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REVIEW



BCG and protection against inflammatory and auto-immune diseases

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ABSTRACT

Introduction: Bacillus Calmette-Guérin (BCG) is the only available vaccine against tuberculosis. Although its protective efficacy against pulmonary tuberculosis is still under debate, it provides protection against other mycobacterial diseases. BCG is also an effective therapy against superficial bladder cancer and potentially decreases overall childhood mortality.

Areas covered: The purpose of this paper is to provide a state-of-the-art summary of the beneficial effects of BCG in inflammatory and auto-immune diseases. As a strong inducer of Th1 type immunity, BCG has been reported to protect against atopic conditions, such as allergic asthma, a Th2-driven disorder. Its protective effect has been well documented in mice, but still awaits definitive evidence in humans. Similarly, murine studies have shown a protective effect of BCG against auto-immune diseases, such as multiple sclerosis and insulin-dependent diabetes, but studies in humans have come to conflicting conclusions.

Expert commentary: Studies in mice have shown a beneficial effect of the BCG vaccine against allergic asthma, multiple sclerosis and diabetes. However, the understanding of its mechanism is still fragmentary and requires further in depth research. Some observational or intervention studies in humans have also suggested a beneficial effect, but definitive evidence for this requires confirmation in carefully conducted prospective studies.

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1. Introduction

The bacille Calmette–Guérin (BCG) is a live attenuated bacterial vaccine developed in the beginning of the last century to protect against tuberculosis. In the course of their studies on the mode of transmission of *Mycobacterium bovis*, Albert Calmette and Camille Guérin realized that upon continued passaging of the *M. bovis* strain 'lait de Nocard' on a medium essentially composed of glycerinated potatoes cooked in ox bile, the organism progressively lost its virulence, yet maintained its protective potential against bovine tuberculosis [1]. After a total of 231 passages over a period of 13 years, the resulting BCG was applied for the first time to a young child born in a family plagued by tuberculosis. Without the BCG vaccine, the child would have had a 25% chance to succumb to tuberculosis within a year. The child survived without any sign of disease, and the BCG vaccine was subsequently tested in over 5000 children in a multicentric trial all over France [2]. The result of this trial showed a more than 90% protection against severe, disseminated, and deadly disease in young children.

Since then, BCG has been recommended as an antituberculosis vaccine for use in young children to be administered at birth. Initially, and in Brazil up to the 1970s, given as an oral vaccine, it is now mostly administered by intradermal injection. Several reasons have led to this switch, including the poor induction of a delayed-type hypersensitivity reaction by the oral route

[3], which is widely used as an indication of vaccine take. However, we know now that the delayed-type hypersensitivity reaction is not correlated with protection. Today, BCG is the most widely used vaccine ever implemented, with an estimated global vaccination coverage of roughly 80% [4]. The protective efficacy of BCG in neonates has been amply documented [5]. However, protection mediated by BCG against pulmonary tuberculosis in adults has been modest, overall reaching only about 50% efficacy [6]. Protection levels in adults appear to follow a gradient from poor protection in countries close to the equator towards higher protection with increasing distance from the equator, a gradient that overlaps with exposure to environmental mycobacteria. There is evidence that environmental mycobacteria limit BCG-induced immunity by several non-mutually exclusive mechanisms (summarized in [7]). They include blocking effects through environmental mycobacteria-induced immunity against BCG, masking effects by the immunity already induced by the environmental mycobacteria, and potentially the induction of regulator T cells by these mycobacteria, which may dampen the protective immunogenicity of BCG. Furthermore, BCG seems to have little effect on preventing the infection by *Mycobacterium tuberculosis*, the principal causative agent of tuberculosis in humans [8]. Therefore, considerable effort is now devoted to the development of new tuberculosis vaccines, destined either to replace BCG or to be used as boosters upon

BCG priming. Several promising vaccine candidates are currently at various stages of preclinical and clinical development, and it is hoped that further knowledge on the pathogenesis of tuberculosis will help to rationally design improved vaccines [9].

As BCG has been developed by serial passages on ox bile-impregnated potato medium, the mechanism of its attenuation is not fully understood. One region on the mycobacterial chromosome, called region of difference 1 (RD1), present in virulent *M. bovis* and *M. tuberculosis*, but absent in BCG, has been thoroughly investigated and found to be involved in virulence [10]. This region contains the genes coding for ESAT-6 and CFP-10 and their secretion apparatus. ESAT-6 and CFP-10 are two important virulence factors involved in vacuolar escape of *M. tuberculosis* into the cytosol [11]. *M. tuberculosis* mutants lacking these genes are attenuated, and insertion of the RD1 region into the BCG chromosome restores virulence to a certain degree, but not fully [12]. These observations indicate that in addition to the RD1 deletion, other genomic changes may also contribute to attenuation of BCG. These alterations include deletions of other RD, such as RD2, RD3, and RD14 to RD16 [13], as well as more subtle changes, including single-nucleotide polymorphisms (SNPs) [14]. How much each of these alterations contributes to attenuation of BCG is currently unknown.

In addition to the genomic differences between *M. bovis* and BCG, further changes between BCG sub-strains also occurred in the course of continued passages since the first BCG strain of 1921 was distributed by Calmette and Guérin. More than 12 different BCG sub-strains have evolved and are used in the various parts of the globe. Comparisons of the genome sequences of these sub-strains have revealed that they differ by several additional deletions or SNPs [15]. Whether any of these additional changes led to further attenuation or may have any impact on the protective potential of the BCG sub-strains is also not yet known.

2. BCG and protection against mycobacterial diseases other than tuberculosis

Although the primary goal of the BCG vaccine is to protect against tuberculosis, early reports also showed a significant protective effect against other mycobacterial diseases, such as leprosy. An 8-year observational study of a large cohort of BCG-vaccinated and non-vaccinated children indicated an 80% protective effect of BCG vaccination against leprosy in Uganda [16]. The protective effect of BCG did not depend on the age of vaccination and was similar for both sexes. Similar protection was later shown in a case-control study performed in Western Kenya, where significant protection against leprosy could be linked to the presence of BCG scar [17]. The protective effect of BCG, as inferred from scars, was again estimated to be roughly 80% against leprosy, whereas the protective effectiveness against tuberculosis was substantially lower in this study. There was no statistical difference between protection against paucibacillary and multibacillary leprosy. In a cohort of more than 80,000 individuals in Northern Malawi for whom the BCG scar status was known, protection was evaluated at 51% against

paucibacillary leprosy and 84% against multibacillary leprosy [18]. No significant protection by BCG was seen against tuberculosis in this study. A meta-analysis based on 7 experimental studies and 19 observational studies showed significant protection by BCG vaccination against leprosy, with an average of 26% protection in the experimental studies and 61% protection in the observational studies [19]. However, there was substantial heterogeneity between the trials. In this meta-analysis, protection was stronger against multibacillary forms of leprosy than against paucibacillary leprosy and stronger among women than among men. Protection waned with age, but the meta-analysis confirmed that BCG efficacy against leprosy was not affected by the age of vaccination. Interestingly, an additional dose of BCG improved the protective effect against leprosy compared to a single dose. This observation is in contrast to what was reported for BCG-induced protection against tuberculosis, where a second dose of BCG did not improve protection against tuberculosis [20]. However, the enhanced protective effect against leprosy by BCG revaccination has more recently been challenged by a Brazilian study, performed on close to 100,000 school children with neonatal BCG vaccination of whom roughly half were revaccinated between 7 and 14 years of age [21]. This study showed no evidence of enhanced protection by the second dose of BCG against leprosy during a 6-year follow-up.

The third mycobacterial disease of numerical importance is Buruli ulcer, a disease caused by *Mycobacterium ulcerans*. The importance of this disease was first recognized in Uganda in the late 1950s although the initial description of a new mycobacterial disease caused by *M. ulcerans* dates back to a medical report from Australia in 1948 [22]. Clinical trials with BCG vaccination to prevent Buruli ulcer initiated in the 1960s. In a first study reported in 1969, BCG was found to prevent the disease, but with a substantial rate of heterogeneity, ranging from 18% in high-incidence areas to 74% in low-incidence areas [23]. A subsequent case-control study performed on Buruli ulcer patients in Benin confirmed that a BCG scar was associated with a lower proportion of *M. ulcerans*-induced osteomyelitis in children [24], a severe form of the disease. However, a case-control study performed in Ghana did not confirm the protective effect of BCG vaccination against Buruli ulcer [25], nor did a case-control study conducted in southern Benin, although a certain level of protection against osteomyelitis was again observed in this study [26]. These observations suggest that the protection induced by BCG may be most effective against severe forms of Buruli ulcer. A more recent large observational study conducted in the Democratic Republic of Congo, Ghana, and Togo found no effect of the presence of a BCG scar on the Buruli ulcer disease status, independently of country, sex, and age [27]. The protective effect of BCG vaccination against Buruli ulcer remains thus controversial. However, most of the studies reported so far were observational studies, with their inherent limitations. Carefully designed prospective studies would be needed to settle this controversy. Studies in mice have suggested that the effectiveness of BCG against *M. ulcerans* depends on the immunogenetic background of the host and on the nature of the bacterial strains that were used for the challenge infection [28]. These parameters as well as the specificity of the BCG strains used for vaccination may have to be taken into account while planning such prospective studies.

3. Nonspecific effects of BCG

Obviously, protective effects of BCG against *M. leprae* and *M. ulcerans*, if confirmed, are likely due to T- and/or B-cell cross-reactivity of the mycobacterial antigens with those of BCG, although the mechanisms have not yet clearly been identified. However, BCG has also shown protective effects in non-mycobacterial diseases. In 1929, Raymond Pearl noted an inverse relationship between tuberculosis lesions and cancer and concluded that there is a marked antagonism between the two diseases, which would only rarely occur together at the same time in the same subject [29]. He suggested that treatment of malignant tumors in humans with tuberculin, a protein preparation from *M. tuberculosis*, should be contemplated. In mouse models, it has been shown that intravenous injection of BCG prior to subcutaneous implantation of solid tumors prolonged the life of the animals and strongly restricted tumor growth [30]. Subsequently, BCG therapy was tempted for treatment of various cancers, such as malignant melanoma, leukemia, colorectal cancer, and lung cancer, and initial successes were reported. However, when subjected to the scrutiny of well-controlled clinical trials, BCG therapy failed to show significant benefit for the patients, especially for patients with advanced disease. The one notable exception is the beneficial effect of BCG for the treatment of bladder cancer. Silverstein et al. [31] noted that intra-tumor injection of BCG resulted in tumor regression of malignant melanoma metastasis in the bladder. Many well-designed clinical trials have followed and shown that intravesical injection of BCG can eradicate and prevent recurrence of superficial bladder tumors (reviewed in [32]). It remains today the most effective local therapy for superficial bladder cancer, most likely because its ability to invoke a strong local immune reaction although the precise mechanism of the anti-tumor effect of BCG has still not been fully elucidated. It has been reported that BCG attaches to bladder tumor cells and urothelial cells via fibronectin and integrin $\alpha 5$ and $\beta 1$ binding and can be internalized by these cells [33], which appears to be crucial for the antitumor activity. BCG induces strong local inflammatory responses with an early granulocyte influx, followed by CD4⁺ mononuclear cells, and increased levels of Th1-type pro-inflammatory cytokines, such as interleukin (IL)-2, IL-12, and interferon (IFN)- γ . The induction of these cytokines can induce tumor necrosis factor (TNF)-related apoptosis-inducing ligand expression, which induces apoptotic cell death of tumor cells, and it has been suggested that this may be one of the underlying mechanisms of the antitumor effect of BCG [34].

A surprising observation was made when the effectiveness of routine childhood vaccination was evaluated in rural West African communities. In an observational study conducted over 6 years on roughly 10,000 children in Guinea Bissau, one of the poorest African countries with relatively low vaccination coverage, BCG vaccination was found to reduce overall mortality [35]. This was especially the case for newborns, between 0 and 2 months of age, whereas at 6 months of age, there was no difference anymore between BCG-vaccinated and non-vaccinated children. A similar observation had already been made by Naeslund more than half a century earlier by studying the effect of BCG vaccination on mortality in children [36]. Such a protective effect was not observed

when the mortality of children was followed after diphtheria, tetanus, and pertussis vaccination. The reduction in overall mortality after BCG vaccination was found to be larger than what could have been ascribed merely to the prevention of childhood tuberculosis. It is thus likely that BCG vaccination conferred nonspecific protection against other infectious diseases, possibly due to its ability to induce strong Th1-type immune responses. The protective effect of BCG against childhood mortality was subsequently confirmed in a small, randomized trial among low-birth-weight children [37] and was mostly due to a reduction in septicemia and respiratory infections in the vaccinated children. A large population-based analysis using national health survey data from 33 low- and middle-income countries over a 25-year period and including over 58,000 survey participants in the primary cohort and more than 93,000 in the secondary cohort showed that BCG vaccination reduced the risk of childhood acute lower respiratory infections [38].

Protection by BCG against lower respiratory infections other than those caused by *M. tuberculosis* probably does not depend on cross-reactive antigens but rather results from induction of innate immunity. That BCG-mediated protection against heterologous pathogens is due to the innate immune arm has been shown by the use of severe combined immunodeficiency (SCID) mice, lacking the adaptive immune system, in which BCG has been demonstrated to confer protection against a lethal fungal infection [39]. Studies with human innate immune cells have shown that BCG vaccination reprograms monocytes to increase the production of pro-inflammatory cytokines, such as TNF and IL-1 β , in response to unrelated microbial stimuli [39], a phenomenon named 'trained innate immunity.' This reprogramming is based on NOD2-mediated epigenetic changes at the level of histone modifications and is accompanied by a strong increase in glycolysis and glutamine metabolism, as has been shown in both mice and humans [40]. The inhibition of glycolysis enzymes abolishes trained immunity, indicating that glycolysis is crucial for the BCG-mediated nonspecific effects and linking innate immunity to metabolic changes.

4. BCG and atopy

A substantial amount of work has been devoted to studying the effect of BCG on the outcome of allergic atopy, an individual or familial tendency to produce IgE in response to low doses of allergens and to develop typical symptoms such as atopic dermatitis, allergic rhinitis, and asthma [41]. Over the last decades, the prevalence of allergic disorders, such as hay fever and bronchial asthma, has increased worldwide, mostly in westernized countries where up to 20% of the population are affected [42]. The risk of developing atopic diseases is strongly influenced by both genetic and environmental factors [43]. Allergic asthma is considered as an inappropriate Th2-mediated immunological response, with elevated IgE levels, increased numbers of allergen-specific Th2 lymphocytes secreting IL-4, IL-5, and IL-13, altered airway reactivity to inhaled allergens, and chronic inflammation of the airways [44]. The 'hygiene hypothesis' suggests that modernized

lifestyles, like improved housing conditions and reduced microbial exposure, lead to decreased Th1 immune responses and a domination of the harmful Th2 type of immune response [45]. Moreover, there is evidence that children who live on farms with livestock in early life present a decreased tendency to suffer from atopy later in life [46]. This protective effect of early-life microbial exposure may be related to the microbial induction of memory T cells secreting anti-allergic Th1 cytokines [47]. As strong inducers of Th1 polarization, characterized by the production of IFN- γ and IL-12, mycobacteria may thus have the potential to prevent the development of allergen-specific Th2 responses and atopy during childhood [48].

The anti-inflammatory properties of BCG have been tested in murine models of atopic asthma, where the mycobacteria were shown to inhibit allergen-induced airway inflammation [49]. In studies of ovalbumin-sensitized mice, allergic responses and airway hyperresponsiveness to methacholine were determined before and after nasal and peritoneal injection of BCG. Intranasal administration of BCG significantly suppressed late allergic reaction, hyperactivity to methacholine, eosinophilia, and IL-5 production in bronchoalveolar lavage fluids [50], potentially via upregulation of IL-12 and IFN- γ production induced by the binding of mycobacterial lipoproteins to Toll-like receptors on antigen-presenting cells [51]. In an independent study, systemic BCG immunization was confirmed to hinder allergic ovalbumin sensitization and to prevent the development of airway reactivity in BALB/c mice [52]. Again, BCG altered the immune balance towards Th1-like activity by decreasing the IL-4 and IL-10 production. The mechanism of IFN- γ -induced inhibition of Th2 responses is not fully understood, but could involve activation of macrophages, direct suppression of developing Th2 lymphocytes, or altered antigen presentation [53].

Studies in murine models have also demonstrated that regulatory T (Treg) cells, generated by a mycobacterial treatment, may inhibit the allergic inflammation, suggesting that the mechanism responsible for the effect of BCG on asthma is more complex than simple changes in the Th1/Th2 balance [54]. Thus, Treg cells may have an essential role in restoring the balance of the immune system to prevent and treat allergic diseases. Akdis et al. [55] reported that allergen-specific Th2 and suppressor (allergen-specific Tr1) T cells exist in both healthy and allergic individuals and that their ratio determines the development of healthy or allergic immune responses. Thus, the existence of potential suppressive allergen-specific Tr1 cells in allergic individuals may perhaps be used in treatment strategies. Ahrens et al. [56] indicated that BCG-induced suppression of Th2-type allergic airway inflammation is associated with enhancement of Treg cell function and Th1-associated neutrophilic airway inflammation, both *in vitro* and *in vivo*. Interestingly, a more recent study demonstrated that the inhibitory effect of BCG in the mouse model of asthma was mediated by dendritic cells (DCs), which induced CD4⁺ CD25⁺ Treg cells [57].

Different BCG strains may have different effects on DC surface expression markers, chemokine and cytokine production. After stimulation with BCG Copenhagen, the cytokine production was less enhanced compared to BCG Pasteur and Chicago. BCG Pasteur induced the strongest IL-12 and BCG Chicago induced the highest TNF- α production. Moreover, in cocultures of BCG-stimulated DC with allergen-specific T cells,

the production of IL-5 and IL-13 was reduced. This effect was probably due to the enhanced regulatory DC functions after BCG stimulation, resulting in strong IL-10 production by Treg cells. The possibility to generate recombinant BCG strains has prompted the development of strains that produce allergens, in order to specifically target the anti-inflammatory responses to allergens of interest. A BCG strain that produced recombinant Der p2 was found to be more effective in suppressing asthmatic airway inflammation than nonrecombinant BCG [58]. This effect was related to Th17 regulation, in which IL-6 secretion and Notch ligand expression by DC were involved. Possibly, recombinant BCG producing Der p 2 affected the differentiation and function of Treg cells by changing the Notch ligands of DC, thereby restoring the Th1/Th2 balance.

The ability of Th1 profiles to downregulate the Th2 type of immune response has led to yet another approach to use recombinant BCG as potential prophylactic or therapeutic agent. This approach is based on strategies to further increase the Th1-promoting properties of BCG by the expression of IFN- γ -inducing factors, thereby directly enhancing allergen-specific Th1 immune response in allergic asthma. This was tempted by generating recombinant BCG strains producing IL-18. This cytokine is a member of the IL-1 family and was described in 1995 as a cofactor for both Th1 and Th2 cells. One of the functions of IL-18 is to act in synergy with IL-12 for Th1 differentiation [59]. IL-18 induces IFN- γ production by activated murine and human T cells [60,61]. The independent observations on the ability of BCG [62] and IL-18 [61] to inhibit the allergic reactions thus led to the development of recombinant BCG strains producing IL-18 [63]. These recombinant strains strongly augmented the long-term production of IFN- γ , tumor necrosis factor (TNF)- α , and granulocyte macrophage colony-stimulating factor (GM-CSF) in a murine model and decreased the secretion of IL-10 by spleen cells stimulated with BCG, and it protected against allergen-induced bronchoalveolar eosinophilia. This effect was correlated with a strong inhibition of IL-5 and IL-13 production [64]. In an independent study, the synergistic effect of BCG and IL-18 on the polarization of Th1 response was observed in a mouse model of bladder cancer [65]. Szpakowski et al. [66] reported that a recombinant BCG strain producing human IL-18 was able to preferentially trigger strong IFN- γ secretion by naive CD4⁺ T cells of healthy human volunteers vaccinated with BCG.

The anti-allergy effect of BCG in humans still awaits definite evidence. An inverse association between mycobacterial infections and atopic disorders has been noted by the demonstration of a reverse correlation between the presence of positive tuberculin skin test response and asthma prevalence in BCG-vaccinated school children aged 12 and 13 years from a tuberculosis-endemic region in Japan [67]. In this study, the prevalence of asthma in children with an induration of >10 mm in the Mantoux skin test in early life was 28% lower than in tuberculin-negative children. In addition to a lower incidence of asthma, the positive tuberculin responses were associated with decreased levels of serum IgE, increased Th1-type cytokines, and decreased IL-4, IL-10, and IL-13 levels. The positive skin test could be the result of BCG vaccination or the exposure to *M. tuberculosis*, to environmental mycobacteria, or to any combination thereof. It was thus hypothesized that

early-life mycobacterial infection promotes the switch from a Th2 to a Th1 profile, thereby inhibiting the expression of atopy.

The work by Aaby et al. [68] supports this hypothesis in their study on 3- to 14-year-old children in Guinea-Bissau, where the absence of evidence of BCG vaccination was related to an increased risk of atopy. Another study indicated that neonatal BCG vaccination has an effect on T-cell allergen responsiveness 7–14 years after vaccination and that among a subgroup of subjects with an inherited predisposition to allergic disease, this is associated with clinically relevant beneficial effects [69].

However, other epidemiological data failed to confirm an association between an asthmatic phenotype and BCG vaccination [70,71], suggesting that this inverse relationship, if real, is much more complex than previously appreciated. Differences in study design, BCG strains, patterns of natural exposure to mycobacteria, and ethnic backgrounds may have had an impact on this discrepancy [72–74]. Thus, it remains still unclear whether live BCG, or recombinant derivatives thereof, may eventually be used as an asthma vaccine in humans. It may be possible that live BCG would have to be applied at the site of allergen exposure to have the most potent suppressive effect on Th2 response, which would imply to apply the BCG directly into the lungs. To our knowledge, this approach has not yet been tempted.

5. BCG and multiple sclerosis

Multiple sclerosis (MS) is a debilitating autoimmune disease of the central nervous system (CNS) with a relapsing and remitting course and affects both sensory and motor nervous functions. It is the result of an immune-mediated demyelination with a strong inflammatory component and is one of the most common causes of nontraumatic neurological disorders in young adults. In a Danish population cohort of 198,000 persons including 92 patients with MS, an inverse relationship was noted between the risk of MS and a positive tuberculin test at the age of 7 years, indicative of exposure to *M. tuberculosis* at young age [75]. However, the same study did not reveal a difference between patients and controls with respect to BCG vaccination. On the other hand, a pilot study on 12 relapsing-remitting MS patients, who had otherwise not been under immunomodulatory treatment, suggested a benefit of BCG treatment [76]. A significant reduction in lesions, as evidenced by magnetic resonance imaging, was observed during a 6-month follow-up after a single intracutaneous dose of BCG. Using a different read-out, i.e. new enhancing lesions that evolve into T1-hypointense lesions, as a measure of tissue damage, on the same patients, confirmed the protective effect of BCG [77]. A reduction of new enhancing lesions was observed after BCG administration, as well as a reduction in the proportion of new enhancing lesions that evolved into T1-hypointense lesions. A subsequent placebo-controlled study on patients with a first demyelinating episode, which is generally reversible, showed that a single intracutaneous BCG administration reduced the proportion of patients with disease progression [78]. The cumulative number of relapses after 18 months was also reduced in the intervention group

compared to the placebo group, and during the 5-year follow-up, significant differences were found in the occurrence of clinically defined MS. At the end of the study, more than half of the vaccinated patients remained relapse-free compared to 30% in the placebo group, indicating a long-term protective effect by BCG.

Although definitive proof of the MS-suppressing effect of BCG awaits further large-scale clinical efficacy trials, these initial studies are certainly encouraging. However, the mechanism of this effect is still not fully understood, but has been approached by the use of a murine model of experimental autoimmune encephalomyelitis (EAE) as a proxy for MS. Intraperitoneal injection of BCG was reported to delay the onset and to reduce the severity of EAE induced by the administration of a myelin oligodendroglial glycoprotein (MOG) peptide in mice in a dose-dependent manner [79]. It also resulted in lower amounts of CNS-infiltrating cells and MOG-specific CD4⁺ T cells in the brain compared to the uninfected mice although the induction of MOG-specific CD4⁺ T cells in the spleen was not affected by the BCG treatment. In fact, BCG appeared to trap the autoantigen-specific T cells in the spleen and in liver BCG granulomas and inhibited their migration to the brain. In addition, infection with BCG can promote apoptosis of activated CD4⁺ T cells, but not of naive CD4⁺ T cells, in an IFN- γ -dependent manner, thereby preventing the overexpansion of these cells [80]. MOG-specific activated CD4⁺ T cells when transferred to mice infected with BCG were indeed shown to undergo apoptosis much more effectively than when they were transferred to uninfected mice [81], and this limited the severity of the immunopathology in the EAE model. BCG-induced apoptosis of these cells did not occur in IFN- γ knock-out (KO) mice and required the expression of the IFN- γ receptor, but not the production of IFN- γ by the activated T cells.

IFN- γ also inhibits the development of IL-17-producing Th-17 T cells [82], which are critical for the induction of EAE. The amount of pathogenic IL-17-producing CD4⁺ T cells is greatly increased in IFN- γ KO mice compared to wild-type mice in the EAE model, and IL-17 and IFN- γ were found to mutually regulate IFN- γ and IL-17 production [83]. Compared to intraperitoneal BCG infection, intracerebral administration of BCG showed a stronger protective effect against EAE. When BCG was injected intracerebrally into mice before the induction of EAE, lower frequencies of IL-17-producing CD4⁺ T cells were found subsequent to EAE induction than in uninfected mice [84]. However, the MOG-specific IFN- γ -producing cells were also less abundant in the BCG-treated mice compared to the uninfected animals, but the decrease in IL-17-producing T cells was more pronounced than that of cells producing IFN- γ . Unexpectedly however, intracerebral administration of BCG provided complete protection against EAE symptoms in IFN- γ KO mice and abolished the cellular infiltration and the generation of demyelinating lesions. In these IFN- γ KO mice, intracerebral BCG resulted in diminished IL-17-producing T-cell frequencies, comparable to wild-type mice, indicating that pathogenic Th-17 cells were inhibited in an IFN- γ -independent manner in this model. These apparently conflicting results suggest that the interaction between IFN- γ and IL-17 depends on the location where these cytokines are induced and that different regulatory mechanism may be at play

depending on the location. The frequencies of FoxP3⁺CD4⁺ T cells were similar between mice infected intracerebrally with BCG and uninfected mice, suggesting that the protective effect of BCG cannot be ascribed to the induction of FoxP3⁺CD4⁺ Treg cells in this model. Other mechanisms may thus play a role here. These may include regulatory B cells or cytokines, such as IL-10, which have been shown to be induced by mycobacterial compounds and to suppress the induction of EAE [85].

6. BCG and diabetes

Another autoimmune disease that has been the focus of BCG treatment is type 1 diabetes, one of the most common genetic disorders affecting children and adolescents, the prevalence of which is increasing globally [86]. Type 1 diabetes is caused by autoimmune destruction of the β cells in the pancreas, leading to a deficiency in insulin production and thus to hyperglycemia, polyuria, and hypoinsulinemia [87]. The disease is influenced by determinants such as sex, age, ethnicity, race, and genetic susceptibility [88]. Several environmental factors including maternal age, enterovirus infections, infant feeding, childhood vaccinations, and infections are also likely to be causes of diabetes. These factors may play a role in initiating autoimmunity or may take part in the destruction of insulin-producing β -cells. The only available treatment for type 1 diabetes is regular insulin administration. Since BCG is known to induce Th1 and Th17 lymphocytes, as well as Treg cells, which are involved in the immune response in type 1 diabetes [89], the BCG vaccine has been proposed as beneficial immunomodulator of the immune response that may limit the development of autoimmune diabetes, both in humans and in animal models [90–92]. However, the mechanism underlying BCG-related immunomodulation is still not fully known and requires further investigations.

In humans, a clinical remission in a late-stage prediabetic patients was noted after administration of a single low dose of BCG. Moreover, remission has been sustained for 6–10 months without any side effects [91]. However, a later study indicated that a single low-dose of BCG vaccination in new-onset diabetic children did not have any beneficial effect when the patients were restudied a year later [92]. Other findings suggest that not a single dose of BCG but repeated BCG administrations during childhood prevent diabetes and autoantibody formation [93]. A more recent pilot study by Faustman et al. [94], involving six subjects with long-standing type 1 diabetes, randomized to receive either BCG or placebo, confirmed functional improvement of the pancreas upon BCG administrations. Two low-dose BCG vaccinations were chosen as a treatment, based on their induction of TNF- α , supposed to kill the disease-causing autoimmune cells. Specific biomarkers, such as the levels and viability of cytotoxic insulin-autoreactive T cells, the induction of protective Treg cells, antibodies against the glutamic acid decarboxylase autoantigen, and the levels of C-peptide, a marker of endogenous insulin production, were monitored for 20 weeks. Alterations in disease-specific T cells and changes in C-peptide secretion were noted in the BCG-treated patients, indeed suggesting brief functional improvements in pancreas function. Importantly, repeated BCG vaccinations at low doses ($1.6\text{--}3.2 \times 10^6$ CFU/injection)

given intradermally to the patients were safe, well tolerated, and caused rapid increases in the number of dead circulating insulin-autoreactive T cells. However, it remains to be established whether the increase in C-peptide levels had any biological significance.

The conclusion of this study was thus in contrast to two previous clinical trials, where BCG failed to preserve β -cell function after a single low-dose BCG administration [92,95]. Instead, in both of these studies, there was a visible trend to greater decline of β -cell function in the BCG-treated group compared to the control group.

In contrast to a study on 137 southern Indian diabetic patients, where the presence of a BCG scar was associated with a significant decrease in autoantibodies [96], large case-control studies in Sweden and Canada failed to provide evidence that BCG vaccination at young age may prevent subsequent type 1 diabetes in children [97–99]. A study conducted by Huppmann et al. [100] in Germany concluded that neonatal BCG vaccination may in fact accelerate progression from autoimmunity to diabetes probably due to the strong immunostimulatory effect of BCG, as manifested by inflammation and enlargement of lymph nodes around the site of injection. Recent data from Québec obtained by Rousseau et al. [101], however, confirmed the lack of association between BCG vaccination and occurrence of childhood diabetes, specifically at the first year of life. These conflicting findings may perhaps be due to the timing of BCG vaccination in early childhood. Vaccination at birth may be associated with protection against type 1 diabetes, whereas vaccination several weeks or months after birth may exacerbate the disease [96].

It should be noted that in the evolution of type 1 diabetes, several immune pathways are involved, which complicates the design of an effective therapeutic strategy to control the immune system and prevent the loss of β -cell function. Thus, the combination of selected anti-inflammatory and immunomodulatory agents targeting innate immunity may bring some beneficial effects in future studies. Although multiple administrations of BCG may be a promising strategy, many questions remain to be answered, such as, for example, whether BCG should be used as a primary, secondary, or tertiary vaccination strategy for type 1 diabetes. Furthermore, the mechanism of the protective BCG effect, if real, requires further elucidation of the cellular and molecular mechanism.

A nonobese diabetic (NOD) mouse model of autoimmune diabetes provided interesting data clearly showing that BCG ameliorated the advanced autoimmune process by stimulating TNF- α , which selectively killed only disease-causing cells [102]. The NOD mice were proposed by Makino et al. [103] as a relevant animal model for studying human type 1 diabetes as these inbred mice spontaneously develop insulin-dependent nonobese diabetes mellitus and insulinitis manifested by mononuclear cell infiltration into Langerhans' islets and destruction of β cells. Insulinitis occurs at around 5 weeks of age and can be observed in the mice of both sexes after 9 weeks of age. Yagi et al. [104] demonstrated that a single intravenous injection of BCG prevented the development of insulinitis in NOD mice over a long period of up to the age of 30 weeks. This protection could be transferred by whole spleen cells from the BCG-treated mice to recipient female mice, suggesting that some kind of suppressor cells were generated as the result

of BCG treatment. It was noted that the T and B lymphocyte responses to concanavalin A (Con A) were reduced for a long period of time in the BCG-treated mice. This effect was accompanied by a reduced IL-2 and IL-2 receptor production by splenic T cells. It was noted that the generation of suppressor macrophages was responsible for this effect, and not a dysfunction of lymphocytes, nor the generation of suppressor T cells. Several possible mechanisms, including a role of prostaglandins, the immunosuppressive effect of lipocortin I or transforming growth factor (TGF)- β , or the production of the reactive nitric oxide radicals, have been suggested [104], but further work is necessary to draw final conclusions.

One of the first indications suggesting that a prime-boost strategy may perhaps enhance the immunomodulatory effect of BCG in autoimmune diseases has been reported by Da Rosa et al. [105], who evaluated the prophylactic potential of a prime-boost strategy based on BCG priming and boosting with the DNA vaccine pVAXhsp65 in diabetes induced by streptozotocin (STZ) in C57BL/6 mice and in NOD mice. Although this vaccination strategy did not protect mice in the STZ model, it was very effective in NOD mice. Although the exact mechanism still awaits clarification; it has been suggested that apoptosis of diabetogenic T cells and the action of Treg cells are probably involved.

7. Expert commentary

Inflammatory and autoimmune diseases are currently on the rise, especially in industrialized countries. Most treatment options are limited due to unacceptable adverse events. BCG is the most widely used vaccine of the last 10 decades and has a remarkably good safety profile. Severe BCG-specific adverse events are almost exclusively due to primary or acquired immune deficiencies. Because of its strong immunomodulatory properties, BCG has been proposed as a prophylactic or therapeutic tool against inflammatory and autoimmune diseases, such as allergic asthma, MS, and type 1 diabetes (see Figure 1). Murine models of these diseases have clearly shown beneficial effects of BCG treatment, and these models have been used in an attempt to determine the

molecular and cellular mechanisms of the BCG effects. However, more investigations are needed to fully understand these mechanisms. In contrast to the murine studies, evidence of a prophylactic or therapeutic effect of BCG against these diseases in humans is less solid. Whereas some epidemiological investigations and clinical trials show beneficial effects, others have failed to confirm them. It is therefore likely that a deeper understanding of the mechanisms is required for better targeted and adapted BCG applications. More studies are thus needed to determine the efficacy, safety, and long-lasting effects of the altered immune response. Appropriate dose finding and the number and frequencies of BCG administrations for optimal effects are some of the challenges for future investigations. Considering the increasing importance of inflammatory and autoimmune diseases, and considering the excellent safety profile of BCG, it is certainly worthwhile to pursue these studies, as they may ultimately lead to a very cost-effective strategy to combat these diseases.

8. Five-year view

Over the past decades, the prevalence of inflammatory and autoimmune disorders has been steadily increasing, especially in industrialized countries. A further increase is likely to occur within the next 5 years. Prophylactic and therapeutic interventions are therefore urgently needed. Several immune interventions have been proposed against these diseases, and some of them are in clinical use. They include immunosuppressive molecules, anti-cytokine antibodies, and other immunoregulatory factors used either alone or in combination with specific auto-antigens. Although these treatments show sometimes promise, their use in humans is often limited due to their inherent systemic toxicity. Undoubtedly, important progress can be expected in that area in the next 5 years. In parallel, the beneficial properties of certain bacterial infections are also being explored, based on the concept of the hygiene hypothesis. In that aspect, BCG is one of the most attractive bacterial agents, due to its almost 100-year-long use as a tuberculosis vaccine, which has shown its excellent safety profile. Murine models have shown a

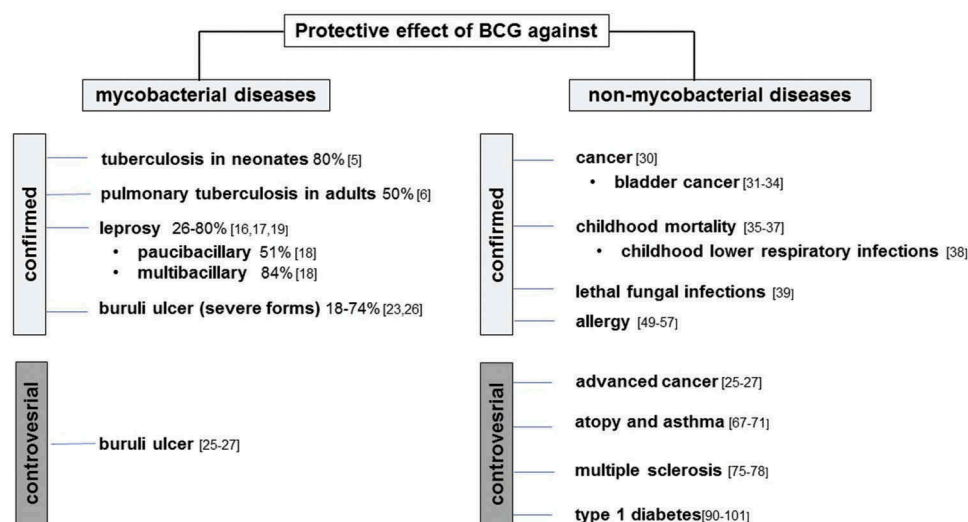


Figure 1. Protective effects of BCG against mycobacterial and non-mycobacterial diseases. Some of the effects have been confirmed, as indicated in the figures, others are still controversial. Percentages indicate percentages of protection in efficacy trials against the indicated mycobacterial diseases. The numbers in brackets refer to the references in the reference list.

good prophylactic or therapeutic effect of BCG administration in several inflammatory and autoimmune diseases, and these models should further be used to gain in-depth understanding of the underlying mechanisms. Studies in other animal models, including nonhuman primates, would also be advisable to confirm the beneficial effects of BCG. As definitive evidence in humans is still lacking, it is expected that carefully conducted prospective studies will clarify the debate on the protective or therapeutic effects of BCG in these diseases. It is conceivable that a combination of BCG treatment with other immune modulating molecules will provide the best clinical outcome. Furthermore, the recognition of the importance of the microbiome on immune responses to vaccines and infections should also foster studies aiming at appreciating its role on the immune modulatory properties of BCG. Recent studies in infants have shown that the abundance of Actinobacteria in stool microbiota is associated with T-cell responses to BCG vaccination [106]. Therefore, within the next 5 years, we can expect important progress in these areas, as long as sufficient resources can be devoted to such studies.

Key issues

- Considering the limited protective efficacy of BCG against tuberculosis, new vaccines are being developed to potentially replace BCG. However BCG replacement strategies should take into account its beneficial non-specific effects.
- Protective effects of BCG against inflammatory and autoimmune diseases have been clearly demonstrated in murine models, but the molecular and cellular mechanisms of this effect needs to be further investigated.
- Definitive evidence of BCG as a prophylactic or therapeutic vaccine against allergic or auto-immune diseases in humans is still lacking and requires carefully designed prospective or intervention studies.
- For some diseases topical applications of BCG should be contemplated, such as for the treatment of allergic asthma.

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Declaration of interest

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