Cellular and Molecular Life Sciences (2026) 83:230
https://doi.org/10.1007/s00018-026-06173-6

REVIEW

Immunological mechanistic action of intravenous BCG-mediated protection against tuberculosis

Sangwon Choi[1] · Jiyun Park[1] · Jung Joo Hong[2,3] · Sung Jae Shin[1,4,5] · Ju Mi Lee[1,4]

Received: 13 September 2025 / Revised: 1 February 2026 / Accepted: 2 March 2026

Abstract

The Bacillus Calmette-Guérin (BCG) vaccine, the only licensed vaccine against tuberculosis (TB), has played a crucial role in mitigating severe manifestations of the disease, particularly in children. However, its effectiveness against pulmonary TB in adults remains variable, largely due to its limited ability to elicit a sustained antigen-specific T_H1 immune response and to generate long-lived tissue-resident memory T cells (T_{RM}) within the lungs. Consequently, innovative strategies that address these immunological shortcomings are needed to enhance BCG immunogenicity and efficacy against pulmonary TB. Recent studies on intravenous (IV)-BCG have emerged as a promising alternative, achieving near-sterilizing immunity in preclinical models such as nonhuman primates by strengthening pulmonary defenses against *Mycobacterium tuberculosis* (Mtb). This review examines how IV-BCG enhances BCG efficacy through a synergistic network of immunological factors, including trained immunity, effector T_H1 cells, lung T_{RM}, inducible bronchus-associated lymphoid tissue (iBALT), and antibody responses. Systemic delivery of IV-BCG induces durable trained immunity, primes robust T_H1 and T_{RM} responses in the lungs, may foster iBALT formation for localized protection, and enhances antibody production to reinforce humoral defenses. Drawing on data from preclinical studies, this review highlights how these components interconnect to sustain long-lasting pulmonary protection and offers insights into optimizing BCG-based TB vaccines.

Keywords Tuberculosis · Intravenous BCG · Trained immunity · T_{RM} · iBALT · Antibody responses

Introduction

Tuberculosis (TB), caused by *Mycobacterium tuberculosis* (Mtb), remains a persistent global health crisis, with an estimated 10.8 million new cases and 1.25 million deaths in 2023, underscoring the urgent need for effective preventive measures [1]. The Bacillus Calmette-Guérin (BCG) vaccine, introduced in 1921, is the only licensed TB vaccine and has been highly effective in preventing severe forms of childhood TB, such as meningitis and disseminated disease [2]. Despite this success, its efficacy against pulmonary TB in adolescents and adults, the primary drivers of Mtb transmission, remains highly variable, ranging from no measurable protection to up to 80% across different populations and settings [3, 4].

This limited efficacy underscores the fundamental immunological limitations of the intradermal (ID) BCG vaccine. Because current ID-BCG vaccines are localized, they do not consistently elicit long-lasting antigen-specific T-helper 1 (T_H1) responses, generate durable tissue-resident memory T cells (T_{RM}) in the lungs, and mount robust

Sangwon Choi and Jiyun Park contributed equally to this work.

✉ Sung Jae Shin
sjshin@yuhs.ac

✉ Ju Mi Lee
jmlee@yuhs.ac

1 Department of Microbiology, Graduate School of Medical Science, Brain Korea 21 Project, Yonsei University College of Medicine, Seoul, South Korea

2 National Primate Research Center, Korea Research Institute of Bioscience and Biotechnology (KRIBB), Cheongju, Chungcheongbuk, South Korea

3 KRIBB School of Bioscience Korea University of Science & Technology (UST), Daejeon, South Korea

4 Institute for Immunology and Immunological Disease, Yonsei University College of Medicine, Seoul, South Korea

5 Department of Biomedical Sciences, Yonsei University College of Medicine, Seoul, South Korea

Published online: 16 April 2026

antibody-mediated immunity [5–7]. Because pulmonary TB prevention requires rapid on-site effector responses and durable system-wide memory, novel strategies are needed to overcome these limitations.

Intravenous (IV) BCG delivery has been identified as an appealing next-generation platform over the past few years. High-dose IV-BCG immunization can trigger nearly sterilizing immunity toward Mtb and boost the protection of the lungs dramatically compared with the ID delivery method, as suggested by preclinical studies within non-human primate (NHP) models [8–11]. Unlike ID administration, which largely confines immunological processes to local draining lymph nodes [12], IV delivery facilitates systemic immune responses to the vaccine. This unique route enables direct access to the bone marrow to induce trained immunity and seeds the lung parenchyma with high frequencies of effector cells, thereby establishing a multi-layered defense system that conventional routes fail to achieve [8, 13]. According to mechanistic studies, IV-BCG strengthens the innate and adaptive arms of host defense by inducing trained immunity, increasing antibody production, and activating dendritic cells (DCs) to prime strong T_H1 and T_{RM} responses in the lungs.

Therefore, this review focuses on the immunological mechanisms by which IV-BCG enhances vaccine efficacy, with particular emphasis on the interplay between antigen-presenting cells, effector T_H1 cells, lung-resident T_{RM} populations, antibody function, and trained immunity. By integrating findings from recent preclinical studies, we aim to provide insights into how IV-BCG can serve as a foundation for developing optimized BCG-based vaccines capable of providing durable protection against pulmonary TB.

BCG vaccination routes

The protective efficacy of BCG depends significantly on the route of administration. While ID administration is currently the only approved route, the emerging mucosal and IV routes of administration produce different immune responses. Figure 1 illustrates the distinct outcomes resulting from different BCG administration routes.

ID-BCG vaccination: established but incomplete protection

ID-BCG is currently the only approved vaccination route, administered to approximately 120 million newborns annually [1]. ID-BCG administration protects children against disseminated TB and meningitis by inducing systemic T cell responses, particularly IFN-γ-producing T_H1 cells [14].

ID-BCG vaccination generates circulating effector T cells and central memory T cells, but these cells are rarely located within the lung parenchyma [7]. Consistent with this, an intravascular staining study in mice demonstrated that more than 95% of $CD4^+$ T cells isolated from lungs after ID-BCG vaccination resided within the blood vessels, with only a small fraction present in the parenchyma. This suggests minimal influx of protective T cells into the tissues [15]. Furthermore, ID-BCG has been reported to weakly activate alveolar macrophages and airway-resident DCs, which are essential surveillance cells for early mucosal defense [15, 16]. This deficiency also delays T cell recruitment, impairs granuloma formation, and suboptimal early bacterial control after Mtb infection [17].

Consequently, ID-BCG provides variable and suboptimal protection against adult pulmonary TB, and its efficacy can be minimal depending on geographic and epidemiological factors [18, 19]. Another limitation is the insufficient establishment of immune surveillance in the respiratory mucosa, which constitutes the primary site of Mtb entry and replication.

Recent data further indicate that high-dose IV-BCG profoundly enhances immune signaling within the airways, with upregulation of IFN-γ, TNF, IL-17, and CXCL9/10 in the bronchoalveolar compartment, alongside increased myeloid–lymphoid crosstalk [10]. This suggests that IV-BCG not only seeds protective T cells but also reprograms the airway microenvironment into a highly activated immune niche, reinforcing its superior protective efficacy. In addition, IV-BCG sustains germinal center reactions within secondary lymphoid organs, leading to the formation of long-lived plasma cells and memory B cells that secrete high-affinity IgG antibodies [43]. IV-BCG may also promote tertiary lymphoid structures such as iBALT, although this possibility remains speculative and requires further validation [8, 44, 45].

Beyond adaptive responses, IV-BCG imprints durable trained innate immunity. By disseminating to the bone marrow, BCG directly educates hematopoietic stem and progenitor cells, leading to long-term epigenetic and transcriptional reprogramming that biases their progeny monocytes and macrophages toward heightened cytokine responsiveness [13]. These stem cell-level changes, together with classical histone modifications such as H3K4me3 at inflammatory loci [28], provide a mechanistic explanation for the persistence of trained immunity months to years after vaccination. Importantly, this mechanism not only enhances Mtb control but also confers heterologous protection against unrelated pathogens through a process recently defined as "integrated organ immunity" in which antigen-specific $CD4^+$ T_H1 cells deliver IFN-γ feedback to lung myeloid and epithelial cells, imprinting prolonged innate antiviral resistance via MyD88-dependent interferon-stimulated gene activation [46].

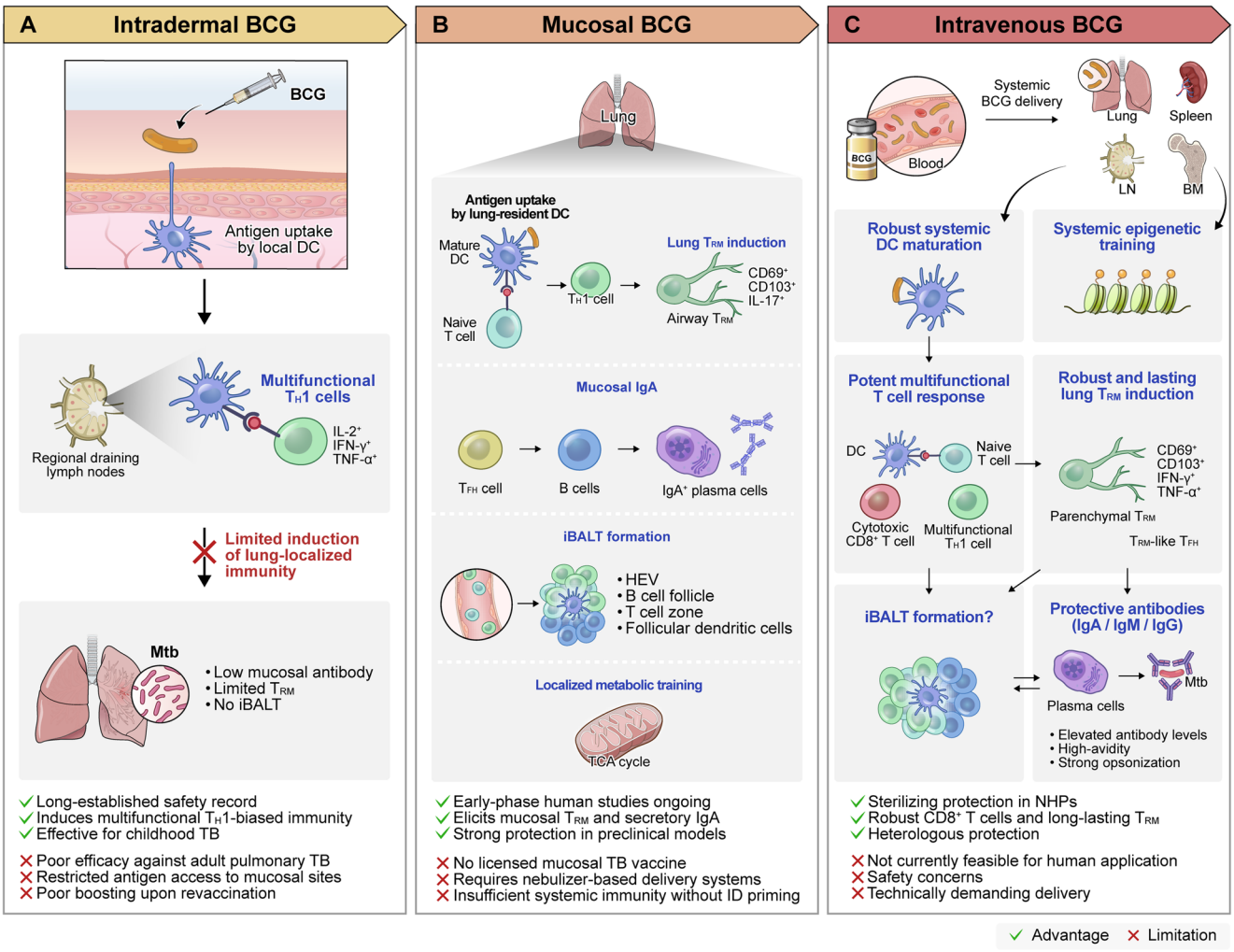

Fig. 1 Distinct immunological outcomes following intradermal, mucosal, and intravenous BCG. The protective efficacy of BCG is highly dependent on the route of administration. Differences in antigen uptake, DC priming, mucosal seeding of T_{RM}, antibody induction, iBALT formation, and trained innate immunity underlie the divergent outcomes of intradermal, mucosal, and intravenous BCG. Arrows indicate directionality of trafficking or signaling; green ticks denote advantages, and red crosses denote limitations. (**a**) Intradermal BCG. The conventional intradermal route enables antigen uptake by skin-resident DCs and subsequent priming of multifunctional T_H1 cells in the draining lymph nodes. This generates systemic T_H1-biased immunity but confers minimal induction of lung-localized responses. Consequently, intradermal BCG elicits only low levels of mucosal antibodies, induces few T_{RM} in the respiratory tract, and fails to promote iBALT formation. (**b**) Mucosal BCG. Direct deposition in the respiratory tract promotes antigen uptake by lung-resident DCs, leading to T_{RM} formation in the airway, T_{FH}–dependent germinal center activity, and mucosal IgA secretion. Mucosal BCG also promotes iBALT formation, which sustains local immune responses through germinal centers and HEVs. In addition, mucosal delivery induces trained immunity via metabolic reprogramming of myeloid cells, including enhanced mitochondrial activity and TCA cycle flux, thereby augmenting innate antimicrobial function. Preclinical and early clinical studies demonstrate strong mucosal protection; however, systemic immunity remains limited, specialized delivery devices are required, and no licensed mucosal BCG product currently exists. (**c**) Intravenous BCG. Systemic administration results in broad dissemination of BCG to multiple lymphoid and non-lymphoid organs, driving robust DC maturation and systemic epigenetic training of innate immunity. This route elicits potent multifunctional T-cell responses, including cytotoxic $CD8^+$ T cells, and establishes durable lung T_{RM} populations with strong effector function. Intravenous BCG further promotes the generation of high-avidity antibodies across multiple isotypes (IgA, IgM, and IgG), thereby enhancing opsonization and bacterial clearance. Notably, intravenous vaccination has achieved sterilizing immunity in non-human primates, mediated by long-lived T_{RM} and protective antibody responses. Despite its remarkable preclinical success, safety concerns and procedural complexity currently preclude its direct translation to humans, highlighting the need for innovative approaches that can capture the protective features of intravenous BCG while ensuring feasibility and safety. BCG, Bacillus Calmette-Guérin; DC, dendritic cell; T_H1, T helper 1 cell; T_{FH}, T follicular helper cell; T_{RM}, tissue-resident memory T cell; HEV, high endothelial venule; iBALT, inducible bronchus-associated lymphoid tissue; IgA, immunoglobulin A; TCA, tricarboxylic acid

Despite these advantages, IV-BCG faces significant challenges for clinical translation in its current form. High-doses of live bacteria administered systemically can raise serious safety concerns, including spleen hypertrophy, systemic inflammation, and disseminated BCGosis, especially in immunocompromised hosts [8, 47, 48]. Moreover, the use of IV-BCG administration for neonatal and infant subjects is not feasible. Variability among species also complicates interpretation. While NHP studies suggest near-complete protection, findings in mouse models are variable and the appropriate human dosage remains unknown [8, 49]. Compounding this uncertainty, certain high-dose regimens have been observed to overstimulate the immune system, leading to tolerance or adverse pathology [10]. Together, these issues highlight key limitations to the safe and effective clinical application of IV-BCG.

For these reasons, IV-BCG should be regarded primarily as a biological benchmark. By uncovering the immune correlates of near-complete protection, massive T_{RM} accumulation, durable germinal center activity, airway-specific immune activation, and long-lived trained immunity, IV-BCG provides a blueprint for rational vaccine design. Current research is focused on IV-mimetic platforms, such as recombinant vectors, liposomal or nanoparticle formulations, and heterologous prime–boost strategies, that aim to recapitulate the immunological benefits of IV-BCG while improving safety, scalability, and regulatory feasibility [50–52]. Combinatorial approaches that integrate ID priming, mucosal boosting, and IV-mimetic platforms may ultimately define the next generation of BCG vaccination strategies.

Mucosal BCG vaccination: enhancement of lung-specific immunity

The conventional ID-BCG vaccination has limitations in establishing robust mucosal immunity. To overcome this, various mucosal administration routes, such as intranasal, intratracheal, and aerosol, are being actively studied as alternatives. Mucosal vaccines directly target the respiratory tract and effectively enhance local lung immunity. In fact, studies in mouse and NHP models have shown that mucosal administration exhibits superior protective effects compared to ID vaccination, reducing the bacterial burden in the lungs by approximately 1.1–1.3 $\log_{10}$ CFU [7, 20, 21]. Unlike ID-BCG, mucosal vaccination induces systemic immunity and establishes a multilayered, localized protective barrier in the lungs. One of its key features is the formation of T_{RM}, the first to respond when infection occurs again, enabling rapid on-site immune activation to suppress bacterial proliferation [7]. T_{RM} have been reported to cooperate with $CD4^+$ T cells that produce IL-17 to promote rapid neutrophil influx and strengthen the airway epithelial barrier, effectively suppressing the spread of pathogens in the early infection stage [22–25]. Furthermore, mucosal BCG administration promotes the generation of inducible bronchus-associated lymphoid tissue (iBALT), in which lymphocytes present antigens and are activated directly at the site of infection without going through lymph nodes (LNs), rapidly mobilizing effector T cells while also promoting mucosal IgA formation, effectively blocking the initial invasion of pathogens. Previous studies of oral BCG have also identified mucosal IgA and lung-resident lymphocytes as important protective factors [26, 27].

The rapid adaptive immune response is further enhanced by the trained immunity induced by mucosal BCG. Compared to ID-BCG vaccination, mucosal BCG induces a more robust functional reprogramming of innate immune cells, enhancing cytokine responsiveness and strengthening the link between innate and adaptive immunity [28]. Separately, recombinant strains such as rBCG-PPE27, exhibit a broader range of bacterial defenses than conventional BCG by altering alveolar macrophage metabolism through the mTORC2/HK1 pathway [29].

Mucosal BCG establishes a distinct surveillance system within the lungs, centered on T_{RM}, iBALT, and IgA, which can function complementarily with ID-BCG, which focuses on systemic immunity. Therefore, a prime-boost strategy, which induces primary immunity through ID-BCG vaccination and then enhances it through the mucosal route, is an immunologically sound approach [8, 30, 31].

Nevertheless, there are numerous hurdles to overcome before clinical application. Major safety concerns, particularly in infants and immunocompromised patients, include the risk of aspiration, local inflammation, and inconsistent delivery doses [32]. Furthermore, the establishment of standardized delivery devices and quality control and regulatory systems is essential [33]. Current clinical trials still lack data on long-term safety and immune sustainability [34]. Therefore, applying mucosal BCG vaccination to clinical and public-health settings will require detailed translational studies and rigorous safety verification.

IV-BCG vaccination: the standard for bactericidal protection

Due to the limitations of ID- and mucosal BCG vaccinations, IV-BCG administration has attracted attention as the most innovative progress in TB vaccine research (Table 1). In NHPs, a single IV dose of BCG achieved an unprecedented level of protection. Specifically, in the landmark study by Darrah et al., 9 out of 10 macaques were highly protected following low-dose aerosol challenge with virulent Mtb (Erdman strain, ~10 CFU) administered 6 months post-vaccination. Most notably, 6 of these 10 animals achieved

Table 1 Summary of IV-BCG examples and major mode of action against Mtb infection

Studies	Key Findings	Detailed Mode of Action	Outcomes	Ref.
Mice (C57BL/6)	IV-BCG vaccination induces rapid and robust activation of splenic APC, leading to systemic T_H1-skewed T cell responses	- Antigen delivery to the spleen- Activation of splenic DC and macrophage markers (MHC-II, CD80, CD86)- Cytokine production (IL-6, IL-10, IFN-g, TNF-α, IL-12)- Robust Ag-specific $CD4^+$ T cell activation in secondary lymphoid organs	- Rapid systemic immune activation- Strong T_H1 responses- Efficient antigen presentation in the spleen	[12]
Mice (C57BL/6)	IV-BCG vaccination confers protection via epigenetically programmed trained immunity, bypassing adaptive pathways	- Transcriptional reprogramming of HSCs and MPPs, favoring myelopoiesis over lymphopoiesis - Epigenetic modifications (H3K4me3, H3K27ac) - Elevated cytokine expression (IFN-g, IL-1β, TNF-α) in macrophages	- Significant reduction in Mtb load - Epigenetic reprogramming - Enhanced innate immunity and long-lasting effect	[13]
Mice (Diversity outbred)	IV-BCG moderately improves survival after Mtb challenge in genetically diverse mice	- Dissemination of BCG to multiple organs (lungs, spleen, liver, bone marrow)	- Evidence highlighting the importance of vaccination route in protective efficacy	[31]
Mice (C57BL/6)	IV-BCG vaccination induces broader and stronger IFN-γ responses against Ag85 and PE/PPE antigens than SC route	- Strong IFN-γ responses in splenocytes - Enhanced recognition of multiple Mtb antigens, including Ag85A/B, PE/PPE family, TB10.4, and Esx-1	- Broader and more robust T cell response	[36]
Mice (BALB/c)	IV-BCG vaccination resulted in systemic dissemination of BCG and induced higher anti-BCG antibody titers than other delivery routes	- Systemic dissemination via bloodstream - Strong humoral immune activation - Histopathologic inflammation in spleen and liver - Recognition of multiple BCG antigens (notably 65 kDa protein)	- IV-BCG-elicited antibody responses of greatest magnitude among all vaccination routes - Systemic organ involvement - Broader antigen exposure and immune priming	[37]
Guinea pigs	IV administration of killed BCG induces granulomatous lesions in tuberculin-sensitized guinea pigs	- Delayed-type hypersensitivity reaction - Granuloma formation in lungs - Transient hepatic inflammation	- Reduced inflammatory responses induced by viable BCG compared with killed preparations - Self-limiting lesion formation with resolution by 4 weeks - Evidence underscoring the risks of IV-BCG in sensitized subjects	[38]
Rhesus macaques	IV-BCG provides robust protection; 9/10 animals protected, 6 with no detectable infection	- Substantially increased antigen-responsive $CD4^+$ and $CD8^+$ T cell responses in blood, spleen, BAL, and lung-draining lymph nodes - High-frequency antigen-responsive T cells distribution throughout lung parenchymal tissues contributing to enhanced protection	- Robust protection against Mtb challenge - Significant reductions in detectable infection and disease pathology compared to other routes of BCG administration	[8]
Rhesus macaques	IV-BCG vaccination protected both SIV-infected and SIV-naive macaques from Mtb challenge	- Induced robust airway $CD4^+$ T cell influx - $CD4^+$ T cells produced IFN-γ, TNF-α, and IL-2 - Elevated plasma and airway antibody titers (specific isotypes not specified)	- Demonstrated protective immunity even in immunocompromised (SIV-infected) hosts - Supported IV-BCG as a viable TB vaccine route in vulnerable populations	[9]
Rhesus macaques	High-dose IV-BCG sustains airway immune activation by recruiting polyfunctional T cells and macrophages, priming the lung mucosa for rapid and coordinated response to Mtb	- Recruitment of polyfunctional $CD4^+$ T cells and macrophages to the airways - Basal activation of alveolar macrophages with IFN and TNF-α signaling - Robust T_H1/T_H17 transcriptional responses upon antigen stimulation	- Sustained immune activation in the airways - Primed airway environment for rapid Mtb clearance - Potential for improved vaccine strategies targeting mucosal immunity	[10]
Rhesus macaques	IV-BCG-mediated protection against Mtb requires both adaptive $CD4^+$ T cells and innate $CD8α^+$ lymphocytes, as depletion of either abrogates protective efficacy	- Essential role of $CD4^+$ T cells in controlling Mtb infection following IV-BCG vaccination - Protective contribution of $CD8α^+$ lymphocytes, particularly innate subsets, through cytokine production and cytotoxic functions	- Increased Mtb burden and dissemination following depletion of $CD4^+$ T cells or $CD8α^+$ lymphocytes, indicating loss of protection - Dominant protective role of innate $CD8α^+$ lymphocytes over adaptive $CD8αβ^+$ T cells	[11]

Table 1 (continued)

Studies	Key Findings	Detailed Mode of Action	Outcomes	Ref.
Rhesus macaques	IV-BCG vaccination induces trained immunity characterized by enhanced innate cytokine production and increased H3K27 acetylation in monocytes	- Epigenetic reprogramming of monocytes mkared by H3K27ac, enhancing cytokine production upon stimulation	- Robust trained immunity in monocytes	[28]
Rhesus macaques	IV-BCG induces robust airway-resident CD4⁺ T cells and NK cell responses together with Fc effector-functional mycobacteria-specific antibodies	- Polyfunctional cytokine production (IFN-γ, TNF-α, IL-17) by airway-resident CD4⁺ T cells - Dose-dependent induction of mycobacteria-specific antibody responses in plasma and BAL fluid - Antibody-mediated complement activation and NK cell engagement as functional immune signatures associated with protection	- Complete protection (sterile immunity) in several macaques - Airway, not blood, immune responses correlated with protection - Identification of humoral immune correlates of protection, highlighting the potential of antibody-based biomarkers to predict vaccine efficacy	[35]
Rhesus macaques	A distinct interferon-driven blood transcriptional module induced within 2 days of IV-BCG vaccination predicts long-term protection, offering early biomarkers of vaccine efficacy	- Induction of module 1 expression (type I interferon and RIG-I-like receptor signaling pathways) at day 2	- Early blood transcriptional responses predict protective efficacy (AUROC≥0.91)- Strong correlation with reduced lung bacterial burden- Lower granuloma counts and lung pathology	[39]
Rhesus macaques	IV-BCG vaccination in rhesus macaques elicits robust antigen-specific IgM responses in both plasma and BAL fluid	- High level induction of antigen-specific IgM, IgG, and IgA antibodies, particularly in the lungs - Functional activity of IgM antibodies, including enhanced Fc receptor binding and promotion of phagocytosis	- Robust lung-compartmentalized antibody responses - Association of elevated IgM responses with enhanced protection against Mtb infection	[40]
Cynomolgus macaques	IV-BCG vaccination in non-human primates induces significantly higher titers of Mtb-specific IgG, IgA, and IgM in serum	- Robust humoral responses with elevated levels of high-avidity IgG antibodies - Superior opsonizing capacity of IV-BCG-elicited antibodies enhancing phagocytosis of Mtb	- Superior humoral immune responses with high-avidity, functionally potent antibodies	[36]
Rhesus macaques	γδ T cells induced by IV/aerosol BCG mediate protection against Mtb, independent of classical T cells	- Systemic expansion of γδ T cells (especially Vγ9Vδ2)- γδ T cells produce IFN-γ and TNF-α, contributing to early protection	- Reduced Mtb burden and lung inflammation- Novel correlate beyond classical adaptive responses	[41]
Rhesus macaques	Network modeling reveals key immune interactions and predicts effects of immune perturbations after IV-BCG	- Markov field-based integration of systems serology, cytokines, and cytometry - Identification of key immune interactions contributing to vaccine efficacy - Prediction and experimental validation of immune perturbation effects on vaccine-induced protection	- Identification of key immune interactions by Markov models - Experimentally confirmed immune network perturbations (e.g., B cell depletion) without loss of protective efficacy	[42]

APC antigen-presenting cell, *HSC* hematopoietic stem cell, *MPP* multipotent progenitor, *DC* dendritic cell, *MHC-II* major histocompatibility complex class II, *CD* cluster of differentiation, *BAL* bronchoalveolar lavage, *γδ T cell* gamma delta T cell, T_{FH} T follicular helper cell T_H1 T helper 1 cell, *NK* natural killer, T_H17 T helper 17 cell, *XCR1* chemokine receptor XCR1, *SIV* simian immunodeficiency virus, *SC* subcutaneous, *TB* tuberculosis, *cDC1* conventional dendritic cell type 1, *Ig* immunoglobulin

"sterilizing immunity," defined as the complete absence of detectable CFU in all examined tissues—including individual lung lobes, thoracic lymph nodes, spleen, and liver [8]. This outcome, characterized by systemic bacterial clearance in the majority of subjects, is substantially greater than that of ID- or mucosal BCG administration [8, 35]. This efficacy results from systemic transmission of live BCG, enabling dissemination to the lung, spleen, liver, and bone marrow, and creating multilayer immune defenses across compartments [8, 29]. IV-BCG delivers universal and prolonged antigen availability, resulting in the elicitation of DC activation and robust T_H1 differentiation [12]. As a result, multiple multifunctional $CD4^+$ and $CD8^+$ T cells are elicited and an antigen-specific T_{RM} pool is formed within the parenchyma of the lung, enabling brisk recall responses upon re-exposure to Mtb [8].

Key immunological components of IV-BCG vaccination

Continuous research has focused on identifying the key immunological components that influence TB vaccine efficacy. Notably, several strategies leveraging these factors have demonstrated enhanced BCG vaccine performance when delivered via IV-BCG administration. Furthermore,

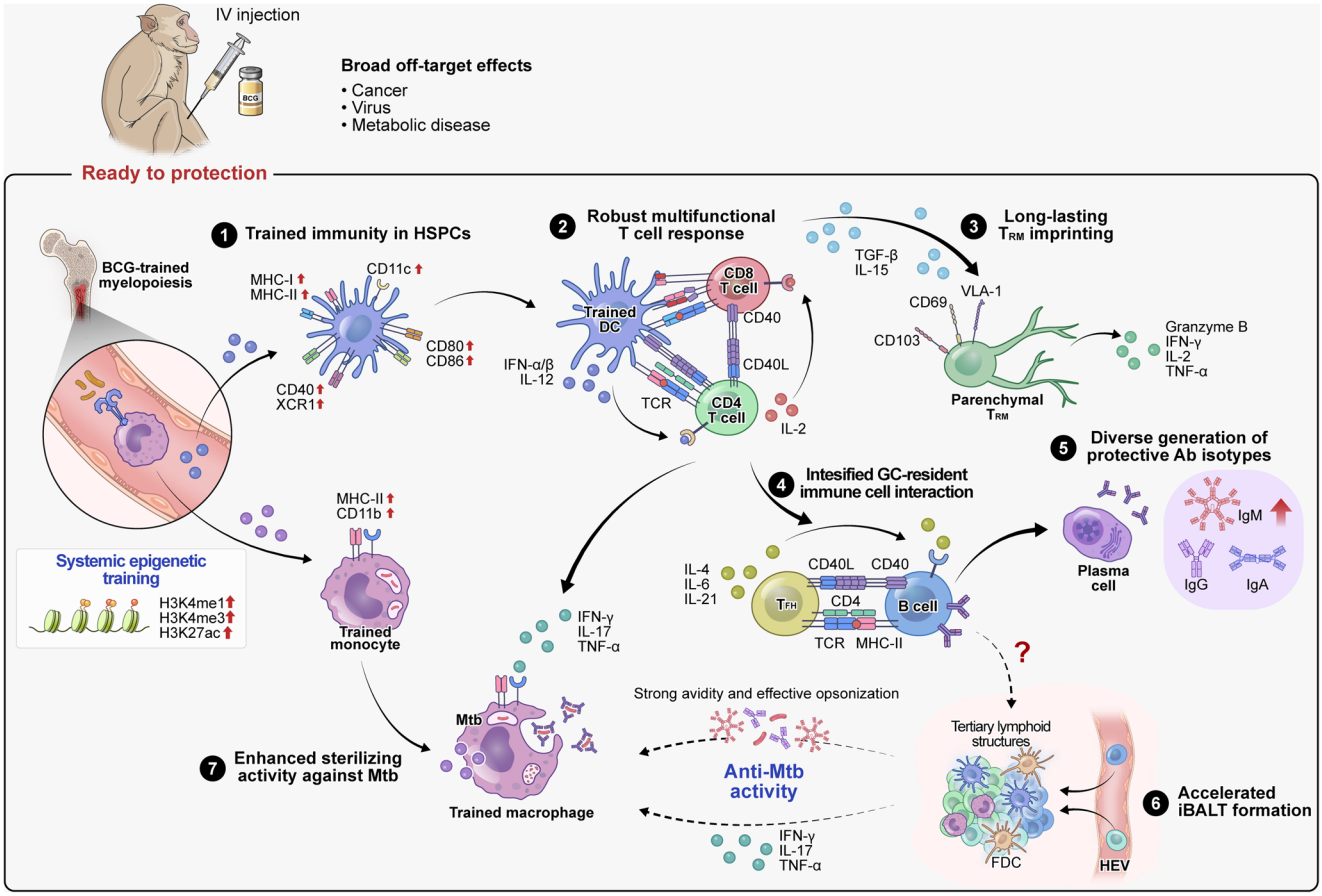

Fig. 2 Intravenous BCG orchestrates multilayered immune programs that drive broad protection. IV-BCG vaccination activates innate, adaptive and tissue-resident compartments in concert, providing durable protection against Mtb and even heterologous pathogens. The schematic highlights interconnected pathways ranging from bone marrow reprogramming to tertiary lymphoid organization. Solid arrows indicate established mechanisms, whereas dashed arrows and question marks mark incompletely defined links. (1) Trained immunity in HSPCs. IV-BCG reaches the bone marrow and imprints HSPCs with histone modifications (H3K4me1/3, H3K27ac). This biases differentiation toward monocytes and DCs with elevated MHC-I/II, CD11c, CD80, and CD86, accompanied by augmented cytokine release such as IFN-α/β, and IL-12. Additional activation markers, including CD40 and XCR1, further reflect enhanced antigen-presenting capacity. (2) Multifunctional T cells. Antigen-loaded DCs present to $CD4^+$ and $CD8^+$ T cells through MHC and CD40–CD40L engagement, promoting IL-2–driven expansion of polyfunctional subsets that secrete IFN-g, TNF-α and IL-17 simultaneously, enhancing effector potency. (3) Tissue-resident memory T cells. Effector T cells traffic to the lung in response to TGF-β and IL-15 secreted by DCs, where they establish T_{RM} characterized by the expression of CD69, CD103 and VLA-1. These cells persist long term and rapidly secrete IFN-g, IL-2, TNF-α and granzyme B upon pathogen encounter, providing immediate frontline protection. (4) Germinal center activity. T_{FH} cells interact with B cells via CD40L and cytokines (IL-4, IL-6, and IL-21), strengthening germinal center reactions, broadening B cell repertoires and generating durable memory and plasma cell populations. (5) Antibody diversification. Plasma cells secrete IgM, IgA and IgG with complementary roles: IgM mediates avid binding and complement activation, IgA protects mucosal surfaces by neutralizing bacteria, and IgG supports opsonization and Fc receptor–mediated clearance. The combined antibody repertoire expands effector mechanisms and synergizes with cellular immunity. (6) Potential iBALT formation. IV-BCG may accelerate the formation of iBALT containing HEVs, B cell follicles and FDCs. These tertiary lymphoid structures could sustain antigen presentation and recall responses, though their contribution to protection remains to be fully defined. (7) Reinforced macrophage activity. Trained monocytes and macrophages infiltrate the lung and express elevated MHC-II and CD11b, reflecting enhanced antigen presentation and phagocytic capacity. Together with cytokines such as IFN-γ, IL-17 and TNF-α from T cells, these myeloid cells exhibit increased phagolysosomal fusion and antimicrobial effector functions that contribute to bacterial clearance. Collectively, IV-BCG coordinates epigenetically trained innate and dendritic cell programs with the generation of multifunctional T cells, durable T_{RM}, expanded antibody repertoires, and potential iBALT formation, thereby achieving sterilizing immunity in non-human primates and underscoring the promise—as well as the challenges—of translation to humans. DC, dendritic cell; HSPCs, hematopoietic stem and progenitor cells; T_{RM}, tissue-resident memory T cell; T_{FH}, T follicular helper cell; GC, germinal center; iBALT, inducible bronchus-associated lymphoid tissue; HEV, high endothelial venule; FDC, follicular dendritic cell; Ig, immunoglobulin

Table 2 Roles of key immunological factors in vaccine efficacy and TB protection

Factor	General Role in Vaccine Efficacy	Specialized Role Against Mtb	Ref.
Trained Immunity	Provides innate immune memory through epigenetic and metabolic reprogramming of monocytes, macrophages, and DCs, enhancing nonspecific and broad protection against diverse pathogens.	Post-IV-BCG, trained immunity strengthens macrophage and DC responses against Mtb by promoting glycolytic reprogramming, Akt-mTOR-HIF1α signaling, and epigenetic modifications, leading to enhanced antimycobacterial activity.	[13, 28, 53–60]
DCs	Act as professional APC that bridge innate and adaptive immunity by sensing antigens, secreting cytokines, and directing T cell differentiation. Maintain immune homeostasis while rapidly initiating protective adaptive responses.	DCs sense Mtb antigens and present them via MHC-II to drive $CD4^+$ and $CD8^+$ T cell priming. In IV-BCG vaccination, lung cDC1s are expanded and activated (CD86, CD40, XCR1, IL-12), enhancing cross-presentation and adaptive $CD8^+$ T cell responses, leading to robust pulmonary immunity and reduced pathology.	[12, 61–64]
Antigen-Specific T Cells	Adaptive immune backbone of vaccine efficacy; $CD4^+$ and $CD8^+$ T cells respond to antigen presentation, providing long-lasting, specific immunity and immunological memory.	IV-BCG induces highly polyfunctional $CD4^+$ and $CD8^+$ T cells (producing IFN-γ, TNF-α, IL-2) enriched in lung parenchyma and lymphoid tissues. Their frequency and polyfunctionality correlate with reduced Mtb burden and protection, suggesting establishment of T_{RM}-like populations.	[8, 10, 12, 35]
Effector T_H1 Cells	Central effector subset of $CD4^+$ T cells that secrete IFN-γ, IL-2, and TNF-α to orchestrate protective immunity. Serve as biomarkers of vaccine efficacy and correlate with pathogen control.	In TB infection, T_H1 cells are indispensable for protection; deficiency in IFN-γ leads to high susceptibility to mycobacterial disease. IV-BCG promotes strong T_H1/T_H17-polarized responses in airway and lung tissue, engaging cross-talk with myeloid cells.	[65–71]
T_{RM} Cells	Provide rapid, localized immune responses at barrier tissues (e.g., lung, mucosa) by remaining in situ after initial infection or vaccination. Defined by $CD69^+CD103^+$ phenotype and supported by cytokines such as TGF-β and IL-15.	IV-BCG induces abundant T_{RM}-like $CD4^+$ and $CD8^+$ T cells (>80% in lung parenchyma), producing IFN-γ, IL-17, and TNF-α. These cells interact with alveolar macrophages, sustain iBALT structures, and provide near-sterilizing pulmonary immunity in NHPs, ensuring rapid lung-specific protection against Mtb.	[7, 8, 35, 72–78]
Antibody Function	Antigen-specific antibodies (IgG, IgA, IgM) complement cellular immunity by enabling effector functions such as opsonization, complement activation, and Fc receptor–mediated responses. B cell–T_{FH} cell interactions drive germinal center formation and antibody diversification.	IV-BCG induces strong lung-compartmentalized IgG and IgA, while plasma and BAL IgM correlate with reduced Mtb burden. IgM monoclonal antibodies can directly limit bacterial survival. Antibodies reduce lung pathology, enhance DC uptake, support T_{RM} and iBALT formation, and synergize with innate and adaptive immunity to contain Mtb.	[9, 40, 43, 79–81]
iBALT Formation	Acts as a TLS in the lung, providing localized hubs for immune coordination. Supports T cell priming, B cell maturation, antibody production, and integration of cellular and humoral immunity.	IV-BCG may induce iBALT through IL-23–ILC3–IL-17–CXCL13 axis, T_H1-derived IFN-γ/TNF-α, and DC activity. Within iBALT, T_H1/T_{RM} cells sustain cytokine output, B cells produce IgA/IgG, and trained DCs enhance antigen presentation. This localized immune hub promotes opsonization, macrophage activation, and containment of Mtb—though its role in human TB remains debated due to variability in granuloma/iBALT presence.	[82–90]

DC dendritic cell, *APC* antigen-presenting cell, *MHC-II* major histocompatibility complex class II, *cDC1* conventional dendritic cell type 1, T_{RM} tissue-resident memory T cell, *iBALT* inducible bronchus-associated lymphoid tissue, *NHP* non-human primate, *Ig* immunoglobulin, T_{FH} T follicular helper cell, *GC* germinal center, *TLS* tertiary lymphoid structure, *ILC3* group 3 innate lymphoid cell

distinct features of innate and adaptive immune responses that correlate with protective outcomes hold promise as potential biomarkers for vaccine efficacy (Fig. 2; Table 2).

Trained immunity: innate amplification

Trained immunity, also referred to as innate immune memory, is a consequence of epigenetic and metabolic reprogramming events in innate immune cells such as monocytes, macrophages, and DCs [53–55, 91]. Unlike adaptive immunity, which generates antigen-specific and long-lasting memory through B and T lymphocytes, trained immunity represents functional adaptations of myeloid cells and natural killer (NK) cells. These adaptations rely on transcriptional, metabolic, and epigenetic remodeling that heightens nonspecific responses against diverse pathogens [56–58, 92, 93].

Mechanistically, IV-BCG promotes epigenetic modifications (e.g., H3K4me3 and H3K27ac) and metabolic shifts (e.g., glycolysis) in macrophages and DCs, thereby enhancing antimycobacterial protection in both murine and NHP models [13, 28]. Xu et al. demonstrated that BCG-induced training depends on the Akt-mTOR-HIF1α axis, glycolytic activation, and NOD-like receptor signaling, with differences in linoleic acid metabolism modulating the degree of trained protection against TB [59]. Similarly, Minute et al. reported that heat-killed Mtb induces trained immunity in human monocytes via epigenetic remodeling in a Syk/HIF-1α-dependent manner [60].

Systemic IV-BCG-induced trained immunity was reported to provide T- and B-lymphocyte-independent protection and broad, nonspecific resistance to infections. DCs can undergo memory-like reprogramming under specific immunization strategies. For example, in a murine model, DCs isolated from immunized hosts displayed enhanced transcriptional activation of interferon and immune signaling pathways, along with sustained cytokine responses upon re-exposure to the same pathogen [94]. Notably, this prolonged response was abolished when histone modifications were inhibited, highlighting the critical role of epigenetic remodeling in establishing a memory-like state in DCs. These reprogrammed DCs not only produce innate cytokines more effectively but also markedly enhance T cell priming, positioning DC epigenetic reprogramming as a potential bridge between trained and adaptive immunity.

Moreover, this epigenetic and metabolic reprogramming of innate immune cells also enhances defense against secondary infections including *C. albicans*, *Brucella abortus*, and virus [13, 57, 95–103]. In addition, BCG-induced trained immunity has also been applied as an immunotherapy for bladder cancer, underscoring its relevance beyond infectious diseases [104, 105]. Consequently, IV-BCG induced trained immunity may offer a promising avenue to improve the efficacy of the current BCG vaccine and to develop novel vaccines that integrate classical adaptive and innate immune memory.

DC: The Initiating Link between innate and adaptive immunity

DCs play a key role in balancing immunity and tolerance by bridging innate information to generate either protective or pathological adaptive immunity in many diseases [61, 106]. DCs constitute a distinct lineage of cell populations with the capacity to seed tissues and maintain immune homeostasis in the steady state, while rapidly responding to local insults and initiating as well as directing innate and adaptive immunity through communication with other immune cells [62]. In addition, DCs are crucial for sensing antigens from Mtb and Mtb-infected cells, then secreting effector signals to drive the differentiation and polarization of T cells necessary to clear infection [107–110]. In the lung, tissue-resident DCs (cDC1s, cDC2s, and pDCs) serve as professional antigen-presenting cells (APC), capturing pathogens, migrating to draining LNs, and priming naïve $CD4^+$ and $CD8^+$ T cells [63]. Following priming, effector and memory T cells, including potential T_{RM}, return to the lung, where local antigen presentation and cytokines, such as TGF-β and IL-15, support tissue retention and durable immunity [63].

Dendritic cells are pivotal in determining the efficacy of IV-BCG, acting as professional APCs that are more efficient than macrophages [12]. This correlates with increased expression of MHC-II molecules and their transcriptional regulator CIITA, underscoring route-dependent differences in the spatial and temporal dynamics of APC activation. Systemic IV-BCG-mediated lung immunity critically depends on cDC1s, which directly present antigen to naive $CD4^+$ T cells [63, 111], and subsequently prime T_H1, T_H1-like T follicular helper (T_{FH}), and regulatory T cells [112]. Additionally, cDC1s are highly efficient at antigen cross-presentation, activating $CD8^+$ T cell through the secretion of IL-12 and type I IFN [39, 113–115]. IV-BCG increases pulmonary cDC1 numbers and upregulates activation markers, including CD80, CD86, CD40, and XCR1, while boosting IL-12 production, collectively promoting T_H1 priming [64]. Therefore, IV-BCG elicits robust systemic and pulmonary T cell responses and confers substantial protection against Mtb challenge in part via trained DC-mediated reprogramming, with reduced lung pathology [8]. However, their precise role in TB remains uncertain, as direct human evidence

is lacking and chronic Mtb exposure could potentially dampen such training effects. By integrating pathogen-derived signals, DCs appear poised to bridge innate sensing and adaptive immunity, but their role in human disease has yet to be fully defined.

Multifunctional T_H1 cells: the adaptive core

Antigen-specific T cells, particularly effector T_H1 cells, form the adaptive backbone of protection induced by IV-BCG. Through DC-mediated antigen presentation, these T_H1 cells mount robust responses that are central to controlling Mtb infection [8, 12, 35].

$CD4^+$ T cells serve as critical effector cells in the host immune response to Mtb, with the T_H1 subset playing a pivotal role by secreting IFN-g, IL-2, and TNF-α to orchestrate protective immunity [65]. In TB vaccine research, reductions in Mtb burden in the lungs and spleens of murine models have strongly correlated with the frequency and proportion of multifunctional $CD4^+$ T cells [66]. Consequently, the cytokine profiles of T_H1 cells specific to Mtb antigens have been widely adopted as immunological biomarkers and investigated as predictors of protective efficacy in TB vaccine assessments [67–69]. In particular, IFN-g is indispensable for eliciting protective immunity, and its deficiency is associated with markedly increased susceptibility to mycobacterial infections [70, 71].

In NHPs, IV-BCG vaccination induced highly polyfunctional $CD4^+$ and $CD8^+$ T cell responses, characterized by IFN-g, IL-2, and TNF-α production, which were particularly enriched in lung parenchyma and lymphoid tissues [8, 10]. These airway-resident T cells displayed strong T_H1/T_H17 polarization and engaged in active cross-talk with airway myeloid cells, as indicated by ligand–receptor interactions [10]. Their differentiation was defined by transcription factors such as *EGR2*, *NR4A3*, *IRF4*, *RPBJ*, *TBX21* (T-bet), *BATF*, and *RORC*, while genes including *IL23R*, *IL21R*, *PIK3CG*, and *TJP2* were associated with long-lived effector states [10]. The frequency and functionality of these polyfunctional T cells strongly correlated with reduced Mtb burden after challenge, establishing them as key immune correlates of IV-BCG–mediated protection [35].

Collectively, these findings demonstrate that IV-BCG elicits broad and durable antigen-specific $CD4^+$ and $CD8^+$ T cell responses that disseminate throughout the lung parenchyma and confer remarkable protection against Mtb challenge [8]. Extensive localization of long-lived T cells suggests the potential establishment of T_{RM}-like populations within the T_H1 subset. Indeed, it has been found that IV-BCG has an increased transcriptional program characterized by tissue maintenance and durable effector status [10], and is also rich in intra-airway multifunctional and conducive $CD4^+$ T cells. Thus, IV-BCG can promote T_{RM}-like features in the lungs as well as induce systemic and pulmonary effector T cell responses, which are increasingly recognized as important correlations of protective immunity to TB.

T_{RM}: lung-specific guardians

T_{RM} play an instrumental role in local immune responses against a wide range of both viral and bacterial infections, rapidly responding to pathogenic insults through their memory of previously encountered pathogens [72, 73, 116, 117]. Phenotypically, T_{RM} are most often defined as $CD69^+CD103^+$ and exhibit increased transcription of markers such as *Itgae* (CD103) in $CD4^+$ T cells and *Itga1* (VLA-1) in $CD8^+$ T cells [7, 74]. Furthermore, specific T_{RM} subsets producing IFN-γ or IL-17 are important in the control of Mtb in mice as well as in the human lung [75, 76].

IV-BCG in NHPs induced > 80% lung parenchyma–derived $CD4^+$ and $CD8^+$ T cells with T_{RM}-like phenotypes, versus 16–35% after ID- or mucosal BCG. Antigen stimulation revealed IFN-g-producing $CD4^+$ T cells across lung lobes and LNs, high expression of CD69 and CD103, indicating IV-BCG establishes abundant antigen-responsive T_{RM}-like cells associated with superior protection [8]. DCs are the major cells that prime T_{RM} by presenting antigens and secreting TGF-β and IL-15, thereby driving their migration to the lungs [77, 78]. After localizing adjacent to alveolar macrophages, T_{RM} secrete IFN-g, IL-17, granzyme B, and TNF-α, which together suppress Mtb growth within days and achieve near-sterilizing immunity in NHPs. Notably, antigen-responsive T cells remain elevated in the lung parenchyma and bronchoalveolar lavage (BAL) for up to six months following IV-BCG vaccination [8, 35, 44, 118]. T_{RM} are derived from T_H1 cells, maintains antigen specificity, and sustains iBALT through cytokine production [82, 119]. T_{RM} have been shown to provide rapid, localized control of Mtb in the lungs following IV-BCG, by integrating DC priming, T_H1 support, iBALT synergy, and innate enhancement, to ensure rapid, lung-specific immunity critical for pulmonary protection [8, 35].

Antibody function: T cell-mediated humoral reinforcement

Several studies have proposed IFN-g independent protective immune responses against TB [79, 120, 121]. Among these, antigen-specific antibody responses have emerged as promising correlates of protection [43, 79, 122–124]. Mechanistically, DCs activate T_{FH} cells, which mature in germinal centers and drive B cell–mediated antibody production [125]. T_{FH} cells can be repeatedly primed and are characterized primarily by their secretion of IL-21 [126]. These IL-21-producing $CD4^+$ T cells also express BCL6,

a transcriptional regulator, and the chemokine receptor CXCR5, both of which are crucial markers of T_{FH} cells [126]. T_{FH} cells further contribute to B cell differentiation and antibody maturation through the production of other cytokines such as IL-4 [126]. Antigen-specific B cells, in turn, orchestrate the positioning of T_{FH} cells into lymphoid follicles. This establishes a B cell-T_{FH} cell axis that supports germinal-center formation and promotes high-affinity IgG1 antibody responses, and contributes to effective control of Mtb [125–131]. Through this pathway, B cells differentiate into plasma cells that generate multiple antibody isotypes, thereby enabling diverse effector functions such as opsonization, complement activation, and Fc receptor-mediated responses [83, 132].

Once considered irrelevant in controlling an intracellular pathogen, antibodies are now recognized to reduce lung pathology in NHP models of IV-BCG [43], while murine aerosol BCG studies have linked anti-Ag85A IgG responses to reduced TB incidence [133]. Mucosal IgA complements T_{RM} and iBALT formation by neutralizing Mtb locally [80], whereas trained macrophages enhance IgG-mediated efficacy [81]. IV-BCG vaccination in NHPs induced stronger antigen-specific humoral immunity than ID-BCG, characterized by robust lung-compartmentalized IgG and IgA responses that contribute to enhanced Mtb containment [9]. In addition, plasma and BAL IgM levels correlated with reduced bacterial burden, and an Mtb-specific IgM monoclonal antibody directly limited bacterial survival, highlighting a protective role for IgM in IV-BCG–induced immunity [40]. Together, these humoral responses integrate with cellular immunity to amplify DC uptake, support T_{RM} development, and promote iBALT formation, collectively strengthening pulmonary containment of Mtb.

iBALT formation: localized immune coordination

iBALT represents a tertiary lymphoid structure (TLS) that emerges in the lung under conditions of infection or chronic inflammation. It is organized into discrete T cell and B cell compartments, often resembling follicular aggregates, and is accompanied by DCs that facilitate local antigen presentation [82, 84, 85]. Functionally, iBALT serves as an ectopic site where T and B lymphocytes interact, enabling the coordination of cell-mediated and humoral immune responses directly within the pulmonary tissue. Lung-derived IL-23 stimulates ILC3s to produce IL-17 and IL-22, of which IL-17 induces CXCL13 expression in stromal cells [86]. CXCL13 attracts $CXCR5^+$ lymphocytes to the lung parenchyma, leading to the structured assembly of iBALT and promoting early immune containment of infection [86]. In parallel, IFN-γ and TNF-α secreted by T_H1 cells initiate lymphocyte infiltration, and DC subsets such as follicular DCs enhance the organization of these aggregates [82, 85]. Once established, iBALT functions as a hub where multiple immune subsets cooperate. T_{RM} provides sustained cytokine release, B cells differentiate into antibody-secreting plasma cells, and memory B cells persist to support long-term recall immunity [87, 88, 134, 135]. Consequently, the collective effect is improved antibody-mediated opsonization, enhanced cytokine-driven macrophage activation, and more effective pathogen uptake.

A hallmark of functional TLSs, including iBALT, is the presence of high endothelial venules (HEVs). These $PNAd^+$ vascular structures enable continuous recruitment of naïve T and B lymphocytes via CCR7–CCL21 signaling, thereby sustaining the immunological activity of iBALT [87]. Murine models of TB consistently demonstrate robust HEV^+ iBALT formation that correlates with reduced bacterial loads [136]. By contrast, human post-mortem studies have reported variable or incomplete iBALT and HEV development, which may reflect differences in host immunity, bacterial strain diversity, or the immunosuppressive environment of advanced TB lesions [137].

In the context of IV-BCG, iBALT induction has not yet been directly demonstrated; nonetheless, the high pulmonary antigen burden and sustained T_H1 activation observed with high-dose IV-BCG raise the hypothesis that this route could foster HEV^+ iBALT [10, 44]. Such ectopic lymphoid niches could amplify protection by sustaining lymphocyte recruitment, promoting local antibody maturation, and integrating opsonization with macrophage effector functions [83, 89, 90]. It is important to distinguish the protective role of vaccine-induced iBALT from the pathological iBALT observed in chronic respiratory diseases [138]. In conditions such as chronic obstructive pulmonary disease and asthma, iBALT formation is often driven by persistent, dysregulated inflammation, leading to aberrant tissue remodeling, airflow obstruction, and tissue damage [139]. Conversely, in the context of IV-BCG vaccination, iBALT is considered a functional immune hub. Instead of driving chronic pathology, it facilitates the organized interaction of T cells, B cells, and APCs directly at the site of potential infection. This localized structure enables a rapid, on-site recall response that contains Mtb within granulomas before it can disseminate, thereby serving as a critical component of sterilizing immunity rather than a driver of disease [137]. Although some reports caution that iBALT may serve as a reservoir for mycobacteria under conditions of dysregulated inflammation [17, 140, 141], the prevailing evidence suggests that when appropriately induced, iBALT can act as a powerful adjunct to granuloma formation, strengthening pulmonary immunity and accelerating protective responses against Mtb.

IV-BCG vaccine application in the context of underlying diseases Although relatively few studies have examined the efficacy of IV-BCG beyond TB, IV administration of BCG appears to broaden its protective capacity, exerting disease-modifying effects in cancer, infectious diseases, and metabolic diseases. BCG is well known to enhance antibacterial defenses through trained immunity, improving host resistance against unrelated bacterial pathogens [99]. It is plausible that IV delivery, by amplifying systemic antigen exposure and myeloid reprogramming, could further potentiate this broad-spectrum antibacterial effect, although direct experimental evidence remains scarce [10].

In lung cancer models, IV-BCG reduced tumor burden and improved survival through cDC1-dependent $CD8^+$ T cell priming and NK cell-mediated immunity, while also synergizing with PD-L1 checkpoint blockade to overcome otherwise resistant tumor microenvironments [64].

In viral infection model, particularly SARS-CoV-2, IV-BCG lowered viral load, mitigated lung pathology, and prevented lymphopenia in hamsters by expanding T_H1, T_H17, cytotoxic T lymphocytes, regulatory T cells, and plasma cells, while reprogramming macrophages and limiting neutrophil-driven pathology, thereby integrating trained innate and adaptive responses. Moreover, in influenza A virus infection model, systemic IV-BCG conferred heterologous protection in mice by expanding $CX3CR1^{hi}$ effector memory $CD4^+$ T cells, whose antigen-independent IFN-g production enhanced alveolar macrophage activity, thereby linking adaptive and innate memory [142].

Additionally, IV-BCG administration in obese, diabetic ob/ob mice alleviated nonalcoholic fatty liver disease by reducing hepatic lipid accumulation, suppressing lipogenic gene expression, and improving insulin sensitivity. Mechanistically, BCG mitigated ER stress and restored insulin signaling in hepatocytes, suggesting that IV-BCG can remodel metabolism and provide therapeutic benefit in obesity-associated fatty liver disease [143].

Collectively, BCG has been associated with heterologous protection against bacterial pathogens, cancers, metabolic disorders, and viral infections [96, 144–147]. While direct evidence for IV-BCG in these contexts remains limited, intravenous delivery—by amplifying systemic antigen exposure and driving myeloid reprogramming—may further potentiate these broad-spectrum effects [10]. Emerging data suggest that IV-BCG can reprogram immune and metabolic networks with potential benefits beyond TB.

Conclusion and perspectives In this review, we summarized the synergistic immune interactions induced by IV-BCG vaccination. IV-BCG modifies the pulmonary protective effect of BCG by forming a synergistic immune network against Mtb. IV-BCG trains monocytes, and trained DCs initiate a chain reaction that activates effector T_H1 cells and T_{RM} in the lungs. T_H1 and T_{RM} cytokines can form a local immune hub, the iBALT. Antibodies induced by activated T_{FH} cells, which opsonize Mtb and complement T_{RM} and iBALT at the mucosal level, enhancing cellular immunity. This trained immunity enhances innate immunity and supports T_{RM} maintenance, iBALT function, and antibody efficacy. IV-BCG achieves near-sterilizing protection over months of follow-up, substantially exceeding ID-BCG in the NHP model, but translation to humans still requires rigorous safety evaluation and dose optimization [8–11]. The synergistic effect relies on systemic administration of IV-BCG, allowing trained DCs to directly link adaptive, humoral, and innate responses in the lung, the primary battleground for Mtb.

To translate the efficacy of IV-BCG to human vaccination, further studies are needed to ensure safety, optimize dose, and consider the heterogeneity of various populations. To this end, TLR agonists and other adjuvants that train DCs or induce iBALT may enhance T_H1 and T_{RM} responses [146–148]. Furthermore, co-administration of IV-BCG with Mtb-specific antigens may expand antigen-specific T cell diversity, overcoming strain-specific variability [149]. In addition, co-administration with existing anti-TB treatments may accelerate bacterial clearance in active TB [9]. However, the extent to which these immune cell-mediated mechanisms operate similarly in humans remains uncertain, as key features such as DC training efficacy, iBALT formation, and the generation and persistence of pulmonary T_{RM} cells differ between animal and humans. While these strategies could be considered for IV-BCG application, they have currently only been demonstrated in non-human primate studies, emphasizing the need for further studies in the human setting. Taken together, IV-BCG vaccination is highly effective against Mtb infection and disease. It orchestrates a multi-layered immune program that integrates innate training, adaptive T cell responses, antibody activation, and localized iBALT formation through a systemic immune response. Although the safety concerns associated with systemic live bacterial administration currently limit the direct clinical application of IV-BCG, its ability to induce near-sterilizing immunity makes it an invaluable biological benchmark [8, 47]. By demonstrating that robust protection requires the synergistic activation of systemic trained immunity and lung-resident memory [13, 35], IV-BCG provides a clear rationale for future vaccine strategies. These insights are now guiding the design of safer alternatives, such as mucosal vaccines or heterologous prime-boost regimens, which aim to mimic the comprehensive immune landscape of IV-BCG without its associated risks

[7, 31, 50]. Collectively, this coordinated immune environment supports durable and broad protection against Mtb, positioning IV-BCG as a critical reference point for next-generation TB vaccine development. While significant challenges remain before human application, preclinical studies highlight the promising potential of BCG to transform the prevention of TB.

Acknowledgements MID (Medical Illustration & Design), as a member of the Medical Research Support Services of Yonsei University College of Medicine, providing excellent support with medical illustration.

Author contribution S.C. and J.P. wrote the first draft of the manuscript. J.J.H. contributed to the organization and structure of the review and assisted in editing the final version. S.J.S. and J.M.L. conceived the project, contributed to writing, and thoroughly revised the manuscript. All authors read and approved the final version of the manuscript.

Funding This work was supported by the Korea Research Institute of Bioscience and Biotechnology (KRIBB) Research Initiative Program (KGM4572532), by a grant of the Korea Health Technology R&D Project through the Korea Health Industry Development Institute (KHIDI), funded by the Ministry of Health & Welfare (RS-2025-02173063), and by a grant from Ministry of Food and Drug Safety (26202MFDS013) in 2026, South Korea.

Data availability Not applicable for review articles.

Declarations

Ethics approval and consent to participate Not applicable.

Consent for publication Not applicable.

Competing interests The authors declare no competing interests.

References

1. World Health Organization (2024) Global tuberculosis report 2024. World Health Organization, Geneva
2. Lange C, Aaby P, Behr MA et al (2022) 100 years of *Mycobacterium bovis* bacille Calmette-Guerin. Lancet Infect Dis 22(1):e2–e12. https://doi.org/10.1016/S1473-3099(21)00403-5
3. Andersen P, Doherty TM (2005) The success and failure of BCG - implications for a novel tuberculosis vaccine. Nat Rev Microbiol 3(8):656–662. https://doi.org/10.1038/nrmicro1211
4. Fine PE (1995) Variation in protection by BCG: implications of and for heterologous immunity. Lancet 346(8986):1339–1345. https://doi.org/10.1016/s0140-6736(95)92348-9
5. Bright MR, Curtis N, Messina NL (2021) The role of antibodies in Bacille Calmette Guerin-mediated immune responses and protection against tuberculosis in humans: A systematic review. Tuberculosis (Edinb) 131:101947. https://doi.org/10.1016/j.tube.2020.101947
6. Kleinnijenhuis J, Quintin J, Preijers F et al (2014) Long-lasting effects of BCG vaccination on both heterologous Th1/Th17 responses and innate trained immunity. J Innate Immun 6(2):152–158. https://doi.org/10.1159/000355628
7. Perdomo C, Zedler U, Kuhl AA et al (2016) Mucosal BCG vaccination induces protective lung-resident memory T cell populations against tuberculosis. mBio 7(6). https://doi.org/10.1128/mBio.01686-16
8. Darrah PA, Zeppa JJ, Maiello P et al (2020) Prevention of tuberculosis in macaques after intravenous BCG immunization. Nature 577(7788):95–102. https://doi.org/10.1038/s41586-019-1817-8
9. Larson EC, Ellis-Connell AL, Rodgers MA et al (2023) Intravenous Bacille Calmette-Guerin vaccination protects simian immunodeficiency virus-infected macaques from tuberculosis. Nat Microbiol 8(11):2080–2092. https://doi.org/10.1038/s41564-023-01503-x
10. Peters JM, Irvine EB, Makatsa MS et al (2025) High-dose intravenous BCG vaccination induces enhanced immune signaling in the airways. Sci Adv 11(1):eadq8229. https://doi.org/10.1126/sciadv.adq8229
11. Simonson AW, Zeppa JJ, Bucsan AN et al (2025) Intravenous BCG-mediated protection against tuberculosis requires $CD4^+$ T cells and $CD8\alpha^+$ lymphocytes. J Exp Med 222(4). https://doi.org/10.1084/jem.20241571
12. Xu Z, Li X, Xia A et al (2023) Activation dynamics of antigen presenting cells *in vivo* against *Mycobacterium bovis* BCG in different immunized route. BMC Immunol 24(1):48. https://doi.org/10.1186/s12865-023-00589-6
13. Kaufmann E, Sanz J, Dunn JL et al (2018) BCG educates hematopoietic stem cells to generate protective innate immunity against Tuberculosis. Cell 172(1–2):176–190 e119. https://doi.org/10.1016/j.cell.2017.12.031
14. Mangtani P, Abubakar I, Ariti C et al (2014) Protection by BCG vaccine against tuberculosis: a systematic review of randomized controlled trials. Clin Infect Dis 58(4):470–480. https://doi.org/10.1093/cid/cit790
15. Bull NC, Kaveh DA, Garcia-Pelayo MC et al (2018) Induction and maintenance of a phenotypically heterogeneous lung tissue-resident $CD4^+$ T cell population following BCG immunisation. Vaccine 36(37):5625–5635. https://doi.org/10.1016/j.vaccine.2018.07.035
16. Koeken V, van der Pasch ES, Leijte GP et al (2020) The effect of BCG vaccination on alveolar macrophages obtained from induced sputum from healthy volunteers. Cytokine 133:155135. https://doi.org/10.1016/j.cyto.2020.155135
17. Lyu J, Narum DE, Baldwin SL et al (2024) Understanding the development of tuberculous granulomas: insights into host protection and pathogenesis, a review in humans and animals. Front Immunol 15:1427559. https://doi.org/10.3389/fimmu.2024.1427559
18. Abubakar I, Pimpin L, Ariti C et al (2013) Systematic review and meta-analysis of the current evidence on the duration of protection by bacillus Calmette-Guerin vaccination against tuberculosis. Health Technol Assess 17(37):1–372. https://doi.org/10.3310/hta17370., v-vi

19. Roy A, Eisenhut M, Harris RJ et al (2014) Effect of BCG vaccination against *Mycobacterium tuberculosis* infection in children: systematic review and meta-analysis. BMJ 349:g4643. https://doi.org/10.1136/bmj.g4643
20. Bull NC, Stylianou E, Kaveh DA et al (2019) Enhanced protection conferred by mucosal BCG vaccination associates with presence of antigen-specific lung tissue-resident PD-1^{+} KLRG1^{-} CD4^{+} T cells. Mucosal Immunol 12(2):555–564. https://doi.org/10.1038/s41385-018-0109-1
21. Dijkman K, Sombroek CC, Vervenne RAW et al (2019) Prevention of tuberculosis infection and disease by local BCG in repeatedly exposed rhesus macaques. Nat Med 25(2):255–262. https://doi.org/10.1038/s41591-018-0319-9
22. Counoupas C, Ferrell KC, Ashhurst A et al (2020) Mucosal delivery of a multistage subunit vaccine promotes development of lung-resident memory T cells and affords interleukin-17-dependent protection against pulmonary tuberculosis. NPJ Vaccines 5(1):105. https://doi.org/10.1038/s41541-020-00255-7
23. Das S, Marin ND, Esaulova E et al (2021) Lung epithelial signaling mediates early vaccine-induced CD4^{+} T cell activation and *Mycobacterium tuberculosis* control. mBio 12(4):e0146821. https://doi.org/10.1128/mBio.01468-21
24. Stylianou E, Paul MJ, Reljic R et al (2019) Mucosal delivery of tuberculosis vaccines: a review of current approaches and challenges. Expert Rev Vaccines 18(12):1271–1284. https://doi.org/10.1080/14760584.2019.1692657
25. Wozniak TM, Saunders BM, Ryan AA et al (2010) *Mycobacterium bovis* BCG-specific Th17 cells confer partial protection against *Mycobacterium tuberculosis* infection in the absence of gamma interferon. Infect Immun 78(10):4187–4194. https://doi.org/10.1128/IAI.01392-09
26. Creighton RL, Woodrow KA (2019) Microneedle-mediated vaccine delivery to the oral mucosa. Adv Healthc Mater 8(4):e1801180. https://doi.org/10.1002/adhm.201801180
27. Hoft DF, Xia M, Zhang GL et al (2018) PO and ID BCG vaccination in humans induce distinct mucosal and systemic immune responses and CD4^{+} T cell transcriptomal molecular signatures. Mucosal Immunol 11(2):486–495. https://doi.org/10.1038/mi.2017.67
28. Vierboom MPM, Dijkman K, Sombroek CC et al (2021) Stronger induction of trained immunity by mucosal BCG or MTBVAC vaccination compared to standard intradermal vaccination. Cell Rep Med 2(1):100185. https://doi.org/10.1016/j.xcrm.2020.100185
29. Peng X, Zhou Y, Zhang B et al (2024) Mucosal recombinant BCG vaccine induces lung-resident memory macrophages and enhances trained immunity via mTORC2/HK1-mediated metabolic rewiring. J Biol Chem 300(1):105518. https://doi.org/10.1016/j.jbc.2023.105518
30. Kollmann TR, Amenyogbe N, Schaltz-Buchholzer F et al (2025) Is systemic dissemination of BCG following neonatal vaccination required for protection against *Mycobacterium tuberculosis*? J Infect Dis 231(6):e1019–e1021. https://doi.org/10.1093/infdis/jiaf051
31. Kurtz SL, Mittereder LR, Lehman CC et al (2023) Intravenous BCG vaccination of diversity outbred mice results in moderately enhanced protection against challenge with *Mycobacterium tuberculosis* compared to intradermal vaccination. Infect Immun 91(7):e0016823. https://doi.org/10.1128/iai.00168-23
32. Fredsgaard-Jones T, Harris SA, Morrison H et al (2024) A dose escalation study to evaluate the safety of an aerosol BCG infection in previously BCG-vaccinated healthy human UK adults. Front Immunol 15:1427371. https://doi.org/10.3389/fimmu.2024.1427371
33. Puri M, Miranda-Hernandez S, Subbian S et al (2023) Repurposing mucosal delivery devices for live attenuated tuberculosis vaccines. Front Immunol 14:1159084. https://doi.org/10.3389/fimmu.2023.1159084
34. Marshall JL, Satti I, Surakhy M et al (2025) Early mucosal responses following a randomised controlled human inhaled infection with attenuated *Mycobacterium bovis* BCG. Nat Commun 16(1):4989. https://doi.org/10.1038/s41467-025-60285-4
35. Darrah PA, Zeppa JJ, Wang C et al (2023) Airway T cells are a correlate of i.v. Bacille Calmette-Guerin-mediated protection against tuberculosis in rhesus macaques. Cell Host Microbe 31(6):962–977e968. https://doi.org/10.1016/j.chom.2023.05.006
36. Singh S, Bolz M, Cornelius A et al (2022) Intravenous BCG driven antigen recognition in a murine tuberculosis model. Comp Immunol Microbiol Infect Dis 87:101838. https://doi.org/10.1016/j.cimid.2022.101838
37. Acosta A, Sarmiento ME, Gonzalez A et al (1994) Histopathologic and humoral study of Balb/c mice inoculated with BCG by different routes. Arch Med Res 25(2):159–163
38. Muggleton PW, Prince GH, Hilton ML (1975) Effect of intravenous B.C.G. in guineapigs and pertinence to cancer immunotherapy in man. Lancet 1(7921):1353–1355. https://doi.org/10.1016/s0140-6736(75)92261-8
39. Liu YE, Darrah PA, Zeppa JJ et al (2023) Blood transcriptional correlates of BCG-induced protection against tuberculosis in rhesus macaques. Cell Rep Med 4(7):101096. https://doi.org/10.1016/j.xcrm.2023.101096
40. Irvine EB, O'Neil A, Darrah PA et al (2021) Robust IgM responses following intravenous vaccination with Bacille Calmette-Guerin associate with prevention of *Mycobacterium tuberculosis* infection in macaques. Nat Immunol 22(12):1515–1523. https://doi.org/10.1038/s41590-021-01066-1
41. Morrison AL, Sarfas C, Sibley L et al (2023) IV BCG vaccination and aerosol BCG revaccination induce mycobacteria-responsive gδ T cells associated with protective efficacy against *M. tb* challenge. Vaccines (Basel) 11(10). https://doi.org/10.3390/vaccines11101604
42. Wang S, Myers AJ, Irvine EB et al (2024) Markov field network model of multi-modal data predicts effects of immune system perturbations on intravenous BCG vaccination in macaques. Cell Syst 15(12):1278–1294e1274. https://doi.org/10.1016/j.cels.2024.10.001
43. Irvine EB, Darrah PA, Wang S et al (2024) Humoral correlates of protection against *Mycobacterium tuberculosis* following intravenous BCG vaccination in rhesus macaques. iScience 27(12):111128. https://doi.org/10.1016/j.isci.2024.111128
44. Jauro S, Larson EC, Gleim JL et al (2024) Intravenous Bacillus Calmette-Guerin (BCG) induces a more potent airway and lung immune response than intradermal BCG in Simian Immunodeficiency Virus-infected Macaques. J Immunol 213(9):1358–1370. https://doi.org/10.4049/jimmunol.2400417
45. Nagatake T, Suzuki H, Hirata SI et al (2018) Immunological association of inducible bronchus-associated lymphoid tissue organogenesis in Ag85B-rHPIV2 vaccine-induced anti-tuberculosis mucosal immune responses in mice. Int Immunol 30(10):471–481. https://doi.org/10.1093/intimm/dxy046
46. Lee A, Floyd K, Wu S et al (2024) BCG vaccination stimulates integrated organ immunity by feedback of the adaptive immune response to imprint prolonged innate antiviral resistance. Nat Immunol 25(1):41–53. https://doi.org/10.1038/s41590-023-01700-0
47. Smith AA, Su H, Wallach J et al (2025) A BCG kill switch strain protects against *Mycobacterium tuberculosis* in mice and non-human primates with improved safety and immunogenicity. Nat Microbiol 10(2):468–481. https://doi.org/10.1038/s41564-024-01895-4
48. Yamazaki-Nakashimada MA, Unzueta A, Berenise Gamez-Gonzalez L et al (2020) BCG: a vaccine with multiple faces. Hum

Vaccin Immunother 16(8):1841–1850. https://doi.org/10.1080/21645515.2019.1706930
49. Joslyn LR, Pienaar E, DiFazio RM et al (2018) Integrating non-human primate, human, and mathematical studies to determine the influence of BCG timing on H56 vaccine outcomes. Front Microbiol 9:1734. https://doi.org/10.3389/fmicb.2018.01734
50. De Voss CJ, Korompis M, Li S et al (2025) Novel mRNA vaccines induce potent immunogenicity and afford protection against tuberculosis. Front Immunol 16:1540359. https://doi.org/10.3389/fimmu.2025.1540359
51. Ding Y, Li Y, Wu Z et al (2025) A novel nanoparticle vaccine displaying multistage tuberculosis antigens confers protection in mice infected with H37Rv. NPJ Vaccines 10(1):173. https://doi.org/10.1038/s41541-025-01216-8
52. Hoseinpour R, Hasani A, Baradaran B et al (2024) Tuberculosis vaccine developments and efficient delivery systems: A comprehensive appraisal. Heliyon 10(4):e26193. https://doi.org/10.1016/j.heliyon.2024.e26193
53. Arts RJ, Joosten LA, Netea MG (2016) Immunometabolic circuits in trained immunity. Semin Immunol 28(5):425–430. https://doi.org/10.1016/j.smim.2016.09.002
54. Cirovic B, de Bree LCJ, Groh L et al (2020) BCG vaccination in humans elicits trained immunity via the hematopoietic progenitor compartment. Cell Host Microbe 28(2):322–334e325. https://doi.org/10.1016/j.chom.2020.05.014
55. Fanucchi S, Dominguez-Andres J, Joosten LAB et al (2021) The intersection of epigenetics and metabolism in trained immunity. Immunity 54(1):32–43. https://doi.org/10.1016/j.immuni.2020.10.011
56. Kang A, Ye G, Singh R et al (2023) Subcutaneous BCG vaccination protects against streptococcal pneumonia via regulating innate immune responses in the lung. EMBO Mol Med 15(7):e17084. https://doi.org/10.15252/emmm.202217084
57. Khader SA, Divangahi M, Hanekom W et al (2019) Targeting innate immunity for tuberculosis vaccination. J Clin Invest 129(9):3482–3491. https://doi.org/10.1172/JCI128877
58. Netea MG, Schlitzer A, Placek K et al (2019) Innate and adaptive immune memory: an evolutionary continuum in the host's response to pathogens. Cell Host Microbe 25(1):13–26. https://doi.org/10.1016/j.chom.2018.12.006
59. Xu JC, Chen ZY, Huang XJ et al (2024) Multi-omics analysis reveals that linoleic acid metabolism is associated with variations of trained immunity induced by distinct BCG strains. Sci Adv 10(14):eadk8093. https://doi.org/10.1126/sciadv.adk8093
60. Minute L, Bergon-Gutierrez M, Mata-Martinez P et al (2024) Heat-killed *Mycobacterium tuberculosis* induces trained immunity *in vitro* and *in vivo* administered systemically or intranasally. iScience 27(2):108869. https://doi.org/10.1016/j.isci.2024.108869
61. Bosteels V, Janssens S (2025) Striking a balance: new perspectives on homeostatic dendritic cell maturation. Nat Rev Immunol 25(2):125–140. https://doi.org/10.1038/s41577-024-01079-5
62. Cabeza-Cabrerizo M, Cardoso A, Minutti CM et al (2021) Dendritic cells revisited. Annu Rev Immunol 39:131–166. https://doi.org/10.1146/annurev-immunol-061020-053707
63. Kawasaki T, Ikegawa M, Kawai T (2022) Antigen presentation in the lung. Front Immunol 13:860915. https://doi.org/10.3389/fimmu.2022.860915
64. Moreo E, Jarit-Cabanillas A, Robles-Vera I et al (2023) Intravenous administration of BCG in mice promotes natural killer and T cell-mediated antitumor immunity in the lung. Nat Commun 14(1):6090. https://doi.org/10.1038/s41467-023-41768-8
65. Gallego A, Vargas JA, Castejon R et al (2003) Production of intracellular IL-2, TNF-alpha, and IFN-gamma by T cells in B-CLL. Cytometry B Clin Cytom 56(1):23–29. https://doi.org/10.1002/cyto.b.10052
66. Caccamo N, Guggino G, Joosten SA et al (2010) Multifunctional $CD4^+$ T cells correlate with active Mycobacterium tuberculosis infection. Eur J Immunol 40(8):2211–2220. https://doi.org/10.1002/eji.201040455
67. Derrick SC, Yabe IM, Yang A et al (2011) Vaccine-induced anti-tuberculosis protective immunity in mice correlates with the magnitude and quality of multifunctional CD4 T cells. Vaccine 29(16):2902–2909. https://doi.org/10.1016/j.vaccine.2011.02.010
68. Lukeman H, Al-Wassiti H, Fabb SA et al (2025) An LNP-mRNA vaccine modulates innate cell trafficking and promotes polyfunctional Th1 $CD4^+$ T cell responses to enhance BCG-induced protective immunity against *Mycobacterium tuberculosis*. EBioMedicine 113:105599. https://doi.org/10.1016/j.ebiom.2025.105599
69. Sakai S, Mayer-Barber KD, Barber DL (2014) Defining features of protective CD4 T cell responses to *Mycobacterium tuberculosis*. Curr Opin Immunol 29:137–142. https://doi.org/10.1016/j.coi.2014.06.003
70. Cooper AM, Dalton DK, Stewart TA et al (1993) Disseminated tuberculosis in interferon gamma gene-disrupted mice. J Exp Med 178(6):2243–2247. https://doi.org/10.1084/jem.178.6.2243
71. Flynn JL, Chan J, Triebold KJ et al (1993) An essential role for interferon gamma in resistance to *Mycobacterium tuberculosis* infection. J Exp Med 178(6):2249–2254. https://doi.org/10.1084/jem.178.6.2249
72. Lange J, Rivera-Ballesteros O, Buggert M (2022) Human mucosal tissue-resident memory T cells in health and disease. Mucosal Immunol 15(3):389–397. https://doi.org/10.1038/s41385-021-00467-7
73. Zheng MZM, Wakim LM (2022) Tissue resident memory T cells in the respiratory tract. Mucosal Immunol 15(3):379–388. https://doi.org/10.1038/s41385-021-00461-z
74. Gebhardt T, Wakim LM, Eidsmo L et al (2009) Memory T cells in nonlymphoid tissue that provide enhanced local immunity during infection with herpes simplex virus. Nat Immunol 10(5):524–530. https://doi.org/10.1038/ni.1718
75. Ogongo P, Tezera LB, Ardain A et al (2021) Tissue-resident-like $CD4^+$ T cells secreting IL-17 control *Mycobacterium tuberculosis* in the human lung. J Clin Invest 131(10). https://doi.org/10.1172/JCI142014
76. Wu Q, Kang S, Huang J et al (2021) Antigen-specific tissue-resident memory T cells in the respiratory system were generated following intranasal vaccination of mice with BCG. J Immunol Res 2021:6660379. https://doi.org/10.1155/2021/6660379
77. Mani V, Bromley SK, Aijo T et al (2019) Migratory DCs activate TGF-β to precondition naive $CD8^+$ T cells for tissue-resident memory fate. Science 366(6462). https://doi.org/10.1126/science.aav5728
78. Strutt TM, Dhume K, Finn CM et al (2018) IL-15 supports the generation of protective lung-resident memory CD4 T cells. Mucosal Immunol 11(3):668–680. https://doi.org/10.1038/mi.2017.101
79. Lu LL, Smith MT, Yu KKQ et al (2019) IFN-g-independent immune markers of *Mycobacterium tuberculosis* exposure. Nat Med 25(6):977–987. https://doi.org/10.1038/s41591-019-0441-3
80. Boyaka PN (2017) Inducing mucosal IgA: A challenge for vaccine adjuvants and delivery systems. J Immunol 199(1):9–16. https://doi.org/10.4049/jimmunol.1601775
81. Kumar R, Kolloli A, Singh P et al (2024) The innate memory response of macrophages to *Mycobacterium tuberculosis* is shaped by the nature of the antigenic stimuli. Microbiol Spectr 12(8):e0047324. https://doi.org/10.1128/spectrum.00473-24
82. Marin ND, Dunlap MD, Kaushal D et al (2019) Friend or foe: The protective and pathological roles of inducible bronchus-associated lymphoid tissue in pulmonary diseases. J Immunol 202(9):2519–2526. https://doi.org/10.4049/jimmunol.1801135

83. Rijnink WF, Ottenhoff THM, Joosten SA (2021) B-cells and antibodies as contributors to effector immune responses in tuberculosis. Front Immunol 12:640168. https://doi.org/10.3389/fimmu.2021.640168
84. Kaushal D, Foreman TW, Gautam US et al (2015) Mucosal vaccination with attenuated *Mycobacterium tuberculosis* induces strong central memory responses and protects against tuberculosis. Nat Commun 6:8533. https://doi.org/10.1038/ncomms9533
85. Koti M, Morales A, Graham CH et al (2020) BCG vaccine and COVID-19: implications for infection prophylaxis and cancer immunotherapy. J Immunother Cancer 8(2). https://doi.org/10.1136/jitc-2020-001119
86. Brazier B, McShane H (2020) Towards new TB vaccines. Semin Immunopathol 42(3):315–331. https://doi.org/10.1007/s00281-020-00794-0
87. Hwang JY, Randall TD, Silva-Sanchez A (2016) Inducible bronchus-associated lymphoid tissue: Taming inflammation in the lung. Front Immunol 7:258. https://doi.org/10.3389/fimmu.2016.00258
88. Silva-Sanchez A, Randall TD (2020) Role of iBALT in respiratory immunity. Curr Top Microbiol Immunol 426:21–43. https://doi.org/10.1007/82_2019_191
89. Dieli F, Ivanyi J (2022) Role of antibodies in vaccine-mediated protection against tuberculosis. Cell Mol Immunol 19(7):758–760. https://doi.org/10.1038/s41423-022-00861-6
90. Foster M, Hill PC, Setiabudiawan TP et al (2021) BCG-induced protection against *Mycobacterium tuberculosis* infection: Evidence, mechanisms, and implications for next-generation vaccines. Immunol Rev 301(1):122–144. https://doi.org/10.1111/imr.12965
91. Netea MG, Quintin J, van der Meer JW (2011) Trained immunity: a memory for innate host defense. Cell Host Microbe 9(5):355–361. https://doi.org/10.1016/j.chom.2011.04.006
92. Netea MG, Joosten LA, Latz E et al (2016) Trained immunity: A program of innate immune memory in health and disease. Science 352(6284):aaf1098. https://doi.org/10.1126/science.aaf1098
93. Netea MG, Dominguez-Andres J, Barreiro LB et al (2020) Defining trained immunity and its role in health and disease. Nat Rev Immunol 20(6):375–388. https://doi.org/10.1038/s41577-020-0285-6
94. Hole CR, Wager CML, Castro-Lopez N et al (2019) Induction of memory-like dendritic cell responses *in vivo*. Nat Commun 10(1):2955. https://doi.org/10.1038/s41467-019-10486-5
95. Arts RJW, Carvalho A, La Rocca C et al (2016) Immunometabolic pathways in BCG-induced trained immunity. Cell Rep 17(10):2562–2571. https://doi.org/10.1016/j.celrep.2016.11.011
96. de Araujo A, de Queiroz N, Marinho FV et al (2023) Bacillus Calmette-Guerin-trained macrophages elicit a protective inflammatory response against the pathogenic bacteria *Brucella abortus*. J Immunol 211(5):791–803. https://doi.org/10.4049/jimmunol.2200642
97. de Araujo A, Mambelli F, Sanches RO et al (2023) Current understanding of Bacillus Calmette-Guerin-mediated trained immunity and its perspectives for controlling intracellular infections. Pathogens 12(12). https://doi.org/10.3390/pathogens12121386
98. Kaufmann E, Khan N, Tran KA et al (2022) BCG vaccination provides protection against IAV but not SARS-CoV-2. Cell Rep 38(10):110502. https://doi.org/10.1016/j.celrep.2022.110502
99. Kleinnijenhuis J, Quintin J, Preijers F et al (2012) Bacille Calmette-Guerin induces NOD2-dependent nonspecific protection from reinfection via epigenetic reprogramming of monocytes. Proc Natl Acad Sci U S A 109(43):17537–17542. https://doi.org/10.1073/pnas.1202870109
100. Koeken V, Verrall AJ, Netea MG et al (2019) Trained innate immunity and resistance to *Mycobacterium tuberculosis* infection. Clin Microbiol Infect 25(12):1468–1472. https://doi.org/10.1016/j.cmi.2019.02.015
101. Mitroulis I, Ruppova K, Wang B et al (2018) Modulation of myelopoiesis progenitors is an integral component of trained immunity. Cell 172(1–2):147–161. https://doi.org/10.1016/j.cell.2017.11.034. e112
102. O'Neill LAJ, Netea MG (2020) BCG-induced trained immunity: can it offer protection against COVID-19? Nat Rev Immunol 20(6):335–337. https://doi.org/10.1038/s41577-020-0337-y
103. Zhang BZ, Shuai H, Gong HR et al (2022) Bacillus Calmette-Guerin-induced trained immunity protects against SARS-CoV-2 challenge in K18-hACE2 mice. JCI Insight 7(11). https://doi.org/10.1172/jci.insight.157393
104. Atallah A, Grossman A, Nauman RW et al (2024) Systemic versus localized Bacillus Calmette Guerin immunotherapy of bladder cancer promotes an anti-tumoral microenvironment: Novel role of trained immunity. Int J Cancer 155(2):352–364. https://doi.org/10.1002/ijc.34897
105. Hilligan KL, Darrah PA, Seder RA et al (2025) Deconvoluting the interplay of innate and adaptive immunity in BCG-induced nonspecific and TB-specific host resistance. J Exp Med 222(4). https://doi.org/10.1084/jem.20240496
106. De Leeuw E, Hammad H (2024) The role of dendritic cells in respiratory viral infection. Eur Respir Rev 33(172). https://doi.org/10.1183/16000617.0250-2023
107. Mayer-Barber KD, Barber DL (2015) Innate and adaptive cellular immune responses to *Mycobacterium tuberculosis* infection. Cold Spring Harb Perspect Med 5(12). https://doi.org/10.1101/cshperspect.a018424
108. Moser M, Murphy KM (2000) Dendritic cell regulation of TH1-TH2 development. Nat Immunol 1(3):199–205. https://doi.org/10.1038/79734
109. Rodrigues TS, Conti BJ, Fraga-Silva TFC et al (2020) Interplay between alveolar epithelial and dendritic cells and *Mycobacterium tuberculosis*. J Leukoc Biol 108(4):1139–1156. https://doi.org/10.1002/JLB.4MR0520-112R
110. Schreiber HA, Sandor M (2010) The role of dendritic cells in mycobacterium-induced granulomas. Immunol Lett 130(1–2):26–31. https://doi.org/10.1016/j.imlet.2009.12.009
111. Semmrich M, Plantinga M, Svensson-Frej M et al (2012) Directed antigen targeting *in vivo* identifies a role for $CD103^+$ dendritic cells in both tolerogenic and immunogenic T-cell responses. Mucosal Immunol 5(2):150–160. https://doi.org/10.1038/mi.2011.61
112. Sulczewski FB, Martino LA, Almeida BDS et al (2020) Conventional type 1 dendritic cells induce T_H 1, T_H 1-like follicular helper T cells and regulatory T cells after antigen boost via DEC205 receptor. Eur J Immunol 50(12):1895–1911. https://doi.org/10.1002/eji.202048694
113. Henry CJ, Ornelles DA, Mitchell LM et al (2008) IL-12 produced by dendritic cells augments $CD8^+$ T cell activation through the production of the chemokines CCL1 and CCL17. J Immunol 181(12):8576–8584. https://doi.org/10.4049/jimmunol.181.12.8576
114. Khan A, Singh VK, Mishra A et al (2020) NOD2/RIG-I activating inarigivir adjuvant enhances the efficacy of BCG vaccine against tuberculosis in mice. Front Immunol 11:592333. https://doi.org/10.3389/fimmu.2020.592333
115. Zhou Y, Wei Y, Tian X et al (2025) Cancer vaccines: current status and future directions. J Hematol Oncol 18(1):18. https://doi.org/10.1186/s13045-025-01670-w
116. Carbone FR (2023) Unique properties of tissue-resident memory T cells in the lungs: implications for COVID-19 and other respiratory diseases. Nat Rev Immunol 23(5):329–335. https://doi.org/10.1038/s41577-022-00815-z

117. Christo SN, Park SL, Mueller SN et al (2024) The multifaceted role of tissue-resident memory T cells. Annu Rev Immunol 42(1):317–345. https://doi.org/10.1146/annurev-immunol-101320-020220
118. Yu S, Lao S, Yang B et al (2021) Tissue-resident memory-like $CD8^+$ T cells exhibit heterogeneous characteristics in tuberculous pleural effusion. J Immunol Res 2021:6643808. https://doi.org/10.1155/2021/6643808
119. Schreiner D, King CG (2018) $CD4^+$ memory T cells at home in the tissue: Mechanisms for health and disease. Front Immunol 9:2394. https://doi.org/10.3389/fimmu.2018.02394
120. Davies LRL, Smith MT, Cizmeci D et al (2023) IFN-g independent markers of *Mycobacterium tuberculosis* exposure among male South African gold miners. EBioMedicine 93:104678. https://doi.org/10.1016/j.ebiom.2023.104678
121. Setiabudiawan TP, Apriani L, Verrall AJ et al (2025) Immune correlates of early clearance of *Mycobacterium tuberculosis* among tuberculosis household contacts in Indonesia. Nat Commun 16(1):309. https://doi.org/10.1038/s41467-024-55501-6
122. Ishida E, Corrigan DT, Chen T et al (2024) Mucosal and systemic antigen-specific antibody responses correlate with protection against active tuberculosis in nonhuman primates. EBioMedicine 99:104897. https://doi.org/10.1016/j.ebiom.2023.104897
123. Jacobs AJ, Mongkolsapaya J, Screaton GR et al (2016) Antibodies and tuberculosis. Tuberculosis (Edinb) 101:102–113. https://doi.org/10.1016/j.tube.2016.08.001
124. Peralta Alvarez MP, Downward K, White A et al (2024) Intravenous BCG vaccination in non-human primates induces superior serum antibody titers with enhanced avidity and opsonizing capacity compared to the intradermal route. Vaccine 42(26):126444. https://doi.org/10.1016/j.vaccine.2024.126444
125. Sage PT, Sharpe AH (2015) T follicular regulatory cells in the regulation of B cell responses. Trends Immunol 36(7):410–418. https://doi.org/10.1016/j.it.2015.05.005
126. Ng TW, Wirchnianski AS, Wec AZ et al (2020) Exploiting pre-existing $CD4^+$ T cell help from Bacille Calmette-Guerin vaccination to improve antiviral antibody responses. J Immunol 205(2):425–437. https://doi.org/10.4049/jimmunol.2000191
127. Crotty S (2015) A brief history of T cell help to B cells. Nat Rev Immunol 15(3):185–189. https://doi.org/10.1038/nri3803
128. Schaerli P, Willimann K, Lang AB et al (2000) CXC chemokine receptor 5 expression defines follicular homing T cells with B cell helper function. J Exp Med 192(11):1553–1562. https://doi.org/10.1084/jem.192.11.1553
129. Soares AP, Scriba TJ, Joseph S et al (2008) Bacillus Calmette-Guerin vaccination of human newborns induces T cells with complex cytokine and phenotypic profiles. J Immunol 180(5):3569–3577. https://doi.org/10.4049/jimmunol.180.5.3569
130. Stebegg M, Kumar SD, Silva-Cayetano A et al (2018) Regulation of the germinal center response. Front Immunol 9:2469. https://doi.org/10.3389/fimmu.2018.02469
131. Swanson RV, Gupta A, Foreman TW et al (2023) Antigen-specific B cells direct T follicular-like helper cells into lymphoid follicles to mediate *Mycobacterium tuberculosis* control. Nat Immunol 24(5):855–868. https://doi.org/10.1038/s41590-023-01476-3
132. Achkar JM, Chan J, Casadevall A (2015) B cells and antibodies in the defense against *Mycobacterium tuberculosis* infection. Immunol Rev 264(1):167–181. https://doi.org/10.1111/imr.12276
133. Xu ZZ, Chen X, Hu T et al (2016) Evaluation of immunogenicity and protective efficacy elicited by *Mycobacterium bovis* BCG overexpressing Ag85A protein against *Mycobacterium tuberculosis* aerosol infection. Front Cell Infect Microbiol 6:3. https://doi.org/10.3389/fcimb.2016.00003
134. Lee H, Park SH, Shin EC (2024) IL-15 in T-cell responses and immunopathogenesis. Immune Netw 24(1):e11. https://doi.org/10.4110/in.2024.24.e11
135. Tran AC, Diogo GR, Paul MJ et al (2020) Mucosal therapy of multi-drug resistant tuberculosis with IgA and Interferon-g. Front Immunol 11:582833. https://doi.org/10.3389/fimmu.2020.582833
136. Mikami Y, Hayatsu M, Kuroda E et al (2025) Characterization of bronchus-associated lymphoid tissue induced by co-exposure to Asian sand dust and ovalbumin: a study using 3D serial section imaging. Front Immunol 16:1578255. https://doi.org/10.3389/fimmu.2025.1578255
137. Camarasa TMN, Iseppi L, Schreiner D et al (2025) Tertiary lymphoid structures in tuberculosis: Persistence, protection, and pathology. Immunol Rev 333(1):e70055. https://doi.org/10.1111/imr.70055
138. Sato Y, Silina K, van den Broek M et al (2023) The roles of tertiary lymphoid structures in chronic diseases. Nat Rev Nephrol 19(8):525–537. https://doi.org/10.1038/s41581-023-00706-z
139. Hwang JY, Silva-Sanchez A, Carragher DM et al (2020) Inducible bronchus-associated lymphoid tissue (iBALT) attenuates pulmonary pathology in a mouse model of allergic airway disease. Front Immunol 11:570661. https://doi.org/10.3389/fimmu.2020.570661
140. Dunlap MD, Prince OA, Rangel-Moreno J et al (2020) Formation of lung inducible bronchus associated lymphoid tissue is regulated by *Mycobacterium tuberculosis* expressed determinants. Front Immunol 11:1325. https://doi.org/10.3389/fimmu.2020.01325
141. Karki R, Sharma BR, Tuladhar S et al (2021) Synergism of TNF-alpha and IFN-gamma triggers inflammatory cell death, tissue damage, and mortality in SARS-CoV-2 infection and cytokine shock syndromes. Cell 184(1):149–168e117. https://doi.org/10.1016/j.cell.2020.11.025
142. Tran KA, Pernet E, Sadeghi M et al (2024) BCG immunization induces $CX3CR1^{hi}$ effector memory T cells to provide cross-protection via IFN-gamma-mediated trained immunity. Nat Immunol 25(3):418–431. https://doi.org/10.1038/s41590-023-01739-z
143. Inafuku M, Matsuzaki G, Oku H (2015) Intravenous *Mycobacterium bovis* Bacillus Calmette-Guerin ameliorates nonalcoholic fatty liver disease in obese, diabetic ob/ob mice. PLoS ONE 10(6):e0128676. https://doi.org/10.1371/journal.pone.0128676
144. Dias HF, Fu JF, Luck TG et al (2024) The spleen assumes a major role in blood glucose regulation in type 1 diabetes patients treated with BCG. Sci Rep 14(1):17611. https://doi.org/10.1038/s41598-024-67905-x
145. Han J, Gu X, Li Y et al (2020) Mechanisms of BCG in the treatment of bladder cancer-current understanding and the prospect. Biomed Pharmacother 129:110393. https://doi.org/10.1016/j.biopha.2020.110393
146. Jurczak M, Druszczynska M (2025) Beyond tuberculosis: the surprising immunological benefits of the Bacillus Calmette-Guerin (BCG) vaccine in infectious, auto-immune, and inflammatory diseases. Pathogens 14(2). https://doi.org/10.3390/pathogens14020196
147. Torracinta L, Gogichadze N, Tanner R (2025) Immune mechanisms mediating the heterologous effects of BCG vaccination: a systematic review. Front Immunol 16:1567111. https://doi.org/10.3389/fimmu.2025.1567111
148. Coler RN, Day TA, Ellis R et al (2018) The TLR-4 agonist adjuvant, GLA-SE, improves magnitude and quality of immune responses elicited by the ID93 tuberculosis vaccine: first-in-human trial. NPJ Vaccines 3:34. https://doi.org/10.1038/s41541-018-0057-5
149. Kwon KW, Choi E, Kim H et al (2025) Adjunctive beneficial effect of c-di-GMP, a STING agonist, in enhancing protective efficacy of TLR4-adjuvanted tuberculosis subunit vaccine formulations. J Biomed Sci 32(1):52. https://doi.org/10.1186/s12929-025-01144-8