^{18}F-FDG PET/CT Mapping of Functional Microbial Niches to Understand Host Glucose Regulation after BCG Vaccinations

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Introduction

- Diverse microbiomes in humans are known to change host energy metabolism
- When the bacillus Calmette-Guérin (BCG) vaccine (live, avirulent *Mycobacterium bovis*) is introduced as experimental therapy into human hosts with underlying type 1 diabetes (T1D), it gradually shifts energy metabolism in host blood lymphoid cells from oxidative phosphorylation to aerobic glycolysis, drawing more glucose out of the blood to fuel intracellular metabolism
  - This metabolic shift may explain the BCG bacillus' therapeutic benefit: Systemic and long-term reduction of excess glucose from the blood of TID patients
- The organ-specific niches where the BCG bacillus alters metabolism and establishes persistent residence are unknown

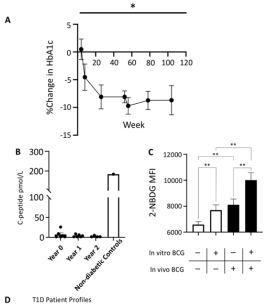
Methods

- In an open-label human clinical trial in participants with T1D (n=6), we mapped organ-specific niches for the BCG-induced shift to aerobic glycolysis using fluorine-18 fluorodeoxyglucose (^{18}F-FDG) positron emission tomography (PET) and X-ray computed tomography (CT)
  - Standardized uptake value ratios were calculated to identify organs with heightened glucose uptake over a 2-year time-period after BCG vaccination and to confirm earlier work that BCG vaccination gradually lowers blood sugar levels without any contribution from endogenous insulin
- We also tested BALB/c mice (n=17) for the presence of BCG colonies in particular organ niches before and after vaccination

Results

- Human and murine studies of the BCG microorganism concurred that the spleen is the major anatomic site of functional metabolic change and residence
- The BCG bacillus also transiently mapped to the bone marrow, liver, circulating lymphocytes as in the descending aorta, and lungs

BCG vaccinations affect lymphoid sugar uptake in humans with T1D



Profile	N	Average Age	Average Age of Onset	Average Duration of T1D
Fig. 1A/B	6	27.3 +/- 4.4	12.5 +/- 4.5	14.8 +/- 1.5
Fig. 1C Left	11	35.1 +/- 5.0	22.2 +/- 4.5	11.5 +/- 2.2
Fig. 1C Right	11	40.3 +/-3.9	27.1 +/- 4.0	13.2 +/- 1.2

Fig. 1. (A) Percent change in HbA1c values over 2 years time after 3 BCG vaccinations in adults with longstanding T1D (n=6). Mixed-Effects Analysis p-value=0·0402; Holm-Šídák's multiple comparisons test vs baseline provided significant p-values for week 0 vs week 52 (0·0006), week 0 vs week 56 (0·0446), and week 0 vs week 78 (0·0237). **(B)** C-peptide, a biomarker of residual pancreatic insulin secretion, was measured in all T1Ds receiving *in vivo* BCG (n=6). The graph shows the sequential values for the 6 patients at baseline, Year 01 and Year 02 after repeat BCG vaccinations. The data show negligible C-peptide production, indicating that residual C-peptide cannot account for restored glucose metabolism. **(C)** Human monocytes harvested from T1D patients who were (n=11) or were not (n=11) treated with *in vivo* BCG vaccination (approx. 2 years' prior) were cultured *in vitro* with or without BCG exposure. Glucose uptake was analyzed using a 2-NBDG assay. Both groups (BCG-naïve/unvaccinated and BCG-vaccinated) had a significant increase in glucose uptake after *in vitro* BCG stimulation (p-values of 0.0026 and 0.0013 respectively, paired t-test). Even without BCG stimulation in culture, vaccinated T1Ds were observed to have a significantly higher glucose uptake (p-value of 0.0062, unpaired t-test). Significantly increased uptake was also seen when comparing both unvaccinated vs vaccinated T1Ds after *in vitro* BCG (p-value of 0.0032, an unpaired t-test. **(D)** Characteristics of the 28 T1D subjects presented in Fig. 1A-C.

^{18}FDG-PET/CT images and gating regions

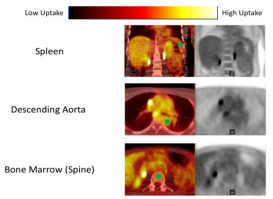


Fig. 2. (A) ^{18}F-FDG-PET/CT images of select tissues showing an accelerated glucose uptake signal with the gating regions used to monitor uptake. Circles represent the areas where the mean SUV was measured. The left column is the CT superimposed onto the ^{18}F-FDG-PETImage (red/yellow), with the brighter the color indicating the greater the glucose uptake. Right image is the ^{18}F-FDG-PET scan alone, with the darker the color indicating the greater the glucose uptake.

^{18}F-FDG-PET/CT scans in BCG-treated participants with T1D identify human organs with accelerated sugar uptake

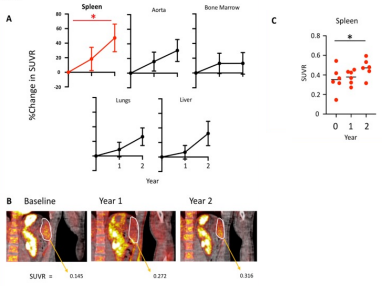


Fig 3. (A) Glucose uptake monitored by ^{18}F-FDG methods at baseline, Year 01 and Year 02 after BCG vaccinations and then compared to baseline glucose uptake in each BCG-treated subject on an organ-per-organ basis (n=6). The graphs show 5 tissues/organs with augmented glucose uptake (out of 10 studied): spleen, aorta, bone marrow, lungs, and liver. The spleen was the only organ to continue through Year 02 to show significantly more glucose uptake, according to a linear mixed effects model ($p = 0.03$). **(B)** ^{18}F-FDG PET/CT images of the spleen over 2 years depicting the increase in standardized uptake value ratios (SUVR). **(C)** Dot plot of the spleen presenting the increase in glucose uptake for open-label patients on an individual subject basis.

Identifying the location and time course of BCG persistence in BCG-vaccinated mice

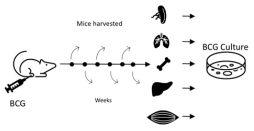


Fig. 4. BCG-treated BALB/c mice were serially sacrificed to look at the tissue niches where the BCG organisms might persist as a live organism. Mice were harvested at 5 separate times over a 22-week time period. Harvested tissues were tested for the presence of BCG using the tissue homogenate and culturing in a media specific for BCG growth. Tissue from the spleen, lungs, bone marrow, liver, and muscle were collected and observed for persistence of the BCG organism through colony-forming units in culture.

BCG vaccinations in mice promote long-term persistence of the BCG organism

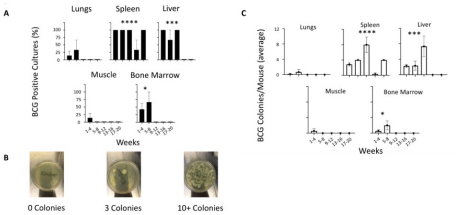


Fig 5. (A) BCG culture experiments using tissues from periodically harvested mice (n=17) show that BCG was significantly detected in the spleen for mice harvested up until week 20 (p-value=<0.0001). There are also significant signs of BCG being present in the liver for mice harvested until week 12 (p-value=0.0002) and in the bone marrow of mice harvested until week 8 (p-value = 0.0312). **(B)** Images showing BCG colonies grown in culture and labelled according to the number of colonies visible; plates with greater than 10 colonies were just counted as 10. **(C)** The average number of BCG colonies per mouse is significantly higher in the spleen up until week 20, the liver up until week 12, and the bone marrow up until week 8. (p-value=<0.0001, 0.0002 and 0.0312, respectively). One-tailed Wilcoxon ranked test was used to test for significance.

Correlation of organ-specific glucose uptake in humans and BCG microbe presence in mice

Tissue	Mouse	Human
Spleen	5/5	5/5
Bone Marrow	3/5	2/5
Muscle	1/5	0/5
Liver	3/5	3/5
Lungs	2/5	3/5

Fig 6. Comparison of increased glucose uptake in humans measured by metabolic changes induced by BCG compared to the physical presence of the BCG microbe in mice. Scoring on a defined scale of 0-5 (based on the amount of BCG microbe present in mice tissue and the degree of glucose uptake observed through the 18-FDG PET scans; 5 reflects the best signal for the presence of BCG or for the best glucose uptake observed), the spleen, bone marrow, muscle, liver and lung tissues correlate with each other.

Conclusions

- These human and murine studies support the spleen as the niche for the BCG vaccine's functional improvement of metabolism
- The spleen is a lymphoid organ massive enough to explain BCG's systemic benefit of lowering blood glucose to near normal levels in T1D

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