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Underlying T Cell Receptor Methylation Defects in Type 1 Diabetes Associated with Quantitative Defects

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Introduction

- The T cell receptor (TCR) is a central regulator of T cell education
- Efficient structural organization and **density** of TCR/CD3 underlies the development and function of T cells
- T cell maturation defects are associated with autoimmunity, suggesting an underlying defect in TCR selection
- Here we explore **quantitative defects** as a cause for altered TCR selection at the level of the TCR/CD3 protein complex

Methods

- We compared TCR densities on CD4 T cells from subjects with type 1 diabetes (T1D, n=80) and non-diabetic controls (NDC, n=37)
- We also investigated a **quantitative defect** in genes in the TCR/CD3 genome for possible underlying overmethylation in T1D
- All 13 gene regions of the TCR segments and all 4 versions of CD3 proteins were studied for methylation and protein differences in T1D vs controls

Results

- **Significant quantitative defects in TCR and CD3 proteins are observed in CD4+ T cells of T1D vs controls (Fig. 1)**
  - Over-methylation of TCR complex genes and CD3 genes resulted in a functional defect: **downregulated cell surface expression**
- Evaluation of TCRαβ expression in CD4+ T cells at the protein level confirmed methylation patterns
- The TCRαβ+ cell population was significantly reduced in T1D vs controls (p=0.005); MFI density of TCRαβ antibody in T1D was also significantly decreased vs controls (p=0.01)
- In T1D, all CD3 genes except CD3ε had hypermethylated patterns, confirmed by RNAseq analysis
- The percentage CD3+ T cells in the CD4+ T cell population was reduced in T1D vs controls (p=0.04), as was the MFI of CD3+ T cells (p=0.02)

TCR-related Genes Are Hypermethylated in CD4⁺ T Cells from T1D Patients vs Non-diabetic Controls

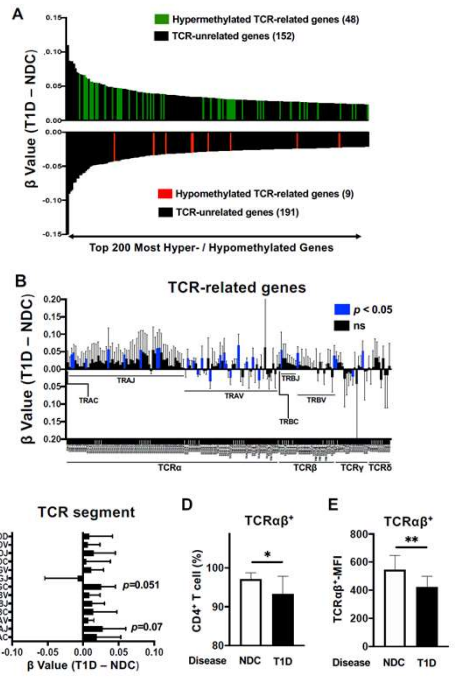

Fig. 1. (**A**) CD4⁺ T-cells were isolated from blood samples of 13 T1D and 8 NDC patients. The β values of all CpGs per gene were averaged and the genes ordered based on the difference in average β value between T1D and NDC. Shown are the top 200 most hypermethylated and top 200 most hypomethylated genes of T1D versus NDC among 35184 total genes. Forty-eight of the top 200 hypermethylated genes (24%) were TCR-related (green bars). Among the 200 most hypomethylated genes, only 9 (4.5%) were TCR-related (red bars). TCR-unrelated genes are shown in black. (**B**) Differences in average β values between T1D at baseline (n=13) and NDC (n=8) (T1D - NDC) are shown for all TCR-related genes (average ± SD, blue bars indicate significant hypermethylation; $p < 0.05$, 2-tailed, unpaired t-test). Positive bars indicate hypermethylation in T1D as compared to NDC, whereas negative bars indicate hypomethylation. (**C**) Overall methylation in TCR-segments at baseline; β values of the local region genes in C-, J-, V- and D-segments were averaged. The differences in β values between NDC (n=8) and T1D (n=13) at baseline (T1D-NDC) are shown at each TCR-segment (average, $*p < 0.05$; $**p < 0.01$; $***p < 0.001$, 2-tailed, unpaired t-test). (**D**) Analysis of the expression of TCRαβ on CD4⁺ T-cells by flow cytometry. The ratio of TCRαβ⁺ cells among CD4⁺ T-cells in NDC (n=11) and T1D (n=10) is shown (average ± SD, $*p < 0.05$; $**p < 0.01$; $***p < 0.001$, 2-tailed, unpaired t-test). (**E**) The mean fluorescent intensity (MFI) of APC in TCRαβ⁺ cells in NDC (n=11) and T1D (n=10) is shown (average ± SD, $*p < 0.05$; $**p < 0.01$; $***p < 0.001$, 2-tailed, unpaired t-test).

Demethylation of TCR/CD3-related Genes in CD4⁺ T-cells from T1D Patients after BCG Vaccination

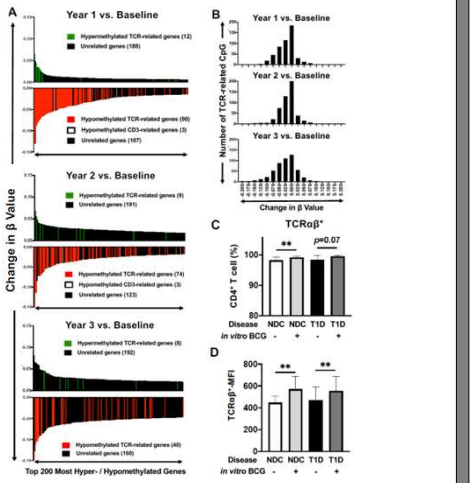

Fig. 2. (**A**) Top 200 most hyper- and hypomethylated genes in CD4⁺ T-cells from T1D treated with BCG vs baseline (prior to vaccination) at Year 1 (top), Year 2 (middle) and Year 3 (bottom). Genes were ordered by the difference in β value versus baseline. TCR-related genes are shown in green (hypermethylated) or red (hypomethylated); CD3-related genes are shown in white (hypomethylated); unrelated genes are shown in black. At Year 1 after BCG vaccination, 90 and 3 of the top 200 most hypomethylated genes were TCR- and CD3-related respectively, whereas only 12 TCR-related genes were hypermethylated. At Year 2, there were 74 TCR- and 3 CD3-related genes among the top 200 hypomethylated genes and 9 TCR-related genes among the top 200 hypermethylated genes. At Year 3, these were 40 (hypomethylated) and 8 (hypermethylated) in TCR-related genes. (**B**) Histograms show the distribution of β values vs baseline for CpGs of TCR-related genes at Year 1 (top), Year 2 (middle) and Year 3 (bottom). A shift of β values toward the left indicates progressive demethylation over time. (**C**) Changes in TCRαβ expression after 7-day culture of CD4⁺ T-cells from NDC and T1D subjects in the presence of BCG. CD4⁺ T-cells were isolated from NDC and T1D patients and cultured for 7 days in RPMI media +/- added BCG, then analyzed by flow cytometry. The percentage of TCRαβ⁺ cells for CD4⁺ T-cells in NDC (n=9) and in T1D (n=8) are shown (average ± SD, $*p < 0.05$; $**p < 0.01$; $***p < 0.001$, 2-tailed, paired t-test). (**D**) The MFI of TCRαβ⁺ cells in NDC (n=9) and T1D (n=8) cultured +/- BCG (average ± SD, $*p < 0.05$; $**p < 0.01$; $***p < 0.001$, 2-tailed, paired t-test).

Abnormally Methylated TCR Genes Observed in T1D Undergo Gradual De-methylation after BCG Vaccine Treatment

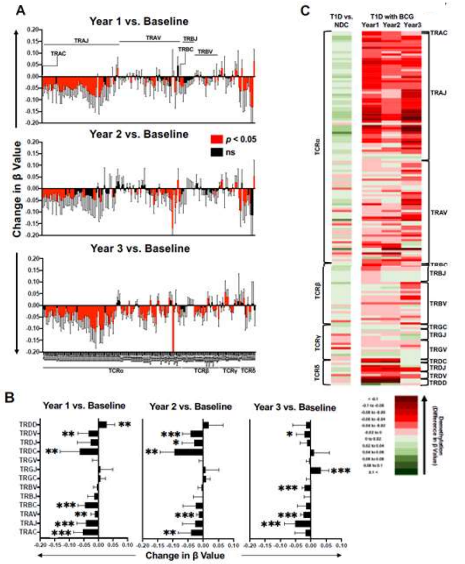

Fig. 3. (**A**) Chronological changes in β values of TCR-related genes from CD4⁺ T-cells of T1D (n=13) after BCG vaccination vs baseline (Year 1 top; Year 2 middle; Year 3 bottom) are shown (average ± SD, red bars mean significant hypomethylation; $p < 0.05$, 2-tailed, paired t-test). The graphs show progressive demethylation of the TCR after BCG vaccination. (**B**) Chronological change of average change in β values vs baseline in T1D (n=13) at the TCR-segment level after BCG vaccination ($*p < 0.05$; $**p < 0.01$; $***p < 0.001$, 2-tailed, paired t-test). (**C**) Heatmap of change in β values for all TCR-related genes. The left lane shows difference in β value between T1D (n=13) and NDC (n=8), and the three right lanes show average methylation vs baseline (before vaccination) at Year 1, 2 and 3 (n=13 vaccinated T1D). Shifts to hypomethylation are displayed in shades of red, and shifts to hypermethylation are displayed in shades of green.

Conclusions

- Patients with T1D have quantitative defects in the TCR/CD3 protein complex, resulting in decreased expression
- TCR triggering is affected by intermolecular distance between TCR proteins; activation diminishes when proximity is increased between TCR surface proteins, leading to faulty T cell selection
- In T1D, altered TCR and CD3 density might be a novel mechanism for failed T cell selection leading to autoimmunity
- BCG therapy causes a gradual de-methylation of the TCR and CD4 genes, leading to partial correction of densities

No conflicts of interest to declare. Contact: dfaustman@mgh.harvard.edu