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REVIEW ARTICLE

Landscape of immunobiological properties of BCG: from vaccine vector to immunotherapy

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Highlights

- BCG is a versatile microorganism as immunobiological agent;
- The protective and non-specific effects of BCG are attributed to its ability to induce trained immunity;
- The BCG immunological properties are responsible for its extensive area of application and its promising results.

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KEYWORDS
Trained immunity; Heterologous immunity; Recombinant BCG; tuberculosis; Bladder cancer; Melanoma.

Abstract: Bacille Calmette-Guérin (BCG) is an attenuated strain of *Mycobacterium bovis*. It is derived from 231 *in vitro* passages conducted at the Pasteur Institute of Lille, France. The first BCG vaccination was performed 100 years ago, and to this day, it remains the only licensed vaccine against tuberculosis (TB). Although it was developed exclusively for conferring protection against tuberculosis, BCG has also shown beneficial effects in several other therapeutic roles, including its use as an immunotherapeutic agent for superficial urothelial carcinoma and melanoma skin cancer. It has also demonstrated potential application as a vaccine vector for the delivery of heterologous antigens and immunological mediators, such as cytokines. Thus, the development of recombinant strains of BCG (rBCG) have been widely explored. Additionally, BCG has demonstrated potential application as an immunotherapeutic agent for autoimmune, allergic, and neurodegenerative diseases, which has been attributed to the induction of memory in innate immune cells (a.k.a. trained immunity). In this review, we provide an update on the immunobiological and immunological aspects involved in bacillus-host interactions in the context of BCG.

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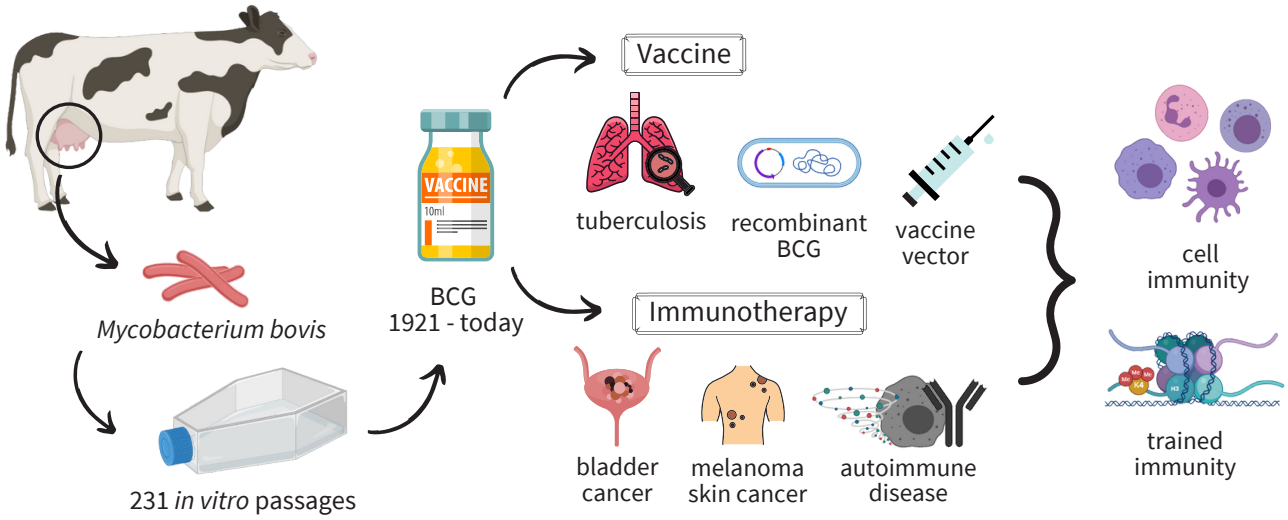
Graphical abstract



Introduction

The attenuated Bacillus Calmette-Guérin (BCG) is derived from the *Mycobacterium bovis* strain through *in vitro* passages made by Albert Calmette and Camille Guérin. In 1921, BCG was first administered to humans and proved to be protective against tuberculosis (TB). It remains to be the only licensed vaccine against TB, and is the most widely used vaccine globally (Abdallah & Behr, 2017). Currently, BCG is also indicated as an immunotherapeutic agent against non-muscle invasive bladder cancer (NMIBC) and melanoma skin cancer; however, its response rates vary and it also evokes side effects in recipients (Larsen et al., 2020). To improve its effectiveness against these diseases, the development of recombinant BCG strains (rBCG) has been extensively explored. Moreover, with the aim of developing novel human and veterinary vaccines, rBCG strains have been used as vectors for expressing heterologous antigens (Nieuwenhuizen & Kaufmann, 2018).

Moreover, BCG has been demonstrated to have non-specific immunostimulatory effects, which reduce overall infant mortality and improve the response to other non-target diseases (Moorlag et al., 2019). These effects are attributed to its ability to induce trained immunity, a mechanism that is strongly driven by epigenetic and metabolic reprogramming of innate immune cells (Divangahi et al., 2021; O'Neill & Netea, 2020). Considering the potential of BCG and rBCG, here, we reviewed their immunological properties and their use as immunobiological agents.

The immune landscape of bacillus-host interaction

Innate immunity

Growing evidence suggests that plants and invertebrates exhibit adaptive immunological characteristics that can be primed by an initial infection, which leads to better protection against subsequent infections (Divangahi et al., 2021). This process resembles the immunological memory of the innate immune cells. Trained immunity, as it is known, is controlled by the mechanisms that have lower specificity and last for a shorter duration compared to the adaptive immune response. Both immune responses fulfill the same goal, which is to provide a faster and stronger response against the pathogens to improve the host survival (Covián et al., 2019; Netea et al., 2020).

The cellular mechanism that underpins BCG-induced trained immunity involves epigenetic reprogramming, including changes in histone acetylation and methylation (Covián et al., 2019; Khader et al., 2019). Kaufmann et al. (2018) demonstrated that BCG reprograms the hematopoietic stem cells (HSCs) in the bone marrow of a mouse model of TB, to initiate myelopoiesis in an interferon-gamma (IFN-γ)-dependent manner. In peripheral blood monocytes, BCG increased the modification of histone H3 with tri-methylation in the fourth lysine residue (H3K4me3), which is associated with the promoters of the genes encoding tumor necrosis factor-alpha (*TNF-a*), interleukin 6 (*IL-6*), and toll-like receptor 4 (*TLR4*), leading to the transcriptional activation of these genes (Covián et al., 2019).

In the context of trained immunity, epigenetic reprogramming is strongly controlled by the cellular metabolic state (Netea et al., 2020; Penkov et al., 2019). The metabolic processes are precisely regulated in immune cells: during resting, monocytes and macrophages recover their energy through oxidative phosphorylation. When exposed to inflammatory stimuli, these cells convert glucose to lactate even in the presence of oxygen, through a mechanism known as the Warburg effect (Penkov et al., 2019). Key metabolites in this pathway can induce rearrangements in the chromatin, increasing the H3K4me3 modifications in *TNF-a* and *IL-6* promoters, which in turn leads to the enhanced secretion of these cytokines. BCG reportedly changes the metabolic reprogramming of the cell (Covián et al., 2019); therefore, metabolic and epigenetic reprogramming together

mediate trained immunity in a mutually dependent manner (Liu et al., 2020).

Humoral immunity

The humoral immune response induced by BCG is considered to be of low relevance in mediating protection against TB. However, a study performed on South African babies vaccinated with BCG showed that specific IgG levels against the Ag85A antigen were associated with a reduced risk of TB (Fletcher et al., 2016). There is also evidence that BCG may increase the production of antibodies in response to unrelated vaccinations during childhood; thus, the BCG vaccine also acts as an adjuvant. Previous or concomitant administration of BCG is associated with significantly higher levels of antibodies against polio type-1, pneumococcal, and influenza viruses (Zimmermann & Curtis, 2018). However, it is important to note that the methodology, host, dosage, and strain of BCG may influence these results. Therefore, the relevance of the humoral response in the BCG-induced protection remains unclear (Tanner et al., 2019).

Cellular immunity

BCG induces a cellular response involving both $CD4^+$ and $CD8^+$ T lymphocytes that mediate cytotoxic activity. In individuals, called responders with high inflammation, BCG response is characterized by polyfunctional $CD4^+$ T cells expressing IL-2, TNF-α, and IFN-γ, and by $CD8^+$ T cells expressing IFN-γ, whereas in responders with low inflammation, the cytokine response is almost absent and is accompanied by the induction of $CD8^+$ regulatory T cells ($CD8^+$ T_{reg}) (Boer et al., 2015). The induction of $CD8^+$ T_{reg} cells over time may be attributed to the long persistence of BCG as a live intracellular bacterium after vaccination (Boer et al., 2014).

IL-10 plays a critical role in BCG efficacy. After primary BCG vaccination, *Il-10* knockout mice show higher IFN-γ, TNF-α, and IL-6 production, which is associated with a higher proportion of IFN γ^+ $CD3^+$, IFN γ^+ $CD4^+$, and IFN γ^+ $CD8^+$ T cells in the spleen. These downregulated T_{reg} responses in IL-10 deficient mice boosted the dendritic cell activation via upregulating surface molecule expression, which resulted in better protection against BCG challenge (Xu et al., 2019). Interestingly, BCG revaccination in young adults boosted circulating frequencies of *Mycobacterium tuberculosis* (*Mtb*)-specific T helper 17 (Th17) $CD4^+$ T cells via IFN-γ and/or IL-2, Ag85A- and BCG-specific $CD4^+$, and $CD8^+$ T cell responses (Rakshit et al., 2019). Specific Th17 responses are characterized by the increased level of Ag85A-, BCG-, and LTAg-specific total IL-$17A^+$, IL-$17F^+$, IL-22^+, and IL-10^+ $CD4^+$ T cell effectors. These results support the hypothesis that the BCG revaccination may expand the pre-existing memory of the T cells and boost the T cell responses through immunomodulation of anti-inflammatory effects, with $CD4^+$ T_{reg}s helping to maintain the balance between pro- and anti-inflammatory $CD4^+$ T-cell responses.

In addition to these mechanisms, BCG was also observed to be capable of stimulating neutrophils to form neutrophil extracellular traps (NETs) both *in vitro* and in a mouse model (Liu et al., 2019; Sun et al., 2020). This process was shown to be involved in suppressing the development of bladder tumors in mice that received intravesical instillation of BCG (Sun et al., 2020).

The versatility of BCG as an immunobiological agent

BCG as a vaccine against tuberculosis

Currently, BCG is still the only licensed vaccine to prevent the development of active TB. Globally, more than 90% of newborns are vaccinated and more than 120 million doses of BCG are administered annually (Abdallah & Behr, 2017). The World Health Organization (WHO) recommends the administration of a single dose of BCG for newborns in regions where TB is endemic or where there is a high risk of exposure to the pathogen. Despite this, each country has its own vaccination policy, and according to WHO-UNICEF (World Health Organization, 2020), the BCG coverage in countries that practice regular vaccination is between 25–99%, which can be partially attributed to the variable effectiveness of the BCG vaccine.

The heterogeneity in the efficacy of BCG has been supported by the blocking and masking hypotheses (Fatima et al., 2020) as well as the genetic variability among BCG strains (Abdallah & Behr, 2017). Interestingly, Trauer et al. (2021) indicated that the large variation in the efficacy of BCG is explained by the timing of *Mtb* exposure. Exposure to the pathogen shortly after receiving the vaccination resulted in better protection, whereas later exposure did not show significant evidence of protection. In addition, the BCG administration route influenced its efficacy.

In a clinical trial (Hoft et al., 2018), healthy adults without known TB exposure risks or immunosuppression were vaccinated with BCG orally (PO) and/or intradermally (ID). In both administration routes, BCG induced systemic Th1 responses with IFN-γ production. The ID route induced a stronger systemic Th1 response, while the PO route induced a stronger mucosal response (TB-specific secretory IgA and bronchoalveolar lavage T cells). These results suggest that the combination of ID and PO vaccinations can be used to induce a synergistic systemic and mucosal immune response against TB.

In a recent study (Darrah et al., 2020), rhesus monkeys that were vaccinated with intravenous (IV) BCG were protected against TB. The IV route led to a large infiltration of T cells in the lungs compared to the administration via the ID and aerosol routes. Memory T cells could still be observed six months after the vaccination when the animals were exposed to *Mtb*. The explanation for this rapid influx and expansion of T cells may be due to the IV route, which leads to the delivery of a high BCG dose to the lungs (Dijkman et al., 2019; Fatima et al., 2020). Despite the heterogeneity in the efficacy of BCG, its safety and immunogenicity support its potential use as an immunobiological agent for the development of new vaccines and for the prevention and treatment of other non-targeted diseases.

BCG as an oncological immunotherapy

Both the European Association of Urology and the American Urological Association recommend the administration of intravesical instillation of BCG as the most effective therapy for patients with an intermediate and a high risk of NMIBC. BCG-positive response rates are 55–65% for Ta and T1 and 70–75% for *Tis* tumors, indicating that 30–45% of patients fail to respond to this therapy. This failure can be associated with the insufficient or excessive BCG instillations, generation of an inadequate immune response, and invasive disease or occult metastasis (Lebacle et al., 2021). In a recently published phase 1b clinical trial (Rosser et al., 2021), patients with BCG-naïve NMIBC were treated with increasing doses of N-803 (an IL-15 superagonist), which was administered in combination with BCG. Six years after the treatment, 100% of the participants were disease-free and demonstrated prolonged antitumor activity. Despite this, due to recurrent reports of side effects and non-responsive patients, rBCG development has been proposed to improve the efficacy and tolerability of bladder cancer therapy.

Additionally, BCG has been the best-known agent for intralesional therapy in inoperable stage III in-transit melanoma cases and is a recommended therapeutic option by the National Comprehensive Cancer Network (NCCN). BCG therapy is a relatively inexpensive option for inoperable cutaneous metastatic melanoma, with high response rates of approximately 80% (Lardone et al., 2017). BCG can be administered alone or in combination with an autologous tumor cell vaccine or intralesional drug, resulting in tumor regression. However, the strain, dose, or time interval for BCG therapy have not been standardized (Benitez et al., 2019).

BCG as a vector for heterologous antigen expression

Live vaccines with recombinant vectors are potential alternatives to increase the protection range and sustained stimulation of the immune system. The use of BCG as a vector for recombinant expression of heterologous antigens has been tested for several pathogens (Dorneles et al., 2020; Goulart et al., 2017; Soto et al., 2018). Goulart et al. (2017) developed rBCG strains that are capable of inducing an effective immune response and protecting against sepsis caused by *Streptococcus pneumoniae*. Some rBCG strains have also been tested in animal models against the human respiratory syncytial virus (hRSV) and human metapneumovirus (hMPV), which are pneumoviruses that affect the lower respiratory tract of young children, the elderly, and immunocompromised patients. Soto et al. (2018) developed rBCG strains expressing the hRSV nucleoprotein (rBCG-N) and the hMPV phosphoprotein (rBCG-P), demonstrating their potential to induce neutralizing antibodies and protect mice from viral replication and lung disease. A rBCG strain has also been evaluated against parasites, such as *Trypanosoma cruzi*, responsible for the Chagas disease. Bontempi et al. (2020) developed a vaccine using the BCG Pasteur strain expressing the N- terminal portion of the enzyme trans-sialidase (NT-TS). BALB/c mice vaccinated with rBCG expressing NT-TS demonstrated 80% of protection with an immune response orientated towards Th1/Th17, indicating that rBCG is a promising platform against *T. cruzi* (Bontempi et al., 2020).

A vaccination strategy based on rBCG was also tested against leptospirosis. Oliveira et al. (2019) developed a chimera containing the antigenic portions of the proteins LigAni, LemA, and LipL32, which were expressed by BCG under the control of different mycobacterial promoters. The chimeric rBCG was capable of providing significant protection and sterilizing immunity in hamsters. The four portions of this chimera were individually cloned into the BCG to assess which one would be involved in the protection against leptospirosis. The results demonstrated 100% protection against leptospirosis and all rBCG strains significantly prevented renal colonization by *Leptospira* bacteria (Dorneles et al., 2020).

BCG as an immunobiological agent to combat non-targeted diseases

Several studies have demonstrated that BCG has beneficial and non-specific protective effects against non-target diseases (Moorlag et al., 2019; Netea et al., 2020). In a phase I randomized clinical trial, two doses of intradermal BCG reduced the HbA1c levels (an indicator of blood glucose levels) to an almost normal range, for up to eight years in long-standing type-1 diabetes patients (Kühtreiber et al., 2018). The reduction of HbA1c levels was related to the induction of T_{reg} cells, death of cytotoxic T lymphocytes that attack the cells of the pancreatic islets, and immunometabolism modulation (Kühtreiber & Faustman, 2019). BCG, which was prepared by extended freeze-drying, also attenuated the severity of experimental autoimmune encephalitis (EAE) in a mouse model, by reducing the infiltration of $CD45^+$ cells in the spinal cord and reducing T_{reg} cells in secondary lymphoid organs (Lippens et al., 2018). The potential of using BCG against non-related viral infections was also explored in a randomized placebo-controlled human challenge study, using yellow fever vaccine as a model of experimental viral infection in humans. The BCG vaccination induced epigenetic reprogramming of monocytes, being highly correlated with the reduction of viremia. The immune responses were mediated by IL-1β, suggesting the induction of trained immunity, and indicating a correlate of protection against a non-related viral infection (Arts et al., 2018).

Considering these non-specific protective effects, several randomized clinical trials are underway to evaluate BCG as a potential measure in the global fight against COVID-19. Clinical trials are being conducted to evaluate whether the BCG vaccination can protect healthcare professionals from COVID-19 and prevent severe SARS-CoV-2 infection in older people. Finally, a study in Germany is testing whether VPM1002, an rBCG vaccine, can protect both healthcare workers and older people against COVID-19 (Redelman-Sidi, 2020). Theoretically, the induction of BCG-trained immunity in healthy individuals should increase antimicrobial defense, inhibit viral replication, reduce viral load, decrease systemic inflammation, and thus reduce the morbidity and mortality associated with SARS-CoV-2 infection (Moulson & Av-Gay, 2021; O'Neill & Netea, 2020).

Conclusions

The use of BCG has demonstrated the versatility of this microorganism as an immunobiological agent. It is a century-old vaccine that was initially developed to be used against TB, but more recently, it has also been used as an effective immunotherapeutic agent for bladder and melanoma cancers. Additionally, BCG is considered to be a potential vaccine vector for the delivery of heterologous antigens. The development of rBCG strains can further improve this microorganism's effectiveness in these applications. Using BCG-induced trained immunity can be an important approach to offer better protection in newborns, the elderly, and immunocompromised individuals, who are otherwise incapable of developing specific and robust adaptive immune responses.

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