# Underlying T Cell Receptor Methylation Defects in Type 1 Diabetes Are Associated with Density Defects

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## Introduction

- In randomized controlled trials, the bacillus Calmette-Guérin (BCG) vaccine frequently used for tuberculosis prevention has been shown to gradually improve type 1 diabetes (T1D) and multiple sclerosis
- We investigated whether these autoimmune benefits are due to an impact on the host T cell receptor (TCR) and TCR signal strength by exploring quantitative defects as a cause for altered TCR selection at the level of the TCR/CD3 protein complex
  - TCR is a central regulator of T cell education; efficient structural organization and density of TCR/CD3 underlies development and function of T cells; T cell maturation defects are associated with autoimmunity, suggesting an underlying defect in TCR selection

## 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 from T1D vs controls (Fig. 1)**
  - TCR complex genes and associated CD3 genes were overmethylated in T1D, resulting in 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)
- All CD3 genes except CD3ε had hypermethylated patterns in T1D, confirmed by RNAseq analysis
- Percentage CD3+ T cells in the CD4+ T cell population was reduced in T1D vs controls (p=0.04), as was MFI of CD3+ T cells (p=0.02)

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

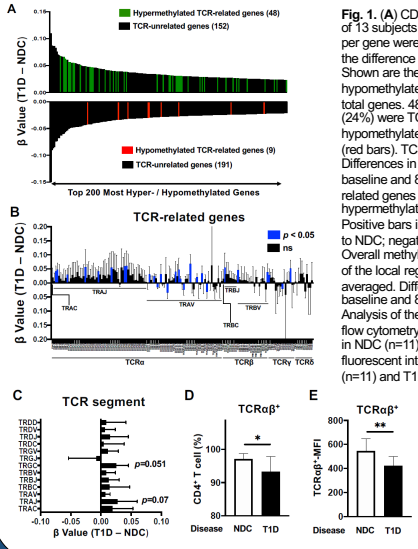


**Fig. 1.** (**A**) CD4⁺ T-cells were isolated from blood samples of 13 subjects with T1D and 8 NDC. β values of all CpGs per gene were averaged and the genes ordered based on the difference in average β value between T1D and NDC. Shown are the 200 most hypermethylated and 200 most hypomethylated genes of T1D versus NDC among 35184 total genes. 48 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 shown in black. (**B**) Differences in average β values between 13 T1D at baseline and 8 NDC (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 compared to NDC; 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. Differences in β values between 13 T1D at baseline and 8 NDC are shown at each TCR-segment. (**D**) Analysis of the expression of TCRαβ on CD4⁺ T-cells by flow cytometry. Ratio of TCRαβ⁺ cells among CD4⁺ T-cells in NDC (n=11) and T1D (n=10) is shown. (**E**) Mean fluorescent intensity (MFI) of APC in TCRαβ⁺ cells in NDC (n=11) and T1D (n=10) is shown.

*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$, 2-tailed, unpaired t-test.

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

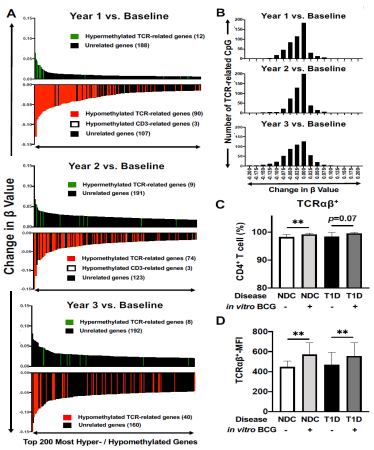


**Fig. 2.** (**A**) Top 200 most hyper- and hypomethylated genes in CD4⁺ T-cells from T1D treated with BCG vs baseline (prior to vaccination) at Year 1 (top), Year 2 (middle) and Year 3 (bottom). Genes were ordered by the difference in β value vs baseline. TCR-related genes are shown in green (hypermethylated) 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 200 most hypomethylated genes were TCR-related and CD3-related, respectively, whereas only 12 TCR-related genes were hypermethylated. At Year 2, there were 74 TCR-related 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. 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 Treatment

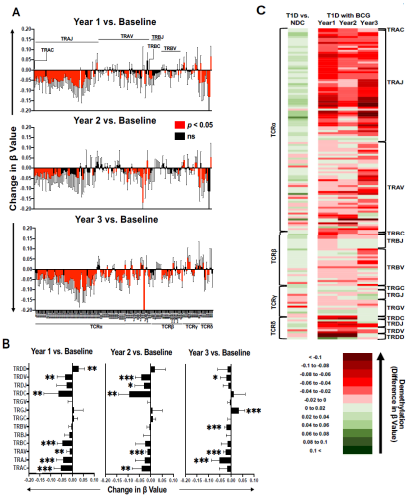


**Fig. 3.** (**A**) Chronological changes in β values of TCR-related genes from CD4⁺ T-cells of T1D (n=13) after BCG vaccination vs baseline (Year 1 top; Year 2 middle; Year 3 bottom) are shown (average ± SD, red bars mean significant hypomethylation; $p < 0.05$, 2-tailed, paired t-test). The graphs 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 TCR defects, consisting of a marked reduction in receptor density on T cells due to hypermethylation of TCR-related genes
  - The TCR sequence is not modified through recombination, ruling out a qualitative defect
- 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 corrects this defect gradually over 3 years by demethylating hypermethylated sites on members of the TCR gene family, leading to partial correction of densities

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