>
<td>Anti-CD5 + C</td>
<td>12</td>
<td>(6)</td>
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<tr>
<td></td>
<td>Anti-CD8</td>
<td></td>
<td>(50.0)</td>
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</tbody>
</table>

FoxP3 is the molecular hallmark of Tregs
Regulatory T cells mediate maternal tolerance to the fetus

Varuna R Aluvihare¹,²,³, Marinos Kallikourdis¹ & Alexander G Betz¹,³

VOLUME 5 NUMBER 3 MARCH 2004  NATURE IMMUNOLOGY

Balb/c Lymphocytes  X  C57BL/6 Male = Normal Litter

Balb/c Lymphocytes CD25 depleted  X  C57BL/6 Male = Small Litter

Balb/c Lymphocytes CD25 depleted  X  Balb/c Male = Normal Litter
Experimental Setup

D.P.C. 0 6.5 12.5 15.5 18.5

C57BL/6 Male
Virgin C57BL/6 Female

ONTAK (30µg/mouse i.p.)
*in vivo* Depletion of Tregs Reduces the Number of Antigenic Fetuses (Males)

Kahn, Baltimore *PNAS* 107:9299 (2010)
*in vivo* Depletion of Tregs Reduces the Weight of the Surviving Antigenic Fetuses (Males)

Kahn, Baltimore *PNAS* 107:9299 (2010)
Experimental Setup

Evaluation of CD4^+ response:

*Dby* = CD4^+ I-A^b restricted H-Y epitope

*Lso* = CD4^+ I-A^b restricted Listeria epitope

<table>
<thead>
<tr>
<th>Non-pregnant (Virgin)</th>
<th>Early (d.p.c. = 6.5)</th>
<th>Mid (d.p.c. = 12.5)</th>
<th>Late (d.p.c. = 18.5)</th>
</tr>
</thead>
</table>

Male Antigen

Control Peptide
Maternal immune response during syngeneic pregnancy

Kahn, Baltimore PNAS 107:9299 (2010)
Effect of Treg depletion on maternal lymphocyte proliferative response to H-Y

Kahn, Baltimore PNAS 107:9299 (2010)
Effect of Treg Depletion on Maternal Proliferative Response to H-Y Throughout Gestation

Kahn, Baltimore PNAS 107:9299 (2010)
KLH/CFA
KLH/CFA

Isolate splenocytes

H-Y
180,000 Treg depleted Splenocytes from KLH/CFA immunized mouse + 20,000 Tregs from pregnant female (B6xB6 day 18.5)
If pregnancy is inducing Tregs in response to fetal antigens, syngeneic tolerance should be transferable to males, but not females.
Model Autoimmune Disease to Test Tolerance.

• EAE
  – Experimental Allergic Encephalomyelitis
  – Model of Multiple Sclerosis
  – Immunize mice with Myelin
  – Progressive paralysis

• 0 = no obvious signs of disease
• 0.5 = partial tail weakness (cannot raise tail against gravity)
• 1 = limp tail
• 1.5 = limp tail and hindlimb weakness
• 2 = limp tail and impairment in righting reflex
• 2.5 = limp tail and hindlimb paresis
• 3 = bilateral hindlimb paralysis
• 3.5 = bilateral hindlimb paralysis and forelimb paresis
• 4 = moribund
• 5 = dead.
♀ C57BL/6-FoxP3-GFP $\times$ ♂ C57BL/6 FoxP3-GFP

Plug Day = 0.5

Sort for GFP+ cells from splenocytes from pregnant and virgin female

Plug Day = 18.5

Transfer Tregs i.p. (100K/mouse)

Day 0 Immunized MOG/CFA

Day 5

EAE Scoring
# Experiments

<table>
<thead>
<tr>
<th>Recipient</th>
<th>Treg (100,000)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C57BL/6 ♂</td>
<td>None, Virgin, Pregnant</td>
</tr>
<tr>
<td>C57BL/6 ♀</td>
<td>None, Virgin, Pregnant</td>
</tr>
</tbody>
</table>
Tregs added
Tregs added
Tregs added

Female + Preg Treg (n=8)

Female + NonPreg Treg (n=6)

Female (n=8)
Tregs added

- Female + Preg Treg (n=8)
- Female + NonPreg Treg (n=6)
- Female (n=8)
Female mice treated with non-pregnant Treg
Female mice treated with pregnant Treg
How are Tregs changing with pregnancy?
Experiment

♀ C57BL/6-FoxP3-GFP
♂ C57BL/6 FoxP3-GFP

Plug Day = 0.5

Sort for GFP+ cells from splenocytes from pregnant and virgin female

Plug Day = 18.5

Transfer Tregs i.p. (100K/mouse)

Day 0
Immunized MOG/CFA

Day 5

End on Day 12

EAE Scoring
Recovered Lymphocytes Male CNS

![Graph showing the %CD4+GFP (FoxP3)+ in No treatment, Virgin Tregs, and Pregnant Tregs for males. The graph indicates significant differences with symbols # and *.]

#, * p < 0.05

Recovered Lymphocytes Female CNS

![Graph showing the %CD4+GFP (FoxP3)+ in No treatment, Virgin Tregs, and Pregnant Tregs for females. The graph indicates significant differences with symbols # and *.]

#, * p < 0.05
Lymphocytes isolated from maternal uterus

\[ p = 0.0078 \]

![Graph showing CD4+FoxP3+ percentages in male, female, and non-pregnant groups. The male group has significantly higher values compared to the female and non-pregnant groups, with no significant difference between female and non-pregnant groups.](image-url)
Chemokine receptor mRNA qPCR from FoxP3+ (GFP) sorted Tregs

virgin n = 3, syngeneic pregnancy n = 3
Chemokine receptor mRNA qPCR from FoxP3+ (GFP) sorted Tregs
 virgin n = 3, syngeneic pregnancy n = 3
CCR6 and Tregs

• Found on both Tregs and $T_H^{17}$ cells

• $CCR6^{-/-}$ resistant to EAE
  – $T_H^{17}$ cells failure to gain entry to the choroid plexus
    

  – Transfer of $CCR6^{+/+}$ Tregs can reduce (further) disease severity in $CCR6^{-/-}$ mice.
    

• $CCR6$ mediated recruitment of Tregs sites of inflammation
  – Glomerulonephritis
    

  – Tumors
    
CD4⁺ Lymphocytes

<table>
<thead>
<tr>
<th></th>
<th>Syngeneic</th>
<th>Allogeneic</th>
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<tbody>
<tr>
<td>Virgin</td>
<td>d.p.c. 12.5</td>
<td>d.p.c. 18.5</td>
</tr>
<tr>
<td>Spleen</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inguinal LN</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pelvic LN</td>
<td></td>
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</tbody>
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FoxP3

CCR6
How does pregnancy induce CCR6?
STAT 5A/B

- Required for FoxP3 expression
  - Double Knockout
- Transduces Prolactin Receptor
- Ligands for Prolactin Receptor:
  - Prolactin
  - Placental Lactogen
Hormone levels during pregnancy

A. Humans

B. Rodents

Hormone levels during pregnancy

A

Humans

B

Rodents

Hormone levels during pregnancy

A. Humans

B. Rodents

Hormone levels during pregnancy

A. Hormone levels in Humans:

- hPL
- E2
- P
- PRL

Weeks of Pregnancy:
4 8 12 16 20 24 28 32 36 40

B. Hormone levels in Rodents:

- PRL
- PL
- E2
- P

Days of Pregnancy:
1 4 7 10 13 16 19 22

Mid Gestation:
Term

Hormone levels during pregnancy

A. Humans

B. Rodents

Effect of Prolactin on Virgin Tregs
Are Tregs being recruited by the fetus?

• Does the fetus produce the CCR6 ligand, CCL20?
Tissue CCL20 expression

Virgin

Pregnancy
Possibilities

- CCL20 production is inhibited by IL-10.
- A hallmark of Treg functionality is IL-10 production.
- Thus, with increasingly antigenic fetuses, CCL20 production is lowered as Tregs arrive on site.
IL-10 levels from FoxP3-GFP+ cells

Relative mRNA expression

Virgin  Syngeneic  Allogenic
Placental CCL20 in IL-10−/−
What about humans?
Tregs in Human pregnancy

• Increase in maternal Treg population peaking in the 2nd trimester

Maternal PBL Proliferative Response to Irradiated Cord Blood Lymphocytes is Augmented with Treg Depletion in Normal Term Pregnancy
Preeclampsia

- 4-5% of all pregnancies world wide.
- second half of pregnancy.
- Typical Manifestations
  - Mild: HTN, Proteinuria
  - Severe: IUGR, liver and renal failure, thrombocytopenia, hemolysis, and ultimately seizures (ECLAMPSIA).
  - Maternal death is not uncommon in the developing world
- Only cure is Delivery
  - Regardless of the gestatational age.
- Risk Factors
  - First pregnancies, extremes of reproductive age, change in paternity, or previous pregnancies affected by preeclampsia.
Maternal PBL Proliferative Response to Irradiated Cord Blood Lymphocytes is High in Preeclamptic Pregnancy and is \textbf{NOT} Augmented with Treg Depletion.
## Patient Characteristics

<table>
<thead>
<tr>
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<th>Normal N=8</th>
<th>Preeclamptic N=8</th>
<th>95% CI</th>
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</thead>
<tbody>
<tr>
<td>Age (yrs)</td>
<td>28.8 ± 2.1</td>
<td>30.8 ± 2.6</td>
<td>(-9.2 to 5.2)</td>
</tr>
<tr>
<td>Gravidity</td>
<td>2.5 ± 0.6</td>
<td>1.8 ± 0.2</td>
<td>(-0.6 to 2.1)</td>
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<tr>
<td>Parity</td>
<td>0.8 ± 0.3</td>
<td>1.8 ± 0.3</td>
<td>(-1.9 to -0.1)</td>
</tr>
<tr>
<td>Gestational Age</td>
<td>39.1 ± 0.2</td>
<td>38.6 ± 0.6</td>
<td>(-0.8 to 1.8)</td>
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<td>NSVD</td>
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<td>4</td>
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<tr>
<td>C/S</td>
<td>5</td>
<td>4</td>
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<tr>
<td>Mild</td>
<td></td>
<td>4</td>
<td></td>
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<tr>
<td>Severe</td>
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<td>4</td>
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<tr>
<td>Magnesium</td>
<td></td>
<td>5</td>
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Overall Maternal Lymphocyte Proliferative Response to Matched Cord Blood (n = 8)

$[^3]H$ TdR uptake (CPM x $10^3$)

<table>
<thead>
<tr>
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<th>Normal</th>
<th>Preeclamptic</th>
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</tbody>
</table>

$^*p < 0.05$
Overall Effect of Treg Depletion on Maternal Proliferative Response to Matched Cord Blood (n=8)

Fold change in proliferation with CD25+ depletion

Normal

Preeclamptic

*p < 0.05
Treg levels are *unchanged* in preeclampsia
Human CCR6

p = 0.00071

CCL20 Whole Placenta

% of CD4+FoxP3+ that are CCR6+

Non-pregnant

Pregnant

3rd Trimester

2nd Trimester

Relative units

[Graphs showing data with error bars]
Conclusions

• Tregs from Pregnancy are activated and capable of transferring tolerance.
• Pregnancy activates a polyclonal population of Tregs with specificity that includes fetal antigens.
• Pregnancy changes the ability of Tregs to traffic through enhanced CCR6 expression
  – Knockout data is in progress
• Potentially, the fetus expresses CCL20 to recruit Tregs which down regulate CCL20 via IL-10.
Conclusions

• Tregs are important in limiting the maternal response to fetal antigens during human pregnancy.
• Treg dysfunction is found during preeclamptic pregnancies.
• Treg composition is not different from normal.
• Acute Maternal Fetal Rejection.
Acknowledgements

Who did the hard work
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  – Margarida Lei

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Thank You!