Immunopathogenesis of type 1 diabetes – the role of IL-17

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Type 1 diabetes

Autoimmune loss of insulin-secreting β-cells

Multiple polymorphisms required for disease

β-cell destruction is facilitated self-reactive CD4 T cells

And mediated by self-reactive CD8+ T cells (CTL)

Breakdown in regulatory T cells (Tregs), natural killer T (NKT) cells, and natural killer (NK) cells

At clinical presentation the majority of β-cells have been lost
Discovery of Insulin

Banting, right and student helper, Best, left, are standing on the roof of the medical building with one of the diabetic dogs used in experiments with insulin, 1921.
Death of Islet Beta Cells in Diabetes

Type 1 (insulin-dependent) diabetes.
Progressive autoimmune destruction of beta cells
Pathogenesis of autoimmunity in type 1 diabetes
T cells

CD4 (Th17)

CTL

Death of self reactive T cell

NK

NKT

Treg

Death of beta cell

Beta

DC

Tolerance
T cells

Death of self-reactive CTL

Survival of autoreactive T cell

Death of beta cell

Helper CD4

DC

CTL CD8

NK

Treg

NKT

CD4

CD8

Treg

Beta
IL-2 (interleukin 2) regulates the balance and plasticity of regulatory T helper cells (Treg)

- **Suppressive**
  - FOXP3+
  - CD127lo
  - CD25hi

- **Th17/reg**
  - FOXP3+
  - RORC2+
  - IL-17+

- **Pro-inflammatory**
  - RORC2+
  - CD161+
  - IL-17+
Hypothesis and Aims

Hypothesis: Pancreatic beta cell destruction in T1D is driven by the conversion of self-reactive Treg cells into a Th17 phenotype due to defective Treg IL-2 signaling in T1D subjects who have polymorphic variants in the \textit{IL2RA} gene.

Aim 1: To determine if subjects with recent-onset T1D have changes in the proportions of Treg and Th17 cells in their peripheral blood.

Aim 2: To determine whether changes in the Treg or Th17 populations occur before the onset of T1D.

Aim 3: To determine whether development of Th17 cells from Treg cells is influenced by T1D-associated polymorphic variants in the \textit{IL2RA} gene.
Hypothesis and Aim

**Hypothesis:** Pancreatic beta cell destruction in T1D is driven by the conversion of self-reactive Treg cells into a Th17 phenotype due to defective Treg IL-2 signaling in T1D subjects who have polymorphic variants in the *IL2RA* gene.

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Aim
To determine if subjects with recent-onset T1D have changes in the proportions of Treg and Th17 cells in their peripheral blood.
### Baseline characteristics of the T1D subject and healthy control groups

<table>
<thead>
<tr>
<th></th>
<th>T1D Subjects</th>
<th>Healthy Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Numbers</td>
<td>64</td>
<td>53</td>
</tr>
<tr>
<td>Females/males</td>
<td>35/29</td>
<td>33/20</td>
</tr>
<tr>
<td>Mean age (y)</td>
<td>9.6 ± 0.6</td>
<td>10.2 ± 0.7</td>
</tr>
<tr>
<td>Mean age of onset (y)</td>
<td>9.4 ± 0.6</td>
<td>N/A</td>
</tr>
<tr>
<td>Mean duration of T1D (m)</td>
<td>2.3 ± 0.3</td>
<td>N/A</td>
</tr>
<tr>
<td>Mean A1C</td>
<td>7.9 ± 0.2</td>
<td>N/A</td>
</tr>
<tr>
<td>WBC (x10⁹/L)</td>
<td>3.0 ± 0.4</td>
<td>3.0 ± 0.2</td>
</tr>
<tr>
<td>% lymphocytes</td>
<td>63 ± 2.4</td>
<td>63 ± 2.8</td>
</tr>
<tr>
<td>% CD4+ (CD14-) T cells</td>
<td>31 ± 1.2</td>
<td>31 ± 1.3</td>
</tr>
</tbody>
</table>

Supplementary Table 1. Characteristics of the T1D subject and healthy control groups from British Columbia's Children's Hospital are presented. Data are shown as the means ± standard deviation. All T1D subjects were being treated with insulin and did not show evidence of other autoimmune diseases. Age-matched subjects with no autoimmune or metabolic diseases were used as healthy controls.
FOXP3 is a marker for Treg cells and there are multiple FOXP3+ subsets:

Human CD4+ FOXP3+ T Cells can be split into 3 distinct subsets based on CD45RA (memory marker) and FOXP3 expressions.

I. CD45RA+FOXP3lo (Naive Treg)

II. CD45RA-FOXP3hi (Memory Treg)

III. CD45RA-FOXP3lo (Th17/reg)

Miyara et al, *Immunity* June 2009
An increase in FOXP3+ cells is restricted to Th17/reg (CD45RA-FOXP3lo)
Th17/reg (CD45RA-FOXP3lo) cells secrete large amounts of IL-17
Th17/reg (CD45RA-FOXP3lo) cells are less suppressive than CD45RA+FOXP3lo or CD45RA- FOXP3hi subsets.
There is a bias toward IL-17 secretion in CD4+ T cells from T1D subjects.
Aim 2: To determine whether changes in the Treg or Th17 populations occur before the onset of T1D.

Samples obtained from TrialNet consortium.

1. Additional recent-onset T1D subjects within 3 months of diagnosis (T1D after diagnosis);
2. Subjects at T1D diagnosis Day 0 (T1D at diagnosis)
3. Subjects prior to the development of T1D (T1D before diagnosis);
4. Autoantibody positive, first-degree relatives of T1D subjects; who did not develop T1D (AB+ controls)
5. Autoantibody negative, first-degree relatives of T1D subjects; who did not develop T1D (AB- controls).
The proportion of Treg/17 cells is reduced only after diagnosis

A. % FOXP3+CD127lo

B. % CD25+CD127lo

Suppressive Treg

Control AB-
Non-prog AB+
T1D before diagnosis T1D at diagnosis T1D after diagnosis

FOXP3+CD127lo

CD25+CD127lo
CD45RA-FOXP3lo (Th17/reg) only elevated after T1D diagnosis.
Th17 cells only elevated after T1D diagnosis.
Why are the IL-17 secreting subsets only increase after T1D diagnosis?
Aim 3:

Do T1D-associated polymorphic variants in the *IL2RA* gene identify T1D subjects before diagnosis with Tregs that signal defectively in response to IL-2 and therefore an increase in IL-17 secreting Tregs.

<table>
<thead>
<tr>
<th>SNP</th>
<th>Region</th>
<th>Association</th>
<th>Minor allele frequency</th>
<th>Publication</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs3118470</td>
<td>Non-coding</td>
<td>Risk (1.3OR)</td>
<td>&gt;10%</td>
<td>1,2</td>
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</table>

2. Kawasaki E. et al. J Clin Endocrinol Metab. 2009*
T1D Patients homozygous for the IL-2RA rs3118470 CC risk haplotype have Tregs with reduced pSTAT5 signaling in response to IL-2.

A. ** MFI pSTAT5

B. ** MFI pSTAT5

rs3118470

rs706778
T1D subjects before diagnosis homozygous for the IL-2RA rs3118470 CC risk haplotype do not have a difference in CD25 expression on the surface of Tregs or T effector cells.
T1D subjects before diagnosis homozygous for the IL-2RArs3118470 CC risk haplotype have a higher proportion of IL-17 secreting FOXP3+ cells.
T1D subjects before diagnosis homozygous for the IL-2RArs3118470 CC risk haplotype do not have a difference in proportion of Tregs or Th17 cells.
Conclusions

- T1D subjects have an increase in IL-17 secreting CD4 and CD8 T cells

- Th17/reg cells from T1D subjects are non-suppressive and secrete IL-17

- (Treg defect may be related to IL-2Ra)
Model:

PANCREAS INSULT

Naive Treg

IL-2

IL-2R gene SNPs

memTreg

IL-2R CD25

FOXP3 hi

FOXP3 loss

Th17/reg

exFOXP3

Th17 cell

FOXP3 int

FOXP3 low

Potential Therapeutics:

1. Anti-IL-17
2. IL-2
3. Treg cellular therapy

Recognise Pancreatic Ag
Ustekinumab (Stelara) is a humanized monoclonal antibody that blocks IL-12 and IL-23 by binding to the shared p40 subunit.
Dr. Tan, the Director of the Immunity in Health Disease research cluster at the Child and Family Research Institute, will use the funding to perform human clinical trials on ustekinumab, a humanized monoclonal antibody currently used for severe cases of psoriasis. Ustekinumab inhibits the immune cells that attack the skin in psoriasis, and Dr. Tan and his team are hoping it will do the same with the T-cells that attack the insulin-producing beta cells in the pancreas, if it’s administered early enough. By doing so, Dr. Tan hopes to postpone the need for insulin treatment.

The year-long pilot study will enroll young adults (18 years or older) starting this spring, and will last a year, examining the drug’s safety in diabetes patients and measuring its effect on immune and blood sugar levels. When that is complete, they plan to extend the trial to adolescents, 13 to 18 years old.

“You never really know what research is going to work,” Dr. Tan said. “Unless you spread the net widely, you’re not going to catch that one project that’s going to finally make the breakthrough.”
UBC Type 1 diabetes drug trial seeks to end injections

Study aims to see if drug ustekinumab could curb or eliminate need for insulin injections

Judith Garcia, 19, fills a syringe as she prepares to give herself an injection of insulin to manage her diabetes. A new UBC study aims to test an alternative to the injections for those with Type 1 diabetes. (Reed Saxon/Associated Press)
UBC Type 1 diabetes drug trial seeks to end injections

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CBC News  Posted: Apr 22, 2015 8:05 AM PT  Last Updated: Apr 22, 2015 9:00 AM PT

Judith Garcia, 19, fills a syringe as she prepares to give herself an injection of insulin to manage her diabetes. A new UBC study aims to test an alternative to the injections for those with Type 1 diabetes. (Reed Saxon/Associated Press)