A humanized mouse model to study insulin-specific tolerance and islet autoimmunity

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The incidence of childhood Type 1 diabetes is increasing worldwide at an alarming rate

Patterson CC et al., Lancet 2009, Ziegler AG et al., JAMA 2013

Nearly 90,000 children newly develop Type 1 diabetes each year

IDF Diabetes Atlas 2015, 7ed. Brussels, Belgium

The number of children affected with Type 1 diabetes is doubling every 12 years

Pathogenesis of autoimmune Type 1 diabetes

modified from Ziegler AG et al., JAMA 2013
Progression from islet autoimmunity to clinical Type 1 diabetes

Plasticity in immune activation and impairments in immune tolerance
Restoration of immune tolerance in Type 1 diabetes

Mediators of tolerance:
Foxp3$^+$ regulatory CD4$^+$ CD25$^+$ T (Treg) cells

Blue helmets - peacekeeping troops - of the immune system
Murine antigen-specific Treg induction

Dendritic cell

MHC class II molecule
Strong agonist ligand
TCR

Naive CD4+ T cell

Treg conversion
Subimmunogenic TCR stimulation

CD4+CD25+Foxp3+
regulatory T cell
(Treg)

Treg expansion
Immunogenic TCR stimulation

CD4+CD25+Foxp3+
regulatory T cell
(Treg cells)


Daniel C et al., PNAS 2010; Daniel C et al., Meth in Mol Biol 2011;
Daniel C et al., Adv Immunol 2011
Human Treg induction using subimmunogenic TCR stimulation in vitro

Serr et al., Nat Comm 2016
Human Treg induction using subimmunogenic TCR stimulation in vitro

Restimulation of human Tregs induced in vitro

Serr et al., Nat Comm 2016
From mouse to man: Human antigen-specific Treg induction relevant for autoimmune Type 1 diabetes

- Insulin autoantibodies are often the first to appear in children.
  - Insulin-specific autoimmunity present in the earliest stages of islet autoimmunity.

- Insulin epitopes function as a main target of the autoimmune attack.
  - Insulin B:9-23 in mice and humans.

- Insulin B:9-23 epitopes are presented by human HLA-DQ8 to human CD4\(^+\) T cells.
How to induce human Foxp3⁺Tregs \textit{in vivo}?
Use of HLA-DQ8 Tg NSG mice

Immune-deficient HLA-DQ8 NSG mice:
Reconstitution with CD34⁺ human hematopoietic stem cells (human HSCs)

5 Weeks post reconstitution

Engraftment in reconstituted HLA-DQ8-NSG mice

\( \text{hu.CD45} \)

\( \text{murine CD45} \)

\( \text{Human CD45} \)

Serr et al., Nat Comm 2016
How to induce human Foxp3$^+$Tregs \textit{in vivo}? Characterization of human CD4$^+$T cells

8 wks post reconstitution

Serr et al., Nat Comm 2016
Ex vivo identification of HLA-DQ8 restricted insulin-specific CD4+ T cells from human peripheral blood
How to induce human Foxp3^Tregs *in vivo*? Characterization of human CD4^T cells

20 wks post reconstitution

Serr et al., Nat Comm 2016
How to induce human Foxp3\(^+\)Tregs \textit{in vivo}? 
Identification of human insulin-specific CD4\(^+\)T cells

20 wks post reconstitution

Identification of HLA-DQ8 restricted insulin-specific CD4\(^+\)T cells in humanized HLA-DQ8 Tg NSG mice.

Serr et al., Nat Comm 2016
How to induce human Foxp3^{+} Tregs \textit{in vivo}? Insulin-specific Treg induction \textit{in vivo}

1. **Naive T cell**
   - strong agonistic insulin mimetopes
   - subimmunogenic conditions

2. **Subimmunogenic application of insulin-mimetopes** (osmotic mini-pumps, sc.)

3. **Treg induction in vivo**

4. **Analyses**
   - mesenteric lymph node
   - blood spleen

- Treg cell

\textit{Serr et al., Nat Comm 2016}
Analysis and functional characterization of human Tregs in humanized mice

Serr et al., Nat Comm 2016
Analysis and functional characterization of human Tregs in humanized mice

Serr et al., Nat Comm 2016
Analysis and functional characterization of human Tregs in humanized mice

Increased frequencies of human Tregs upon Treg induction with insulin-mimetopes in humanized HLA-DQ8 Tg NSG mice.
Efficient human insulin-specific Treg induction in humanized mice

Treg signature genes mRNA abundance 6 months after Treg induction in vivo

<table>
<thead>
<tr>
<th>Gene</th>
<th>Phosphate buffered saline</th>
<th>Insulin-mimetopes [5 μg/d]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Foxp3</td>
<td><strong>15 ± 1</strong></td>
<td><strong>20 ± 2</strong></td>
</tr>
<tr>
<td>CTLA4</td>
<td><strong>25 ± 2</strong></td>
<td><strong>30 ± 3</strong></td>
</tr>
<tr>
<td>IL-2Ra</td>
<td><strong>20 ± 2</strong></td>
<td><strong>25 ± 3</strong></td>
</tr>
<tr>
<td>TIGIT</td>
<td><strong>15 ± 1</strong></td>
<td><strong>20 ± 2</strong></td>
</tr>
<tr>
<td>RTKN2</td>
<td><strong>10 ± 1</strong></td>
<td><strong>15 ± 2</strong></td>
</tr>
</tbody>
</table>

T cell effector genes mRNA abundance 6 months after Treg induction in vivo

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<thead>
<tr>
<th>Gene</th>
<th>Phosphate buffered saline</th>
<th>Insulin-mimetopes [5 μg/d]</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-17Rα</td>
<td><strong>15 ± 1</strong></td>
<td><strong>20 ± 2</strong></td>
</tr>
<tr>
<td>IL-21</td>
<td><strong>20 ± 2</strong></td>
<td><strong>25 ± 3</strong></td>
</tr>
<tr>
<td>RORγt</td>
<td><strong>15 ± 1</strong></td>
<td><strong>20 ± 2</strong></td>
</tr>
<tr>
<td>T-bet</td>
<td><strong>10 ± 1</strong></td>
<td><strong>15 ± 2</strong></td>
</tr>
<tr>
<td>NFATc2</td>
<td><strong>5 ± 1</strong></td>
<td>not detectable</td>
</tr>
<tr>
<td>IFNγ</td>
<td><strong>10 ± 1</strong></td>
<td><strong>15 ± 2</strong></td>
</tr>
</tbody>
</table>

Phenotypes of induced human Tregs in humanized HLA-DQ8 Tg NSG mice are stable.
Analysis and functional characterization of human Tregs in humanized HLA-DQ8 Tg NSG mice

Induced human Tregs from humanized mice are functional and suppressive.
Methylation analyses of Foxp3 Treg-specific demethylated region (TSDR) from humanized mice

Ex vivo human CD4⁺T cells from peripheral blood

Ex vivo human CD4⁺T cells from humanized mice

human Foxp3 TSDR methylation status
[male donors]

human Foxp3 TSDR methylation status
[female donors]

human Foxp3 TSDR methylation status
upon insulin-mimeticpe specific Treg induction
in T cells from humanized HLA-DQ8 NSG mice
[female donors]

Induced human Tregs from humanized mice are functional and stable.
Is there evidence in children with islet autoimmunity that insulin-specific Treg cells can impact the progression to Type 1 diabetes?
High frequencies of insulin-specific Foxp3+ Tregs do associate with slow progression to Type 1 diabetes.
Summary

Human insulin mimetopes induce insulin-specific Treg cells in a pre-clinical setting of humanized mice.

Induced human Tregs in humanized HLA-DQ8 Tg NSG mice are functional and suppressive.

In children, high frequencies of insulin-specific Tregs or low frequencies of insulin-specific TFH precursor cells do associate with slow progression to Type 1 diabetes.

Critical next steps:
Understand identified T tolerance defects mechanistically
Dissect T tolerance defects and mechanisms *in vivo*
→ Modelling of islet autoimmunity in humanized mice
Study insulin-specific Treg induction using combination strategies to interfere with autoimmune activation.
Acknowledgements, cooperations and network

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[Logos and institutions]
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