Modulation of immunogenicity by engineered antigen-specific regulatory T cells: Fighting fire with fireman or police CARs

David W. Scott
Department of Medicine
Uniformed Services University of the Health Sciences
Bethesda, MD, USA

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Our lab has focused on developing novel approaches to induce specific unresponsiveness ("immune tolerance") and applying these to modulate human diseases and prevent/reverse adverse immune responses.

One such approach is regulatory T-cell therapy, which has been proposed to treat autoimmune diseases, allergy and transplant rejection, as well as to suppress undesirable antibody responses to biotherapeutics, such as FVIII.

Dealing with immunogenicity and adverse responses
Clinical studies with expanded human regulatory T-cell therapy are already in progress. However, these are polyclonal T cells that include a diverse repertoire of relativities.

**Caveats:**

- The frequency of relevant regulatory T cells (Tregs) may be quite low.

- Expanded polyclonal Tregs (multiple specificities) may be non-specifically immunosuppressive.
Approaches

• Enrich and expand Tregs with antigen/tetramer, etc.

Possible solution based on chimeric antigen receptor (CAR) therapy for cancer:

- Engineer specificity into polyclonal Tregs via retroviral transduction of specific “receptors”, e.g.:
  - T-cell receptor (TCR)
  - CAR (scFv) or Antigen (B-cell antibody receptor=BAR)
Approach to transduce expanded polyclonal Tregs
Transduced antigen-specific polyclonal Tregs
Engineering antigen-specificity into polyclonal T cells: Four flavors

**TCR Treg**

**CAR Treg**

*BAR Treg*

*BAR CD8*

*B-cell antibody receptor = BAR*
Engineering antigen-specificity into polyclonal T cells: TCR V-regions

TCR Treg effects

Aihong (Allan) Zhang
Yong Chan Kim

FVIII in hemophilia A
MBP in multiple sclerosis
Systems and targets

- Multiple systems:
  - Hemophilia inhibitors (FVIII)
  - Autoimmunity, e.g., MS (MBP) or Type 1 diabetes
  - Allergy (OVA)
  - Future targets (ADA’s)
Hemophilia

X-linked blood clotting disorder

FVIII mutations cause Hemophilia A*

FIX mutations cause Hemophilia B

*Deletions, inversions, missense, stop codons
What is standard treatment for bleeds?
The unwelcome response to a human protein, FVIII

Hemophilia A patients can mount an immune response to FVIII depending, in part, on the nature of their mutation.

**Because they lack FVIII, they did not develop immune tolerance to therapeutic FVIII**

Specific antibodies against FVIII *inhibit* clotting by binding to domains required its bio-activity ("inhibitors")
Application in Hemophilia A

IMMUNOBIOLOGY

Engineered antigen-specific human regulatory T cells: immunosuppression of FVIII-specific T- and B-cell responses

Yong Chan Kim,1 Ai-Hong Zhang,1 Yan Su,1 Sadiye Amcaoglu Rieder,2 Robert J. Rossi,1 Ruth A. Ettinger,3 Kathleen P. Pratt,1 Ethan M. Shevach,2 and David W. Scott1

1Department of Medicine, Uniformed Services University of the Health Sciences, Bethesda, MD; 2Laboratory of Immunology, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Rockville, MD; and 3Puget Sound Blood Center Research Institute, Seattle, WA

Key Points

- Generation and function of specific human Tregs.
- Specific regulation of FVIII responses by engineered human Tregs.

Expansion of human regulatory T cells (Tregs) for clinical applications offers great promise for the treatment of undesirable immune responses in autoimmunity, transplantation, allergy, and antidrug antibody responses, including inhibitor responses in hemophilia A patients. However, polyclonal Tregs are nonspecific and therefore could potentially cause global immunosuppression. To avoid this undesirable outcome, the generation of antigen-specific Tregs would be advantageous. Herein, we report the production and properties of engineered antigen-specific Tregs, created by transduction of a recombinant T-cell receptor obtained from a hemophilia A subject’s T-cell clone, into expanded human FoxP3+ Tregs. Such engineered factor VIII (FVIII)-specific Tregs efficiently suppressed the proliferation and cytokine production of FVIII-specific T-effector cells. Moreover, studies with an HLA-transgenic, FVIII-deficient mouse model demonstrated that antibody production from FVIII-primed spleen cells in vitro were profoundly inhibited in the presence of these FVIII-specific Tregs, suggesting potential utility to treat anti-FVIII inhibitory antibody formation in hemophilia A patients. (Blood. 2015;125(7):1107-1115)
**Immunology 101: The immune Response to FVIII**

**Antigen presenting cells**
- FVIII protein
- FVIII peptide

**Activated FVIII-specific T cells**
- FVIII-specific helper T cell
- FVIII-specific regulatory T cell aka “Police CARs”

**Activated FVIII-specific B cells**
- FVIII-specific plasma cells

**Cytokines**
- FVIII-specific plasma cells

**FVIII-specific helper T cell**
- Activated FVIII-specific helper T cell

**FVIII-neutralizing inhibitor antibody**
- FVIII-neutralizing inhibitor antibody
Isolation of naive T cells and regulatory T cells from normal donor PBMC

Treg
(CD4⁺CD25^{hi}CD127^{l})

T naive (helper)
(CD4⁺CD25⁻CD45RA⁺)
Antigen-specific upregulation of Treg markers in 17195 Tregs

**Induction of Foxp3 and GARP**

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<tr>
<th>OVA peptide</th>
<th>FVIII peptide</th>
<th>Anti-CD3ε</th>
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<tbody>
<tr>
<td>17195</td>
<td>17195</td>
<td>17195</td>
</tr>
<tr>
<td>9.59%</td>
<td>9.59%</td>
<td>90.1%</td>
</tr>
<tr>
<td>84.0%</td>
<td>84.0%</td>
<td>5.62%</td>
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**Induction of LAP and GITR**

- LAP
- GITR

- Mock
- GFP+ Mock
- GFP+ 17195

- pC2<sub>(2191-2210)</sub>

- No antigen
FVIII-C2-specific immunosuppression by Treg17195

Can this approach work to prevent or reverse inhibitor responses in hemophilia A mice?
Protocol of FVIII-specific suppression of secondary antibody formation by engineered FVIII-specific human Tregs

**Immunization in vivo**

- HLA.DR1XE16
- Immunized mice

**Reactivation in vitro**

- rFVIII
- ELISPOT assay to detect anti-FVIII antibody

<table>
<thead>
<tr>
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<th>No FVIII</th>
<th>+ FVIII</th>
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<td>7 days</td>
<td>![Image]</td>
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**Data**: The ELISPOT assay results show a significant reduction in the number of anti-FVIII antibody spots in the reactivated immunized mice treated with rFVIII compared to the control group without FVIII.
A Treg 0

B

1/2
1/4
1/8
1/16

E

Mock

Spots per 10 x 10^6 splenocytes

17195 vs. C2 domain peptide

Engineering antigen-specificity into polyclonal T cells: Single chain (scFv) CARs
What about CAR (chimeric single chain Fv) Tregs?

Antigen on B cell or APC or endothelial cell surface

ScFv vs. antigen

Signaling domains

CAR Treg
Both TCR- or scFv engineered human Tregs suppress the secondary anti-FVIII response in vitro

TCR- or scFv engineered human Tregs suppress the anti-FVIII response \textit{in vivo}
Scheme for bystander suppression of multiple T-cell clones by a single Treg

- C2
- A2
- C1
- xxx
- 17195 TCR

APC

T effector

Treg

Suppression
Can antigen-expressing “BAR” T-cell therapy modulate antibody responses by directly engaging antigen-specific B cells?

“BAR” = B-cell antibody receptor
FVIII-specific B cell

Structure of BAR (B-cell Antibody Receptor) Treg or CD8 cell

Kalpana Parvathaneni
Engineering antigen-specificity into polyclonal T cells: Targeting the B cell

TCR Treg

CAR Treg

BAR Treg

BAR CD8
A2/C2 BAR mCD8-mediated elimination of anti-FVIII B cells from E16-mouse spleen cells stimulated with LPS

A. αFVIII IgM+ ELISPOT assay by LPS-stimulated E16 B cells

LPS (1 μg/mL) E/T ratio: 5:1

B. Quantification of number of spots

FVIII-coated wells
Survival of 2JLO-injected NSG mice with BAR CD8 T-cell therapy

Day 10

Day 18

Day 25
Can “BAR” engineered CD4 Tregs target and suppress FVIII-specific B cells
Prevention of anti-FVIII antibody development in naïve E16 mice by BAR human Tregs in vivo

- rFVIII (2 μg) in IFA, s.c.
- hTregs, $1 \times 10^6$, i.v.
- rFVIII (1μg) in PBS, i.v.
- 10% TNP-SRBC, i.v.

Anti-FVIII antibody (µg/ml)
Systems and targets

- Multiple systems:
  - Hemophilia inhibitors (FVIII)
  - Autoimmunity, e.g., MS (MBP) or Type 1 diabetes
  - Allergy (OVA)
  - Future targets (ADA’s)
Can BAR Tregs be used to modulate allergy?

- OVA-specific B cell
- Mast cell with FcεR
- BCR
- OVA protein
- CD28
- CD3ζ
- OVA-specific BAR Treg
- OVA-specific IgE
Future: CAR or BAR cell therapy not only for hemophilia, but also for allergy, transplantation, autoimmunity or other monogenic diseases (and ADA?)
Summary

• Antigen-specific TCRs, single chain Fvs and antigen domains (BARs) have now been engineered for retroviral transduction into human T effectors and human (mouse) Tregs.

• These Tregs specifically suppressed both proliferation and cytokine production by antigen-specific T effectors, and antibody formation *in vitro* and *in vivo* in multiple model systems.

• Recent data with “BAR” CD8’s and Tregs (expressing antigen domains) may allow multiple approaches to regulate adverse immune responses.
  ✓ e.g., Ovalbumin-BAR iTregs are able to suppress both active and passive anaphylaxis.

• Expansion of these studies to Tregs in a larger species (hemophiliic dogs) is in progress, with human clinical studies on the horizon.
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