Title: Historic and Emerging Applications of BCG in Prevention and Treatment of Disease

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Abstract

Bacillus Calmette Guerin (BCG) was introduced 100 years ago for vaccination against tuberculosis and 40 years ago for treatment of bladder cancer. The last decade has witnessed a surge of new uses of BCG in the prevention or treatment of autoimmune disease, allergy and respiratory disease unrelated to tuberculosis. This article reviews the efficacy and safety of the two main uses of BCG, tuberculosis prevention and bladder cancer treatment. It discusses the animal and human studies underlying the new uses of BCG. Finally, it covers the two avenues of basic science propelling the new uses of BCG, both of which rely on BCG role as an inducer of the cytokine Tumor Necrosis Factor (TNF): the selective destruction of autoreactive T-lymphocytes and the induction of T-regulatory cells.

Introduction

Bacillus Calmette Guerin (BCG) was first introduced into humans nearly 100 years ago as a vaccine against tuberculosis (TB). French microbiologist Albert Calmette and veterinarian Camille Guerin made the vaccine by isolating a tuberculosis-related microbe from a cow (*Mycobacterium bovis*) and then by passaging it in culture over several years to weaken its virulence (1). Decades of research have established that BCG is highly effective (>50-80%) in children against severe forms of tuberculosis, namely TB meningitis, and miliary TB, but less so against pulmonary TB (2, 3). Since BCG's introduction, an estimated 3 billion people worldwide have received it, considering that each year 100 million children are vaccinated (4). Because of declining incidence of tuberculosis in Europe and the US, BCG vaccination has been discontinued there and is primarily administered in developing countries.

Another application of BCG is for a treatment of the most common forms of bladder cancer. In recent years, there has been an explosion of research investigating BCG's immunotherapeutic value in preventing or treating autoimmune diseases, allergy, and respiratory infections. BCG's mechanism of action for many of these new disease applications stems from its induction of the cytokine tumor necrosis factor (TNF) (5, 6).

1.1 BCG and Tuberculosis

The first BCG vaccination against TB was administered in 1921 to an infant born to a woman who died of TB a few hours after giving birth. From 1924 to 1928, 114,000 infants were given BCG without serious adverse events (7). The death rate in Paris from TB was cut dramatically, from 25-36% among the unvaccinated to 1.8% among the vaccinated population (8). Over the next 90 years, hundreds of clinical trials and case-

control studies of varying quality were performed. One of the first modern reviews of efficacy, analyzing data from eight high-quality randomized controlled trials, found 0-80% protective effect of BCG against pulmonary TB (9). Evidence from several meta-analyses indicates that BCG shows greater efficacy against miliary TB (also known as disseminated TB) and TB meningitis in children than in pulmonary TB. The meta-analyses calculate the protective effect by 1 minus RR (relative risk) or 1 minus OR (odds ratio). A meta-analysis by Colditz and colleagues (2) found an overall protective effect of the BCG vaccine at 74% in five BCG clinical trials and 52% in 11 case-control studies. With stratification of the data, the investigators found a 65% protective effect against death from TB, a 64% protective effect against the development of TB meningitis, and a 78% protective effect against development of miliary TB. A subsequent meta-analysis by Trunz and colleagues (3) found a 73% protective effect for TB meningitis and 77% for miliary TB. The authors estimated that the 100 million doses of BCG given to children each year prevented 40,000 cases of TB meningitis and miliary TB. This figure translates to one case prevented for every 2,500 inoculations.

The variable efficacy of BCG vaccines is attributed to multiple factors. The first factor is strain differences. There are, worldwide, at least 20 different strains of BCG that differ in terms of genotype and phenotype (10). Other factors contributing to the vaccines' variable efficacy are the exposure of clinical populations to environmental, non-tuberculosis mycobacteria, nutritional or genetic differences, differences in clinical trial methodology, and interference by concurrent parasitic infection (11, 12).

The duration of vaccine efficacy has been determined by an influential study of American Indians and Alaska Natives who were given BCG in a clinical trial from 1935-1938. Analyzing medical records, TB registries, death certificates and supplemental interviews with trial participants, the study found that efficacy of a single dose of BCG persisted for 50-60 years (13).

Safety of BCG Vaccine

The BCG vaccine has a longstanding record of excellent safety and tolerability. The most common adverse event is suppurative lymphadenitis, which is self-limiting and does not require treatment. Its occurrence across a broad array of developing and developed countries is 0.1-38 cases per 1000 vaccinations (14). A more serious adverse event is osteitis, which occurs more rarely (0.01-330 cases per million vaccinations) and occurs when the vaccine is administered subcutaneously or intramuscularly instead of intradermally (14). The variability in these adverse events is also tied to BCG strain differences, country, manufacturing process, surveillance methods, the person administering the vaccine and body site of vaccination (12). For example, there was a striking rise in the incidence of osteitis in Sweden and Finland coinciding with the replacement, in 1971, of the Gothenburg strain of BCG with one produced by the State Serum Institute. Another rare complication is disseminated BCG infection, which occurs primarily among immunocompromised patients with underlying immune genetic defects (15). The occurrence of a fatal reaction to BCG is extremely rare, estimated at 0.19-1.56 per million vaccinations, almost all of which are tied to compromised immunity (4). Disseminated BCG infection can also be

idiopathic, accounting for an estimated 0.59 cases per million vaccinations (16).

BCG for Bladder Cancer Treatment

The utility of BCG for cancer traces back to 1929, when a pathologist noticed a reduction in cancer among patients with TB at autopsy. Its application to other cancers was thwarted by the death, in 1935, of 70 infants in Germany, which was falsely attributed to BCG instead of inadvertent live virulent tuberculosis (7). It was not until the 1970s that BCG was first considered for non-muscle-invasive bladder cancer (NMIBC). This is the most prevalent form of bladder cancer and consists of superficial tumors that may have invaded the bladder's lamina propria but not yet invaded the detrusor muscle (stages Ta, T1, and Tis (carcinoma *in situ*)). After standard surgery to remove the tumor (transurethral resection) and without further treatment, an estimated 15-70% of tumors will recur, and 7-40% will advance to invade the detrusor muscle (17, 18). The first clinical trial of BCG for bladder cancer in a small, uncontrolled study was published in 1976 (19). BCG was introduced directly into the bladder (today typically at doses of 80-120 mg, or $10.5 \pm 8.7 \times 10^8$ colony forming units) once a week for six weeks. The study found 12-fold lower tumor recurrence. The results spurred the onset of two randomized controlled trials, both of which showed significant reductions in tumor recurrence when compared with standard therapy, which at the time was cystoscopy and fulguration (20, 21). The success of these trials led to dozens of other clinical trials. At least five meta-analyses were subsequently conducted, the results of which prompted BCG as the standard of care. The current guidelines from the National Comprehensive Cancer Network endorse BCG as a preferred therapy for high-grade Ta, T1, and Tis tumors following tumor resection (22).

A meta-analysis by Shelley and colleagues (23) of 25 clinical trials consisting of 1,901 patients found that, compared to the chemotherapy agent mitomycin C, BCG was found superior. It found a 31% reduction in tumor recurrence over mitomycin C. A second meta-analysis (24) analyzed 25 clinical trials with 4,767 patients. Forty percent of patients had tumor recurrence compared to 49.7% in the non-BCG group. A third meta-analysis, performed for the Cochrane Collaborative, combined five clinical trials totaling 1,111 patients (25). It found BCG superior to the chemotherapy agent epirubicin: overall, 35.5% of BCG-treated patients had tumor recurrence versus 51.4% of the epirubicin group. Furthermore, 8.02% of BCG-treated patients progressed to T2 or higher stage bladder cancer versus 10.32% in the epirubicin group.

Maintenance therapy with BCG was introduced after the six weekly instillations, in part because without maintenance, BCG efficacy was no longer significant at 15 years (1). One of the largest trials of maintenance therapy, of 660 patients, found that the median recurrence-free survival was 35.7 months in the no maintenance arm and 76.8 months in the maintenance arm (26). The maintenance regimen in this trial consisted of one BCG instillation each week for three weeks given at three, six, 12, 18, 24, 30, and 36 months after initiation of induction therapy. A meta- analysis sought to determine whether BCG plus maintenance was more effective than BCG alone (27). It combined results from 24 trials with 4,863 patients. It found a significant reduction in disease progression by 27% with maintenance BCG versus BCG alone (OR=0.73, p=0.001). Another meta-analysis

studied maintenance BCG (for a minimum of six months) against BCG combined with chemotherapy in four clinical trials that included 801 patients (28). It found no significant reduction of recurrence between BCG plus maintenance and BCG with chemotherapy groups.

Safety of Multi-dose High Dose BCG for Bladder Cancer

The dose of BCG in bladder cancer is about 250 times higher than that used in the vaccine. The safety record of BCG and bladder cancer shows a high likelihood of local effects on the bladder, yet only mild systemic effects, and less than 1% severe systemic effects. Most local and systemic adverse events resolve on their own within 48 hours but also are treatable. In 1986, a study combining 1,278 BCG-treated bladder cancer patients seen in Canada, US, and Europe found cystitis in 91% of patients, hematuria in 43%, low-grade fever in 28%, malaise in 24%, and nausea in 8% (29). That case series was combined with another 1,324 patients (for a total of 2,602) to examine the rate of complications (30). The results covering systemic complications are in Table 1.

Table 1: Systemic complications of BCG in 2,602 bladder cancer patients

High fever	2.9%
Pneumonitis/hepatitis	0.7%
Arthralgia	0.5%
Rash	0.3%
Renal abscess	0.1%
Sepsis	0.4%
Cytopenia	0.1%

Source: (38)

The safety findings are consistent with those from several other studies (31, 32) and a recent study of 607 patients with a median follow-up of 45 months (33). The latter study found BCG- induced sepsis at 0.2% of patients. The study sought to reduce the rate of local and systemic toxicity by cutting the BCG dose by half and replacing it with the chemotherapy agent epirubicin. Studying patients for a median duration of four years, the study (33) found similar efficacy yet lower toxicity. In animal models and rare human cases, BCG used in bladder cancer can cause reactive arthritis (34, 35).

BCG for Autoimmune Disease

BCG's application to the treatment of autoimmune disease has been inspired by research on animal models (36) and by the "hygiene hypothesis," which attributes the increased incidence of human autoimmunity (and allergies) over the last decades to the reduction in exposure to natural pathogens (37) . It follows that exposure to attenuated pathogens, such as the BCG vaccine, should reduce autoimmunity. Consequently, BCG has been evaluated for its capacity to control Type 1 diabetes, multiple sclerosis, Sjogren's Syndrome, and allergy.

2.1 **Type 1 Diabetes**

The first study to determine if BCG could prevent type 1 diabetes was conducted in non-obese diabetic (NOD) mice (38, 39). It found that the earlier the injection, the greater was the suppression of type 1 diabetes onset (40). Two other small studies found that Complete Freund's Adjuvant (CFA, the non-Good Manufacturing Equivalent of BCG) blocked the onset of type 1 diabetes in NOD mice (41) and BB diabetes-prone rats (42). CFA also reversed full-blown type 1 diabetes in NOD mice (43, 44).

In humans newly diagnosed with Type 1 diabetes, single-dose BCG administration

led to remission in 65% of patients for a period of 1-8 months (41). However, subsequent clinical trials (45, 46) and epidemiology studies (47-49) could not repeat these findings. The reason for the negative findings may be due to insufficient dosing and the use of various strains. A case-control study from Turkey in 2012 found a protective effect of BCG on diabetes onset with at least two vaccinations, but not with a single vaccination, of BCG. Cases with three BCG vaccinations, especially one in infancy, had the greatest protection (50, 51). In the NOD mouse model, repeat BCG dosing is more effective than a single dose at diabetes prevention (52).

A proof-of-concept, double blind, placebo-controlled trial of repeat doses of BCG was conducted in established type 1 diabetes (mean duration: 15.3 years) (53). The trial showed that the two doses of BCG, delivered one month apart, triggered transient and very small restorations of stimulated pancreatic insulin activity measured by sensitive C-peptide assay (C-peptide, which is co-released with insulin by B-islet cells, is used as a marker for insulin production because it is unaffected by exogenous insulin administration). The study, which also measured three other biomarkers of BCG activity, sheds light on the mechanism of action of BCG and is discussed in the final section of this review.

2.2 Multiple Sclerosis

The use of BCG or immune adjuvants in animal models of multiple sclerosis traces back to the 1970s (54). But there were no studies in humans until 1999, with the publication of a Phase I clinical trial showing fewer MRI lesions in BCG-treated patients with multiple sclerosis (55). BCG was subsequently tested as a preventative treatment for multiple sclerosis in a double-blind, long-term phase II clinical trial (56). In the trial, 33 subjects with early symptoms of MS -- but not yet a definitive diagnosis -- were given a single dose of BCG, while another 40 subjects were given the placebo. Over the next six months, vaccinated subjects were significantly less likely to develop gadolinium-enhancing lesions and new and enlarging T2-hyperintense lesions. The number of T1-hypointense lesions was lower in the BCG group at 6, 12, and 18 months. At the end of 5 years, 58% of subjects who received BCG did not progress to MS, compared with 30% of those who received the placebo (56). No major adverse events were reported, and the frequency of all adverse events did not differ between treated and placebo groups. Based on the successful outcome of the Phase II trial, BCG is now being evaluated in a Phase III clinical trial (Table 2).

2.3. Sjogren's Syndrome

Sjogren's Syndrome, an autoimmune disease that targets and destroys exocrine glands such as salivary and lacrimal glands, affects up to 4 million people in the US. BCG administration restores salivary flow in NOD mice with advanced type 1 diabetes, which also display a Sjogren's-like syndrome (57). This animal study was an outgrowth of research on the pathophysiology of Sjogren's syndrome. Two decades ago it was established that NOD mice were deficient in a protein that forms one of the catalytic subunits of proteasomes, the organelles that degrade cytosolic proteins (58). The identical defect was later found in humans with Sjogren's syndrome (59). The defect renders proteasomes unable to perform two essential immune functions: the degradation of self-peptides for presentation on the MHC class I groove on the cell membrane, and the cleavage of transcription factor nuclear

factor kappa B (NFkB) from its chaperone protein in the cytoplasm (60). Based on the success of the animal study showing reversal of Sjogren's-like syndrome with BCG (57), it is being tested in humans with Sjogren's Syndrome in new, Phase I clinical trials at the NIH and in Norway (Table 2).

3. Infant Mortality and Hospitalizations

As early as 1927, a physician in Sweden observed that children receiving BCG at birth experienced a mortality rate that was nearly three times lower than that in unvaccinated children (61). Because the reduction in mortality was seen in the first year of life, yet tuberculosis kills mostly older children, the physician hypothesized that BCG triggers a "nonspecific immunity" for infectious diseases other than TB. In the 1940s and 1950s, several controlled trials in the US and the UK found that BCG vaccination was associated with fewer nonaccidental deaths from causes other than TB (62). Research in the modern era focused on third world countries where BCG is still routinely used. Several observational epidemiology studies from third world countries found positive non-specific effects of BCG immunization on childhood mortality (63-65). A retrospective epidemiological study from Spain found that BCG vaccination, which is still conducted at birth in the Basque region, was associated with a 40% decrease in hospitalization rates due to respiratory infections and sepsis compared to unvaccinated children in the other geographic regions of Spain where BCG was discontinued in 1982 (66). Two randomized controlled clinical trials were performed in West Africa, finding that low birth weight children vaccinated at birth with BCG had 40 percent lower mortality than low birth weight children with delayed vaccination (67, 68). The lowered mortality was from fewer neonatal respiratory infections other than TB and less sepsis. In West Africa, low birth rate babies are not ordinarily vaccinated until they reach normal birth weight, typically around 4 months of age (62).

The success of BCG against childhood mortality in the third world led to the initiation of a randomized clinical trial in a first world country, Denmark, which had discontinued BCG vaccination in the 1980s because of declining rates of TB. The Denmark trial is a multicenter study in which infants are randomized to be vaccinated with BCG within the first seven days of life or to receive no intervention (69). The primary study endpoint is hospitalization within the first 15 months of life. Data collection for this trial will be completed by the end of 2015.

BCG and Atopy Prevention

Atopy refers to the tendency to be "hyperallergic," typically presenting with one or more of the following: eczema (atopic dermatitis), allergic rhinitis (hay fever) or allergic asthma. Atopy is mediated by CD4 T helper (Th) cells-type 2 release of IL-4, which activates IgE antibodies, and release of IL-5, which activates eosinophils (70). In the past few decades, the rates of asthma and other atopic diseases have risen dramatically, particularly in developed nations. The hygiene hypothesis is an attractive explanation for the increase (71). BCG to prevent onset of atopy has been studied in animal models, epidemiology studies, and clinical trials.

In one of the first animal studies, Balb/c mice were given BCG two weeks before sensitization with ovalbumin. BCG blocked production of IgE/IgG1 antibodies, secretion of IL-4 and IL-10 by splenocytes, airway hyperresponsiveness, and eosinophilic influx

into the airway (72). In another animal model, intranasal BCG given four weeks before allergen challenge led to 90-95% reduction in lung eosinophilia and reduced IL-5 secretion by T cells draining the lymph nodes (73). Finally, neonatal Balb/c mice vaccinated with BCG revealed a significant reduction in airway inflammation, fewer eosinophils in lung fluid, and reduced serum ovalbumin-specific IgE levels (74).

The epidemiological evidence is mixed. One of the first studies of atopy did not find an effect; it was a retrospective cohort study of schoolchildren with atopic heredity who had received BCG younger than 6 months and were compared with unvaccinated age-matched controls (75). Since then numerous studies were performed, including a meta-analysis on 23 epidemiological studies of childhood asthma (10 cohort, 5 case-control, and 8 cross-sectional studies). It found that BCG was associated with an overall pooled OR of 0.86 (95% CI 0.79-0.93), leading the authors to conclude that exposure to BCG early in life prevents asthma (76).

But a subsequent meta-analysis, conducted five years later, found that the protective effect of BCG on development of asthma was only transient (protection was found through age 11, but not through ages 13-17). It also found no protective effect of BCG for sensitization, eczema, rhinoconjunctivitis or allergy (77).

Most clinical trials have uncovered a protective effect of BCG. In a double-blind, placebo-controlled trial of asthma patients, BCG induced improved lung function, decreased symptom scores, and reduced medication use (78). In a follow-up study by the same investigators, repeat BCG vaccination one year later was compared with single vaccination; repeat BCG showed greater lung function and a significant increase in the peripheral blood interferonγ/IL -4 ratio (indicating a shift to Th-1 responses (79). BCG was given in three different dosages and compared with saline in a randomized, double-blind controlled clinical trial of adult asthmatics with allergic rhinitis (80). The study found no differences for asthma symptom scores, use of bronchodilator, and blood IgE levels, but did find a decline in allergic rhinitis symptoms and less loratadine consumption that were temporary in all but the highest dose group. In the only negative trial, BCG was given to asthmatic schoolchildren in a placebo-controlled trial. Serum IgE levels did not change in the BCG group but rose in the placebo group. IL-4 and γ-interferon levels were unaltered, and most BCG-vaccinated patients exhibited the same asthma severity and number of emergency room visits as placebo patients (81). An ongoing randomized trial in Denmark of BCG at birth is evaluating the effects on wheezing, eczema as secondary outcome measures (69). Two other ongoing Phase I/II clinical trials are being conducted in Australia

5. Mechanism of Action

BCG's new application to autoimmune diseases, allergies, and non-tuberculosis respiratory diseases are traceable to its well-established role as an inducer of TNF (82). In animal models, TNF administration has been found to prevent the development of type 1 diabetes, lupus, and experimental autoimmune encephalomyelitis, a murine model of multiple sclerosis (60). But using TNF as a therapeutic or preventative agent in humans is problematic because of its systemic toxicity, largely due to widespread expression of the TNF Receptor 1 (TNFR1) (83). The TNF-inducer BCG is a safer alternative to direct administration of TNF. BCG, like TNF, has therapeutic value by

virtue of acting through at least two mechanisms: selective death of autoreactive T cells and induction of T regulatory (Treg) cells.

5.1 Selective Death of Autoreactive T Cells

The mechanistic rationale for BCG's use in autoimmune disease stems from the paradox that anti-TNF therapies, which are widely used in treating rheumatoid arthritis and several other autoimmune diseases, trigger or exacerbate other forms of autoimmunity (60). This paradox led us to hypothesize that the opposite therapeutic strategy – the administration of TNF – may be beneficial based on its selective death of autoreactive but not normal T lymphocytes (T cells) in blood of patients with type 1 diabetes, multiple sclerosis, lupus, psoriasis, Crohn's disease and Grave's disease (84, 85). Autoreactive T cells, but not normal T cells, are selectively vulnerable to TNF-induced apoptosis as a result of genetic or functional defects that prevent the transcription factor NFkB from entering the nucleus to express pro-survival genes (60, 85). Genetic and protein processing defects in the NFkB pathway are found in type 1 diabetes, Sjogren's syndrome, lupus, Crohn's disease, rheumatoid arthritis, scleroderma, and ulcerative colitis (86). In the case of the NOD mouse and humans with Sjogren's syndrome, there is reduced or absent expression of the LMP2 subunit of the proteasome (59, 84). Defective proteasomes are unable to cleave NFkB in the cytoplasmα. From its chaperone molecule IkB T cells are selectively vulnerable to TNF-induced cell death because, unlike B lymphocytes and monocytes, they do not constitutively express the active form of NFkB (87). NFkB normally protects against TNF-induced cell death by inducing expression of pro-survival genes (60).

There is now evidence directly linking BCG to selective death of autoreactive T cells. In the NOD mouse model, CFA selectively triggers death of autoreactive T cells (43, 44). In a human clinical trial, BCG-treated type 1 diabetic patients displayed massive death of insulin autoreactive T cells, as detected by flow cytometry (53). Interestingly, massive death of insulin autoreactive T cells in this trial also was detected in a placebo patient that turned out to have been infected with Epstein-Barr Virus (EBV). EBV infection, like BCG, triggers robust release of TNF (6, 88). But unlike BCG, EBV infections are limited, and the added benefit of BCG may be the long-term synergy with the host.

5.2 Induction of T regulatory Cells

BCG may be therapeutic for autoimmune diseases, allergy and infectious diseases by another mechanism, the induction of Treg cells. Tregs are a rare subset of CD4 T cells with roles in prevention of autoimmunity by maintaining self-tolerance, and by suppression of allergy and asthma and pathogen-induced immunopathology (89). Genetic elimination of Tregs triggers massive autoimmunity (90, 91).

In humans with type 1 diabetes, BCG administration induced proliferation of Tregs in a phase 1 clinical trial (53). The mechanisms underlying this finding are being studied, and may occur only during the quiescent phase of BCG infection. During that phase, BCG-infected macrophages secrete both soluble and transmembrane TNF (92-94), the two active forms of TNF. Transmembrane and soluble TNF trigger distinct signaling pathways (95). Transmembrane TNF is the primary ligand for Treg proliferation by virtue of its preferential binding to TNFR2 (96), which

is highly expressed on Tregs. TNFR2 induces proliferation of Tregs that are highly suppressive (97, 98). Using a TNFR2 agonist, TNFR2 was identified as a master control switch on Tregs, triggering their homogeneous expansion rather than heterogeneous expansion through TNFR1 (99). The TNFR2 receptor in CD4 T cells activates the NF-kB pathway through TRAF2/cIAP, yielding transcription of pro-survival genes (100). Additionally, agonism of TNFR2 through either agonistic antibodies or through transmembrane TNF induces IL2R (CD25), TNF, and TRAF2 expression, all of which are elements of the TNFR2 signaling pathway for Treg expansion (99).

Conclusion

BCG is showing promise for widespread applications beyond its roles as a safe and effective vaccine for TB and as treatment for bladder cancer. Its new uses include autoimmune disease, atopy, and infectious disease. Considerable animal, epidemiological, and clinical trial evidence reviewed here supports these new applications. Fueling these applications is a burgeoning knowledge of how BCG exerts its therapeutic effects. The mechanisms underlying these applications involve BCG's role as an inducer of TNF, which is selectively toxic to autoreactive T cells and which triggers expansion of Treg cells.

Conflicts of Interest

The authors report no conflicts.

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