Bacillus Calmette-Guérin (BCG) and Prevention of Type 1 and Type 2 Diabetes: Observational Studies from Bladder Cancer Therapy and Neonatal BCG Vaccination

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Introduction

- A double-blind Phase I clinical trial and open-label trial have shown that adults with type 1 diabetes (T1D) receiving repeat bacillus Calmette-Guérin (BCG) vaccinations experience statistically significantly lowered HbA1c values over a multi-year time course
- BCG has also been used in the United States and Europe for early-stage bladder cancer, administered commonly as 6 intravesical doses for 6 weeks
- We investigated whether BCG therapy for bladder cancer might improve blood sugar levels in those with comorbid T1D or type 2 diabetes (T2D)

Methods

- We investigated whether BCG therapy for bladder cancer via intravesical instillation improves blood sugar levels in patients with comorbid T1D or T2D by retrospectively analyzing three large patient databases in the United States, identifying subjects with documented T1D (N=19) or T2D (N=106) who underwent BCG therapy for bladder cancer and assessing BCG's subsequent year-by-year impact on HbA1c
  - Databases were Management Sciences Associates/Quest Diagnostics (MSA, N=263 million adults), Massachusetts General Brigham (MGB, N=6.5 million) and Optum Labs (OL, N=45 million)
- We also performed an ecological study to determine whether BCG exposure reduces onset of T1D and T2D by examining country-by-country associations between mandatory neonatal BCG vaccination programs and T1D and T2D incidence
  - Global Health Data Exchange (GHDx) and International Diabetes Federation (IDF) datasets; children ages 0 to 14 years; countries divided into those with and without a policy of mandatory neonatal BCG vaccination

BCG therapy in adults with comorbid diabetes and bladder cancer was associated with multi-year and stable lowering of HbA1c in T1D, but not in T2D

% Change HbA1c after BCG Instillation for Bladder Cancer

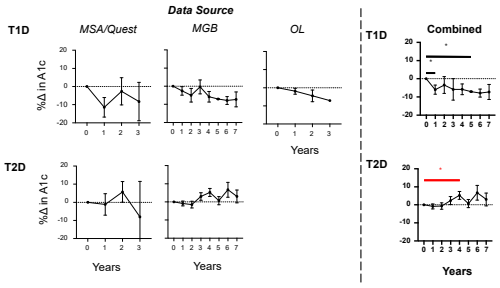

Fig 1. Lower HbA1c in T1D, but not T2D, post-BCG treatment for bladder cancer. In T1D, all three datasets show a reduction in HbA1c values (% change in HbA1c post-BCG instillation) and a near 10% decrease in HbA1cs at differing time points. The combined T1D data shows a statistically significant decrease in HbA1C at Year 1 and Year 5 post-BCG (p = 0.0304, 0.0136). In T2D, MSA data shows no change and MGB data shows an increase in HbA1c values post-BCG instillation. The combined T2D data indicates a significant increase in Year 4 after BCG instillation (p = 0.0223). (N for each dataset: T1D: MGB=4, MSA=9, OL= 6 for T1D; T2D: MGB=97, MSA=9.)

Average Change HbA1c after BCG Instillation for Bladder Cancer

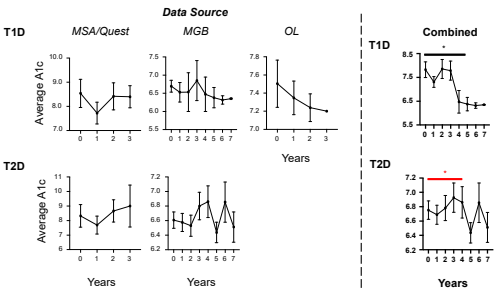

Fig 2. Decreasing trends in average HbA1c for T1D, but not T2D. In T1D, lower HbA1cs are seen in all three databases for subjects treated with BCG for bladder cancer. When all three T1D datasets were combined, a Student's paired t-test showed significance when comparing year 0 to year 5 (p = 0.0133). In T2D, the trend is in an upwards direction post BCG instillation, a significant increase when comparing year 0 to year 4 (p = 0.0460). (N for each dataset: T1D: MGB=4, MSA=9, OL=6; T2D: MGB=97, MSA=9)

Countries with mandatory neonatal BCG vaccination policies had a lower T1D incidence in two international databases and a lower T2D incidence in one database

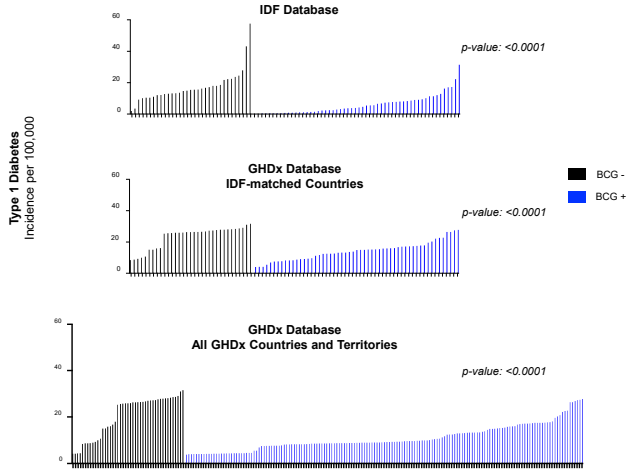

Fig. 3. In global population data, BCG neonatal vaccinations consistently correlate with reduced incidence of T1D. From the IDF list of countries, those with current BCG vaccination programs (blue lines) have significantly reduced incidence of T1D in children (p = <0.0001). Countries with a mandatory BCG vaccination policy had an average of 65% reduced incidence. An almost identical trend was observed with a repeat search of T1D incidence for children in the GHDx database using the same countries as the IDF database. Countries with a childhood BCG program are observed had an average of 39% lower incidence for T1D (p = <0.0001). Using all the countries and territories available in the GHDx dataset, the incidence of T1D in children is reduced by an average of 47% if they earlier received neonatal BCG vaccinations (p = <0.0001). A Mann-Whitney U test was used to compare the two groups of countries with (blue) and without (black) newborn BCG vaccinations based on the country-by-country policies. (N = 33 for BCG- countries, N = 56 for BCG+ for the IDF database.) (N = 45 for BCG-, N = 159 for BCG+ countries and territories in the GHDx database).

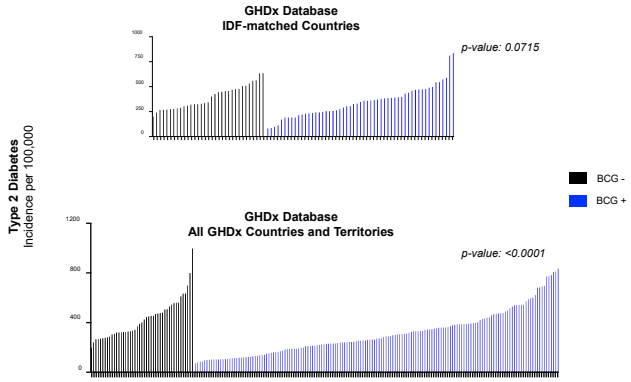

Fig. 4. In global population data, neonatal BCG vaccinations might confer some protection from T2D onset. Using IDF-matched countries in the GHDx database, the T2D incidence was not significantly different between countries with a childhood BCG program (in blue) and those without neonatal BCG vaccinations (in black) (p = 0.0715). However, using all countries and territories available in the GHDx dataset, the T2D incidence was reduced by an average of 28% in countries with neonatal vaccine programs (p <0.0001). A Mann-Whitney U test was used to compare the two groups of countries with and without newborn BCG vaccinations policies. (N = 33 for BCG- countries, N = 55 for BCG+ for the IDF database.) (N = 45 for BCG-, N = 159 for BCG + countries and territories in the GHDx database).

Conclusions

- Elderly patients with longstanding T1D who received BCG bladder cancer treatment show a gradual lowering of HbA1c, but this is not seen in those with T2D
- The bladder cancer data suggests that high-dose intravesical BCG therapy may contribute towards a drop in HbA1c values in T1D, but not T2D (likely due to metformin interference in BCG's ability to change glycolysis pathways)
- Ecological analysis of global data suggests a role for neonatal BCG in the prevention of T1D and, to a lesser extent, T2D
- The epidemiological evidence analyzed here suggests that BCG may play a role in the prevention and treatment of T1D and in the possible prevention, but not treatment, of T2D

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

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

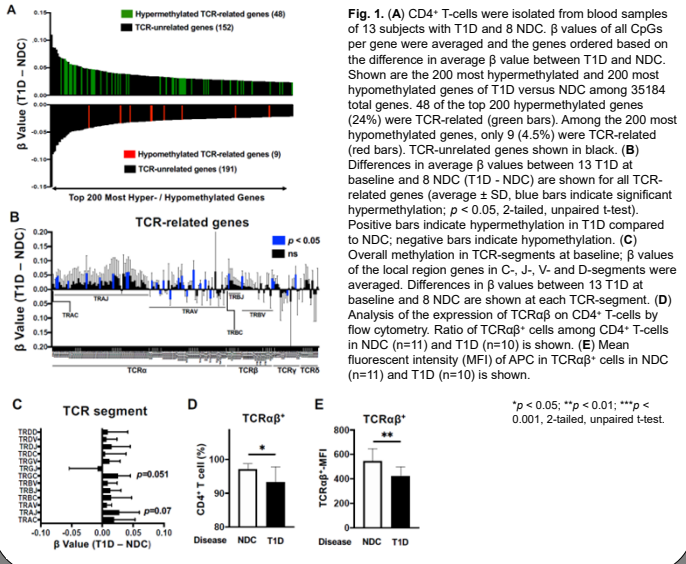

Fig. 1. (**A**) CD4⁺ T-cells were isolated from blood samples of 13 subjects with T1D and 8 NDC. β values of all CpGs per gene were averaged and the genes ordered based on the difference in average β value between T1D and NDC. Shown are the 200 most hypermethylated and 200 most hypomethylated genes of 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

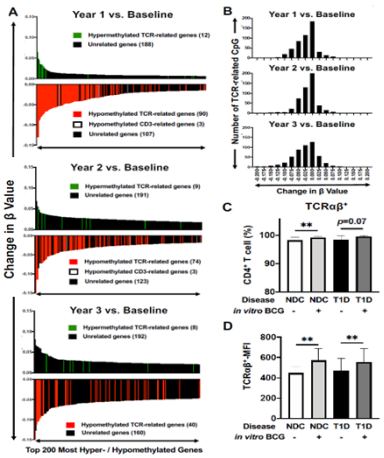

Fig. 2. (**A**) Top 200 most hyper- and hypomethylated genes in CD4⁺ T-cells from T1D treated with BCG vs baseline (prior to vaccination) at Year 1 (top), Year 2 (middle) and Year 3 (bottom). Genes were ordered by the difference in β value vs baseline. TCR-related genes are shown in green (hypermethylated)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

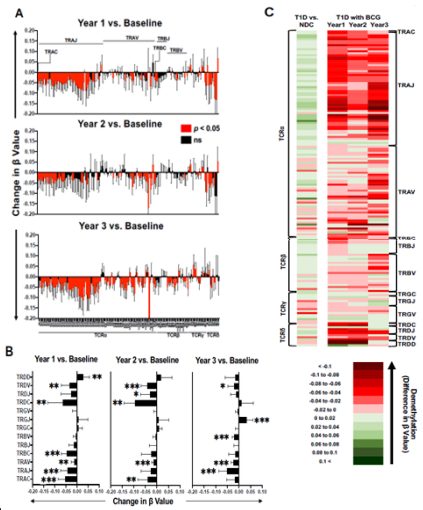

Fig. 3. (**A**) Chronological changes in β values of TCR-related genes from CD4⁺ T-cells of T1D (n=13) after BCG vaccination vs baseline (Year 1 top; Year 2 middle; Year 3 bottom) are shown (average ± SD, red bars mean significant hypomethylation; $p < 0.05$, 2-tailed, paired t-test). The graphs show 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