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Treatment of Diabetes type 1 using (BCG) vaccine

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Abstract:

The attenuated *Mycobacterium bovis* Bacillus Calmette Guérin (BCG) strain has been administered globally for 100 years as a vaccine against tuberculosis. examination of type 1 diabetic subjects with long-term disease who received two doses of the BCG vaccine. After year 3, BCG lowered hemoglobin A1c to near normal levels for the next 5 years. We observe a systemic shift in glucose metabolism from oxidative phosphorylation to aerobic glycolysis, a state of high glucose utilization. To prove BCG could induce a systemic change to promote accelerated glucose utilization and impact blood sugars mice data demonstrated reduced blood sugars and aerobic induction in non-autoimmune mice made chemically diabetic. BCG via epigenetics also resets six central T-regulatory genes for genetic re-programming of tolerance.

Introduction:

The bacillus Calmette-Guérin (BCG) vaccine is one of the oldest vaccines in the world, developed for tuberculosis (TB) protection and for early stage bladder cancer therapy. BCG is an attenuated version of the virulent *Mycobacterium bovis*.

Type 1 diabetes is generally thought to be precipitated by an immune-associated, if not directly immune-mediated, destruction of insulin-producing pancreatic β cells.

In the past 10 years has seen a surge of clinical trials that re-introduce BCG for a diversity of autoimmune, allergic, and induced adaptive immune responses to childhood infections. In multiple sclerosis(1), BCG halts new onset disease, yet the clinical effect is most dramatic nearly 5 years later. In type 1 diabetes, three BCG vaccines administered in childhood lowered the incidence of T1D by age 12.(2)

Many autoimmune NOD (non-obese diabetic) murine (mice) studies have shown a beneficial effect of in preventing the onset of autoimmune diabetes and even reversing full blown established disease in mice.(3)

The rational of this study was to investigate in established T1D subjects (average disease duration of 19 years), the possible long term immune, metabolic and clinical benefits of two doses of BCG vaccine as the Connaught strain (given two times, 4 weeks apart). This 8-year-long Phase 1 trial shows the long-term lowering of blood sugars after year 3 measured by HbA1c. The lowering of blood sugars to a range near normal was maintained for the next 5 years of monitoring. Repeat BCG appears to reset the immune system by de-methylating in CD4 T cells all six key T-regulatory genes by 8 weeks, resulting in enhanced mRNA expression, according to epigenetic analysis. T regulatory cells are critical for immune balance and are believed to facilitate a decrease in inflammation and to prevent or slow the autoimmune process. BCG appears to switch the immune system of T1Ds from high oxidative phosphorylation to increased early aerobic glycolysis, a systemic metabolic shift that occurs gradually and allows cells to consume in a regulated fashion large amounts of glucose to safely lower hyperglycemia to near normal levels.(4)(5)

Material and methods:

Clinical trial and research study participants

This is a study of 282 human research participants for both in vivo BCG vaccine 87clinical trial studies ($n = 52$) and in vitro mechanistic studies ($n = 230$). Of these total research subjects 211 had type 1 diabetes and 71 were non-diabetic control subjects.(2)

The in vivo study of BCG vaccinations is comprised of adult T1D subjects receiving BCG, receiving placebo vaccinations reference vaccination with the same standard of care. (2)

T1D and control subjects in this study also included volunteers who donated blood for in vitro studies under Study and were compared to the in vivo studied subjects receiving BCG or placebo vaccination or compared to in vitro studied subjects. (2)

mice

BALB/c male mice were and housed at five animals per cage at the MGH animal facilities (four animals per cage after they reached a weight of >25 g).

the HbA1c blood samples from the mouse has been determined using the A1CNow + kit from PTS Diagnostics (Indianapolis, IN). For mouse blood sugars the ACCUCHEK Aviva blood glucose meter has been used (Roche, Indianapolis, IN). BCG was administered by hind footpad injection (0.10 mg BCG in 25 μL saline in one footpad). Induction of hyperglycemia was performed using a single

intraperitoneal injection of Streptozotocin (Sigma-Aldrich, Milwaukee, WI) at 150 mg/kg in PBS. (3)

Clinical chemistries

All human HbA1c, glucose levels were processed directly from fresh blood by certified diagnostic laboratories approved by The Massachusetts General Hospital. Human serum samples were assayed for C-peptide using regular or ultrasensitive C-peptide ELISA kits using −80 ℃ frozen serum.(2)

Intravenous glucagon stimulation test

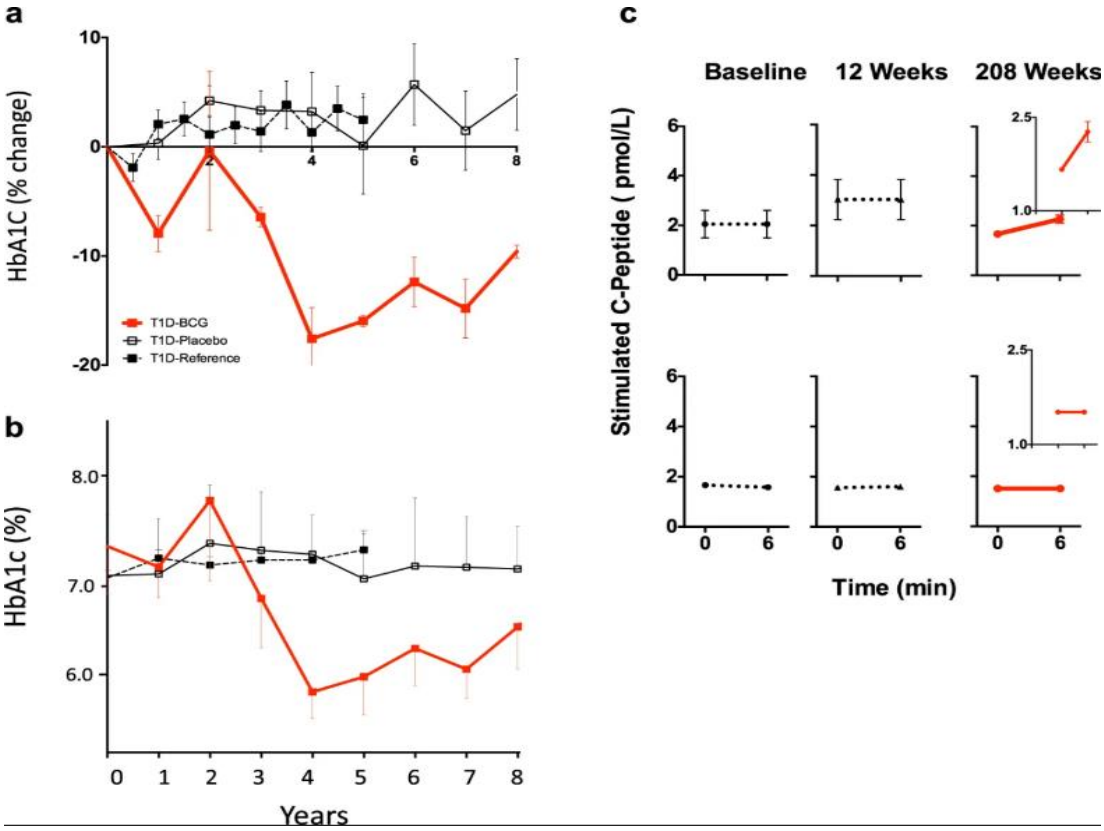
Subjects were asked to consume at least 150 g of carbohydrates for the 3 days prior to the intravenous glucagon stimulation test (GST) and fast for 8 h the night before. On the morning of the GST, subjects were asked to withhold insulin until after they had completed the test. The GST was only administered when blood sugars were between 3.9 mmol/L (70 mg/dL) and 12.5 mmol/L (225 mg/ dL). A 20-22-gauge angiocatheter was placed and a 5 mL blood sample taken for baseline glucose and for baseline C-peptide levels. Six minutes later, 1 mg of glucagon was administered intravenously. An additional 5 mL blood sample was then collected at 6 min. Serum was prepared from the blood samples and frozen at −80 ℃ for subsequent C-peptide Elisa as previously described. (2)

Result:

The 8 year long followed and BCG-treated T1Ds showed a reduction in HbA1c levels of greater than 10% after year 03, 18% at year 04, and the HbA1c remained low for the next 5 years (Fig. 1a, b)

in contrast, the placebo group and the reference T1D groups had consistently higher HbA1c over the entire monitoring period of 8 years and 5 years, respectively (Fig. 1a, b). The efficacy of BCG was also apparent with raw HbA1c values, which

show the BCG-treated TIDs by year 8 declined.(2)



(Fig. 1a, b)

After BCG vaccinations, regulatory T cell signature genes are de-methylated in vivo resulting in enhanced mRNA expression

The beneficial effect of BCG in humans, as previously documented in mouse experiments, could be due to an induction of the beneficial Treg cells. The co-evolution of *Mycobacterium* and humans has resulted in *Mycobacterium*-modulated host cell machinery, including de-novo host gene expression by de-methylation of important immune response genes. Treg cells are believed deficient in numbers or function in diverse autoimmune diseases and induction through BCG therapy would be a first step in restoring the immune balance that has been quantified by only flow cytometric methods after BCG. Transcriptional start site (TSS) clusters are located within the Treg-specific demethylation region (TSDR) that is critical for Treg function and that are modulated by de-methylation as was monitored in this study.(6)

C-peptide was measured with a glucagon challenge in the BCG and placebo type 1 diabetic subject groups at three time points (pre-BCG, post-BCG 12 weeks, and 208 weeks) to look for pancreas recovery or regeneration (Fig. 1c,)C-peptide is co-

secreted with insulin from the pancreas and can be used to selectively detect the secretion of endogenous insulin. Insulin levels cannot be used to look for pancreas regeneration since all subjects take exogenous insulin. In this study there have been a long term and stable lowering of blood sugars in humans after BCG vaccinations. In the human, this stable blood sugar control was not driven primarily in these human subjects by pancreas recovery or regeneration. The human pancreas after BCG even at four years after repeat vaccinations did not secrete significant insulin as clinically measured by C-peptide.(2)

Discussion:

Our 8-year long clinical trial of monitoring subjects receiving *Mycobacterium* re-introduction through the BCG vaccine triggers two clinical effects in humans with established T1D: stable and long-term reductions in blood sugar and epigenetic changes in Treg signature genes for restored tolerance. Both beneficial effects appear to be driven by a systemic metabolic shift from oxidative phosphorylation towards increased and early aerobic glycolysis. The significant clinical effects, using BCG with intradermal dosing, took three years to occur but then held steady for at least five additional years without further interventions.(7) (4)

BCG also demethylated Treg signature genes. Treg-specific DNA hypomethylation is central in gene upregulation for Treg function. The demethylation of Treg signature genes also correlated with increased mRNA expression of the genes involved in the de-methylation process. Published studies confirm that Treg-specific DNA hypomethlylation is instrumental in gene upregulation in steady state Tregs.(7)(4)

The health benefits of the BCG vaccine reported here suggest reconsideration of vaccine policy in areas of the world without endemic tuberculosis. Not one, but multiple, BCG doses are necessary to prevent T1D, based on epidemiological and animal studies. Studies have already reported that the BCG vaccine alters the susceptibility to infections with unrelated organisms at later times during life. Animals injected with *Mycobacterium bovis* as the BCG vaccine demonstrate acquired resistance to *Stapylococcus aureus, Herpes virus, Salmonella, Listeria monocytogenes and Candida albicans*. This has also been observed in humans since 1928. (8)

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