Bridging the gap between vaccination with Bacille Calmette-Guérin (BCG) and immunological tolerance: the cases of type 1 diabetes and multiple sclerosis
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At the end of past century, when the prevailing view was that treatment of autoimmunity required immune suppression, experimental evidence suggested an approach of immune-stimulation such as with the BCG vaccine in type 1 diabetes (T1D) and multiple sclerosis (MS). Translating these basic studies into clinical trials, we showed the following: BCG harnessed the immune system to ‘permanently’ lower blood sugar, even in advanced T1D; BCG appeared to delay the disease progression in early MS; the effects were long-lasting (years after vaccination) in both diseases. The recently demonstrated capacity of BCG to boost glycolysis may explain both the improvement of metabolic indexes in T1D, and the more efficient generation of inducible regulatory T cells, which counteract the autoimmune attack and foster repair mechanisms.

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WE studied the effect of BCG vaccine in patients with early MS. A single cross-over magnetic resonance imaging (MRI)-monitored trial [8] was performed on 14 participants. Comparing monthly MRI scans of pre-vaccination and post-vaccination period we found that MRI activity, expressed as gadolinium (Gd)-enhanced lesions plus new and enlarging lesions in T2-weighted (T2W) images, significantly dropped after BCG. No major adverse events were reported [9**].

In this same cohort of MS patients we evaluated the effect of BCG vaccine on the evolution of new Gd-enhancing lesions to hypointense lesions on T1-weighted MRI images through three new MRI scans over 24 months after vaccination [10]. This allowed to quantify the so-called black holes (BH), expression of irreversible tissue damage, to evaluate the vaccine influence on axonal loss, that underlies the development of clinical disability [11]. Once again we compared the two phases of the trial (the run-in versus the post-BCG percentage of NEL that evolve into BH), and found a significant result: new enhancing lesions at MRI persisted less and less frequently evolved to black holes after the BCG vaccine. This post-hoc analysis seemed to indicate that vaccination with BCG might decrease the tissue damage. Moreover, the dynamics of BCG effects over time prompted us to hypothesize its long-term benefit and to design subsequent studies on a longer temporal scale.

The suggestion found confirmation in a third study on subjects with the first demyelinating episode (usually
referred to as clinically isolated syndrome—CIS), that is considered, in the presence of a brain and spinal cord MRI compatible with MS, the onset of clinical disease [12]. In these parallel groups trials we met several significant end points after a single vaccination with BCG in people with CIS: reduced disease activity with MRI monitoring, less propensity to develop black holes at 18 months, lower occurrence of the second demyelinating event (that is conversion to clinically definite MS) at 60 months, without major adverse event due to vaccine (Figure 1) [13**].

Underway currently is our trial with the BCG vaccine in people with ‘radiologically isolated syndromes’ (RIS): a new subclinical entity, that occurs when an MRI of the central nervous system shows results compatible with MS, but without any symptom or sign of disease [14]. The trial stemmed from reasoning that safe and manageable approaches, that can be used with the (biological) onset of the disease, are important unmet needs in MS. In fact, the last-decade therapeutic progress for the overt relapsing-remitting form was reached at the cost of safety, quality of life and overtreatment. Being safe, cheap and available, the BCG vaccine seemed appropriate as a frontline immune-modulatory approach for people with RIS.

The mechanisms of action of BCG vaccine in MS are not clear. However, clinical observations along with genetic and functional studies suggest a net beneficial role of TNF in human neuroinflammation, that may be deficient in MS patients, and seems to be at variance with more complex effects seen in EAE [15]. In fact, diseases worsening occurred when therapies antagonizing tumor necrosis factor (TNF) were tried in MS [16], contrary to other autoimmune disorders where anti-TNF is beneficial, but bears risks of demyelinating episodes. Moreover, an MS-associated genetic variant directs increased expression of a soluble protein that acts as an analogous of anti-TNF agents [17]; notably, this variant is not associated with autoimmune disorders where TNF antagonism results beneficial. A potent TNF inducer, such as BCG vaccination, may be useful to rescue TNF deficit in MS, and this is in keeping with evidences coming from type 1 diabetes (see next section).

Another promising field for adjuvant approach seems to be that of more common neurological disorders, such as Alzheimer’s and Parkinson disease, classically considered of degenerative nature. Recent evidences consistently

Figure 1

Long-term effects of a single BCG vaccination in people with first demyelinating episode. (a) Mean change in the total T1-hypointense lesions, including ‘black holes’ that express irreversible tissue damage, over 18 months. (b) Proportion of people free from the second clinical event (that is not converting to clinically definite multiple sclerosis) over 60 months.
showed a neuroinflammatory component in these diseases, with a prevalent role for glial cells and innate immune system [18]. In this context a neuroprotective action of BCG vaccine emerged in experimental models of neurodegenerative diseases. Two works in models of PD suggested a BCG-mediated immune stimulation, that may limit deleterious microglial response to a neuronal insult [19*], may promote T regulatory protective responses [20]. Other two works in models of AD elucidated additional mechanisms of neuroprotection: activation of tumor necrosis factor receptor 2 (TNFR2), that block neuroinflammation and promote neuronal survival [21], as well as recruitment of inflammation-resolving monocyte (producing anti-inflammatory cytokines and neutrotrophic factors) at the choroid plexus and perivascular spaces of plaque pathology [22]. Along the same line of research several works showed a beneficial effect of neonatal BCG vaccination on neurodevelopment and on countering neurobehavioral impairment and neuroinflammation due to secondary immune challenge in adult mice [23,24].

**BCG and type 1 diabetes**

Our 20-year journey towards using BCG to treat type 1 diabetes (T1D) started off in a paradoxical direction. Studying both human and mouse models, we discovered T-lymphocyte (T cell) imbalances, both an overabundance of pathogenic cytotoxic T cells (CD8 CTLs), and a paucity of suppressive T-regulatory (CD4 Treg) cells. Importantly these immune cell imbalances could be corrected with tumor necrosis factor (TNF), a well-known pro-inflammatory cytokine with immune-stimulatory properties. At the time, however, the prevailing view was that treatment of T1D and other forms of autoimmunity required immunosuppression, not immunostimulation, the same belief that also dominated the multiple sclerosis field.

Through basic science, we discovered that the antigen-presenting cells of T1D patients had interruptions in CD8 T cell education due to a paucity of self-peptides being generated for the HLA class I groove, a process controlled by intracellular processing in antigen-presenting cells [25* ,26,27]. This was some of early T1D data implementing CD8 T cells in the field; prior studies thought CD4 T cells were the central cells in T1D. The intracellular antigen presenting process normally utilized the TAP and proteasome genes and this presentation of self antigens was altered in T1D. Interestingly these HLA class I presenting genes were located in the high-risk HLA region [28–33]. Our research suggested that HLA class I presentation of self-proteins was necessary for tolerance [30]. With proteasome dysfunction, additional intracellular processing defects were also detected in T1D. NFkB activation, a proteasome-dependent process for T cell education pathways, was also dysfunctional [34,35]. TNF is the cytokine that normally stimulates the NFkB signaling pathway. This TNF-stimulated pathway promotes antigen presentation, T cell maturation and proper education of T cells. Interrupt of this pathway would be predicted to cause autoimmunity. If there was perhaps too little TNF or at least sluggish TNF-mediated signaling in the immune system, could something as simple of adding back TNF be therapeutic for T1D? Indeed, TNF selectively killed autoreactive T cells causing T1D and multiple sclerosis and also in other autoimmune diseases such a multiple sclerosis antigen presenting defects in HLA class I have been reported [36–39]. The data started to accumulate that this inflammatory cytokine, TNF might be therapeutic. Animal models of autoimmunity also demonstrated the therapeutic benefit of TNF. Disease causing cytotoxic T cells, harvested from the spleens of diabetes affect mice, normally transfer disease to naive animals. If these splenocytes were first in vitro treated with TNF, disease transfer was prevented [36]. TNF itself or TNF induction with the BCG vaccine was found to prevent onset as well as reverse even end-stage disease in the NOD mouse model when administered in vivo [40,41]. This suggested that autoimmune diseases needed TNF, not immunosuppression, as a therapeutic approach (Figure 2). As further refinements of these experiments continued, TNF or TNF stimulation through the TNFR2 receptor was the central pathway for disease modification [42,43].

Translating these basic studies into clinical trials of T1D could be accomplished by two routes: manufacture and start a TNF drug program (TNFR2 agonistic antibodies were not available at the time) or develop and recycle a safe 100 year-old drug, BCG, an inactive version of *Mycobacteria bovis* originally developed to protect from tuberculosis and a known inducer of TNF. Because of monetary limitations, we decided to launch a T1D trial with BCG as a method to induce and restore TNF.

In 2012, we published the Phase I results from multi-dosing BCG in long-term T1Ds (>10 years disease duration) [44**]. This was a safety trial that also studied important biomarkers of efficacy. We established that BCG was safe in this patient population, and showed consistent trends with the proposed mechanism of action of BCG: death of autoreactive T cells, induction of Tregs, and minor increase of C-peptide secretion (a marker of insulin production by the pancreas), suggesting some restored activity [44**]. In support of BCG in T1D, an epidemiology study from Turkey has since showed that multi-dosing BCG may not only be beneficial in T1D treatment, but also in its diabetes prevention. Participants who received greater than 2 childhood vaccines of BCG had diminished lifelong risk for developing T1D [45].

Our 12-week-long Phase I trial showed that C-peptide production was not increased in a clinically meaningful way, although seeing any restored C-peptide in T1D with this long standing disease was unusual. We asked, had we followed our patients long enough? We subsequently
learned from the multiple sclerosis trials by Ristori et al. that BCG’s benefit, while slow to start, showed measurable disease improvement over the next 2–3 years. With this in mind, we re-opened our Phase I trial to follow our T1D patients for another 8 years. Similar to the timeframe with multiple sclerosis, we found that, by year 3, blood sugar began to drop and hemoglobin A1c (HbA1c) gradually returned to near normal levels. This slow-to-materialize, but remarkable, correction of blood sugars towards normal by BCG persisted for 5 more years [46**]. Repeat BCG vaccines were a powerful way to harness the immune system and alter metabolism to ‘permanently’ lower blood sugars, even in advanced T1D. In 2018, we showed that the mechanism for better blood sugar control by BCG was due to a systemic shift in metabolism from oxidative phosphorylation to aerobic glycolysis, a metabolic state with accelerated transport and utilization of serum sugar for energy. BCG therapy in human autoimmunity thus opened up the exciting possibility of a safe and an effective vaccine for resetting the immune system even in existing T1D disease states. The BCG vaccine confirmed and verified an entirely novel way to regulate blood sugars. Fully enrolled and ongoing human clinical trials continue to test the validity of BCG lowering of blood sugars in large patient populations and unlike most immune intervention trials is T1D, the BCG vaccine was potent enough to have these beneficial clinical effects in patients with long standing diabetes. Before this time, immune interventions were exclusively tried in only new onset T1D, a clinical setting thought easier to reverse.

**Immunometabolic effects of BCG in autoimmunity**

BCG, as therapeutic opportunity to induce immunological tolerance, is a very attractive one. At mechanistic level, despite compelling experimental evidence suggests the capacity of BCG to affect immune responses, the cellular and molecular determinants controlling this process are still not fully understood. Originally, BCG was considered a powerful tool to stimulate immunity against *Mycobacterium tuberculosis* (MTB); indeed BCG infection is characterized by a classical strong induction of Th1/Th17 responses in immunized individuals, able to exert protection against MTB infection, particularly in very young individuals. The induction of this type of response is in ‘apparent contrast’ with the capacity of BCG to protect from induction of mouse EAE and the development of T1D in the NOD mouse, both models of disease caused by pro-inflammatory Th1/Th17 switches [47,48].

A possible explanation for this ‘apparent paradox’ comes from recent studies linking immunometabolic pathways...
to the capacity of BCG to induce glycolysis in T cells and innate immune cells such as monocytes/macrophages [49**]. Glycolysis fuels proliferation and differentiation of CD4+ T cells towards Th1 and Th17 responses, but it is also necessary to generate specific populations of inducible regulatory T cells (iTreg) cells from conventional T (Tconv) cells in humans [50*]. While differentiated Treg cells show a mixed metabolic profile being able to engage both glycolysis and lipid oxidation, engagement of glycolysis appears to be necessary to generate iTreg cells from Tconv cells [50*,51*]. Specifically, this process appeared tightly linked with the timing and strength of T cell receptor (TCR) engagement; indeed only TCR stimulation determined a consistent engagement of glycolysis able to sustain the appropriate induction and maintenance overtime of the Foxp3 gene, whose expression is crucial for Treg cell maintenance and suppressive functions [50*,51*,52*].

Figure 3

Hypothetical pro-tolerogenic capacity of BCG vaccination via enhancement of glycolysis. (a) In autoimmune disorders such as MS and T1D there is a reduced engagement of glycolysis in Tconv cells which leads to reduced iTreg cell induction and consequent enhancement of pro-pathogenic Th1/Th17 responses. (b) BCG vaccination is able to boost engagement of glycolysis in Tconv cells upon TCR activation and consequently increase iTreg cell generation which in turn control pro-pathogenic Th1/Th17 responses. deleterious microglial response to a neuronal insul
It is also interesting to note that autoimmune disorders such as MS and T1D associate with reduced engagement of glycolysis by T cells [50,53**]. From these considerations, it is possible to speculate that glycolysis is part of a ‘double edged’ process in which while effector responses towards BCG require glucose oxidation to warrant Th1/Th17 differentiation, also iTreg cells generate in the same process, whose function is necessary to control the inflammatory process and avoid possible ‘collateral damage’ during inflammation driven by BCG infection. Thus, with this view, immunological tolerance could be considered as an inducible process during ongoing immune responses, and thus infections and inflammatory processes that consistently engage glycolysis warrant also a better induction of Foxp3+ T cells which in turn control inflammation and tissue damage (Figure 3). These cells, can contribute to improvement of inflammation in autoimmune diseases such as MS and T1D, but also could enhance tissue-remodeling and regeneration in both myelin and beta-cells, through mediators such as TGF-β. It is clear that further research is needed to better dissect at cellular and molecular level this hypothesis, but the ongoing direction of current basic and pre-clinical research is highly suggestive of this novel concept.

We ‘converged’ on the clinical evaluation of BCG in MS and T1D from different premises. This is remarkable, as it supports the broad rationale that a benign exposure to microbes (especially the ‘old friends’ that have co-evolved with human beings for millennia) [54] may help antagonize conditions sharing chronic inflammation and tissue damage. The beneficial effect of BCG vaccination, at least in some autoimmune disorders, adds an intriguing viewpoint for studying the host-microbe interplay in the pathophysiology and treatment of immune-mediated diseases.

Conflict of interest statement
Nothing declared.

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References and recommended reading
Papers of particular interest, published within the period of review, have been highlighted as

- of special interest
- of outstanding interest


This was the first paper on BCG vaccine use in multiple sclerosis: a reduction of disease activity at cerebral magnetic resonance imaging was evident.


This paper showed that in patients with early multiple sclerosis (first demyelinating episode) BCG vaccine may delay the second episode over a 5-year period, suggesting long-term action of a single vaccination.


This paper and the next one suggested a wider than anticipated beneficial effects of BCG vaccine, including anti-inflammatory and pro-repair effects in neurodegenerative diseases.


25. Faustman D, Li X, Lin HY, Fu Y, Eisenbarth G, Avruch J, Guo J: Linkage of faulty major histocompatibility complex class I to autoimmune diabetes. Science 1991, 254:1756-1761. This is the first paper demonstrating that HLA class I presentation of self peptides was an important pathway for T cell tolerance: furthermore this pathway was interrupted in autoimmunity.


This is the first paper showing the value of multi-dosing BCG in type 1 diabetes with the drug inducing cytotoxic T cell death, inducing Treg cells and also restoring a very tiny amount of insulin secreting from the pancreas.


This paper shows the first long term and safe reversal of blood sugars in T1D who received multiple doses of the BCG. T1D subject were either followed for 5 or 8 years showing the durability of the BCG reset of the immune system and the permanent tolerance re-established at the gene level with BCG.


This is a key report showing that BCG is able to boost glycolysis in T cells.


This is the first paper linking glycolysis engagement and induction of iTreg cells in humans, particularly those containing the Foxp3 exon2 splicing variants.


This report, suggests that ex vivo human Treg cells engage glycolysis and when put in vitro change dynamically their metabolism engaging both glycolysis and lipid synthesis.


This paper suggests metabolism as key novel determinant in the control of immune tolerance to self.


This is the first report showing that in MS there is a reduced engagement of glycolysis which is reversible by treatment with beta-interferon.