Regulatory T cell genes and pathways associate with islet AGER expression in individuals with type 1 diabetes

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INTRODUCTION
Type 1 diabetes (T1D) results from the autoimmune destruction of the pancreatic islets by invading immune cells. Changes in islet gene expression could reveal new insights into this process. The receptor for advanced glycation end products (RAGE), encoded by the AGER gene, is expressed in the islets and immune cells, and AGER polymorphisms affect T1D risk1-4.

AIMS
We analyzed islets from control, non-diabetic autoantibody-positive and T1D donors, to determine if changes in islet gene expression could differentiate these groups when stratified by AGER expression.

METHODS
Islets were obtained by laser-capture microscopy from human pancreatic cryosections from the Network for Pancreatic Organ Donors with Diabetes (nPOD) biobank. RNA was hybridized on the Affymetrix Human Gene 2.0ST microarray.

Targeted analyses were performed on 118 genes, with known association with AGER or the immune response. Raw intensity values were RMA corrected, quantile normalized, median polish summarized, and log2 transformed.

Principal component analyses (PCA) and Gene Ontology enrichment were performed to detect differences among groups, using pcaGoPromoter and limma in R. Groups were bisected by AGER expression into AGERhi and AGERlo subsets, and reinterrogated by PCA and Gene Ontology enrichment.

Table 1. Donor characteristics in nPOD study.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Control (N = 18)</th>
<th>Auto-Ab+ (N = 12)</th>
<th>T1D (N = 20)</th>
</tr>
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<tbody>
<tr>
<td>Female : male (number)</td>
<td>9:9</td>
<td>5:7</td>
<td>10:10</td>
</tr>
<tr>
<td>Age (year)</td>
<td>36.0 ± 16.16</td>
<td>37.21 ± 18.24</td>
<td>19.97 ± 9.672*</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>25.26 ± 4.817</td>
<td>24.89 ± 6.015</td>
<td>24.05 ± 4.249</td>
</tr>
<tr>
<td>Diabetes duration (year)</td>
<td>N/A</td>
<td>N/A</td>
<td>5 ± 6.815</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>5.688 ± 0.372</td>
<td>5.425 ± 0.1708</td>
<td>11.12 ± 1.983*</td>
</tr>
<tr>
<td>C-peptide (ng/mL)</td>
<td>2.955 ± 3.89</td>
<td>5.43 ± 11.71</td>
<td>0.05 ± 0.05***</td>
</tr>
</tbody>
</table>

Comparisons were by 1-way ANOVA/Tukey’s post-hoc (mean ± SD). Proportions were analyzed by Fisher’s test. * P<0.05 vs. other groups; ** P<0.001 vs. other groups; † P=0.02 vs. autoantibody-positive group.

RESULTS AND DISCUSSION
PCA revealed separation between autoantibody-positive and T1D donors (20 of 20 T1D donors excluded from autoantibody-positive group’s 95% CIs; Fig. 1). T1D donors in the upper-right quadrant were outliers due to differences in age (inset, Fig. 1).

REFERENCES

Fig 1. PCA of islet gene expression (inset, age of T1D donors, t-test). Ellipses are 95% confidence intervals.

Fig 2. Gene Ontology Enrichment for PC2 in PCA. Bonferroni P-values are shown.

Gene Ontology enrichment of PCA found 14 overrepresented biological pathways (P<0.05; Fig. 2). The most extensive branch described genes that regulate T cell proliferation (P=2.49x10^-3). The ten highest ranked PCA genes (FASLG, CTLA4, TLR9, IL1R2, NFKB2, MMP9, FOXP3, BBC3, STAT6, IL2RA) influence T cell function. FOXP3 and IL2RA modulate T regulatory cells (Tregs).

In the AGERhi subgroups, FOXP3 expression was reduced in autoantibody-positive islets (vs. control; P = 0.04), and increased in T1D (vs. autoantibody-positive; P = 0.03; Fig. 3).

CONCLUSIONS
Differences in islet gene expression between autoantibody-positive and T1D donors encompass pathways involved in the activation and regulation of immune cells. Stratification by AGER expression revealed differential expression of FOXP3 among groups. Thus, our findings suggest that changes in AGER may influence islet autoimmunity.