100 years of *Mycobacterium bovis* bacille Calmette-Guérin

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***Mycobacterium bovis* bacille Calmette-Guérin (BCG), an experimental vaccine designed to protect cattle from bovine tuberculosis, was administered for the first time to a newborn baby in Paris in 1921. Over the past century, BCG has saved tens of millions of lives and has been given to more humans than any other vaccine. It remains the sole tuberculosis vaccine licensed for use in humans. BCG provides long-lasting strong protection against miliary and meningeal tuberculosis in children, but it is less effective for the prevention of pulmonary tuberculosis, especially in adults. Evidence mainly from the past two decades suggests that BCG has non-specific benefits against non-tuberculous infections in newborn babies and in older adults, and offers immunotherapeutic benefit in certain malignancies such as non-muscle invasive bladder cancer. However, as a live attenuated vaccine, BCG can cause localised or disseminated infections in immunocompromised hosts, which can also occur following intravesical installation of BCG for the treatment of bladder cancer. The legacy of BCG includes fundamental discoveries about tuberculosis-specific and non-specific immunity and the demonstration that tuberculosis is a vaccine-preventable disease, providing a foundation for new vaccines to hasten tuberculosis elimination.**

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Introduction

Tuberculosis is the leading cause of death by a bacterial infectious disease worldwide.[1] Global tuberculosis control is limited by the absence of a vaccine that effectively protects people exposed to *Mycobacterium tuberculosis* from infection and disease.[1] The only vaccine licensed for the prevention of tuberculosis is *Mycobacterium bovis* bacille Calmette-Guérin (BCG), a vaccine based on attenuation of bacteria naturally causing tuberculosis in cattle, other animals, and occasionally humans. Analogous to the development of the smallpox vaccine by Edward Jenner in 1796, Léon Charles Albert Calmette (1863–1933; a physician) and Jean-Marie Camille Guérin (1872–1961; a veterinarian) applied the *similia similibus curentur* (like cures like) principle to develop a tuberculosis vaccine (figure 1). First administered to a neonate in Paris in 1921,[2] BCG has probably been administered to more humans than any other vaccine for the prevention of infectious disease. In 2019, 88% of children globally received BCG vaccination during their first year of life.[3] The BCG vaccine offers greater than 70% protection against disseminated tuberculosis and tuberculous meningitis in neonates and school-age children. Nevertheless, vaccine efficacy is much lower in adults.[4] In addition to preventing tuberculosis and reducing tuberculosis-specific morbidity and mortality, epidemiological studies show non-specific benefits of childhood BCG vaccination for prevention of other communicable and non-communicable diseases and improved overall survival.[5–7] The mechanism underlying these heterologous or non-specific effects, mediated by a combination of trained innate and adaptive immunity, has only begun to be unravelled.[8–10]

In celebration of the 100th anniversary of BCG use in humans, we provide a historical review of the development and application of the BCG vaccine and a perspective on the current status of and future prospects for tuberculosis vaccination.

The origin of BCG: from Koch's tuberculin to BCG

Robert Koch's identification of *M tuberculosis* as the causative agent of tuberculosis in 1882 clearly demarcated a new era.[11] Nevertheless, only in 1901 did Koch accept that two different organisms caused human and bovine tuberculosis (*M tuberculosis vs M bovis*). Although it was clear that *M bovis*-contaminated cow's milk caused a substantial proportion of tuberculosis cases, notably in young children, Koch considered *M bovis* transmission from cattle to humans to be negligible. More serious was Koch's 1890 claim to have discovered a tuberculosis vaccine, termed tuberculin, that he considered effective for tuberculosis prevention and therapy.[11] Tuberculin was a concentrated liquid culture of *M tuberculosis* with bacteria removed by filtration; today it would be defined as a subunit vaccine comprising *M tuberculosis* proteins and glycolipids serving as antigen and adjuvant, respectively. Unfortunately, in numerous clinical studies, tuberculin was ineffective. Emil von Behring, Koch, and their colleagues attempted to reduce the virulence of *M tuberculosis* without success.[2,12–14] Mycobacteria from

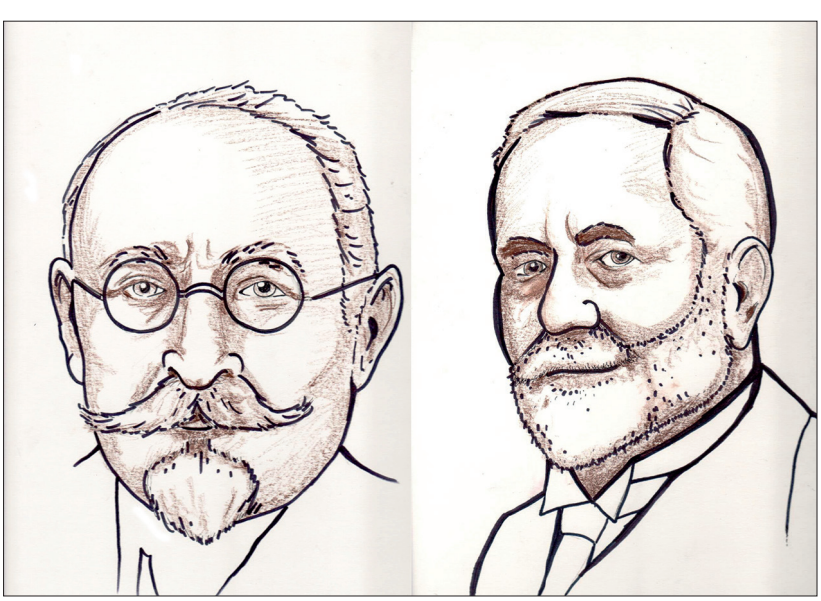

Figure 1: **The founders of BCG**
Jean-Marie Camille Guérin (1872–1961), left, and Léon Charles Albert Calmette (1863–1933), right.

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other host species, including avian-derived bacilli or mycobacteria derived from cold-blooded animals, such as turtles or blind worms, were investigated, but their efficacy was low and short-lived.[2,15] Researchers repeatedly failed to inactivate experimental vaccines by heat, chemical treatment, and solvents.[2,16,17]

Calmette and Guérin finally concluded that the only way forward was development of a live attenuated vaccine. They followed the strategy developed by their mentor, Louis Pasteur, of performing serial passages of the bacterium on a medium thought to affect virulence and testing the success of attenuation in animal models.[18] In 1906, development of BCG began with the treatment of *M bovis* with ox bile that altered the appearance of the bacilli.[2] When *M bovis* was cultured on potato slices soaked with ox bile and glycerine, the dry-crumbly colonies became smooth with a greenish-brownish colour. The mycobacteria grew on these solid cultures for 3–4 weeks; thereafter, transfer to new cultures was required. Accordingly, between 1908 and 1920, Calmette and Guérin renewed their cultures every 3 weeks, reaching 230 passages in 1920. The resulting strain was innocuous for a variety of animal species usually susceptible to *M bovis*. During these years, the bacterium lost a nine-gene locus (encoding for proteins Rv3871, PE35, PPE68, ESAT-6, CFP-10, Rv3876, Rv3877, Rv3878, and Rv3879c), referred to as region of difference 1 (RD1) as it is present in virulent *M bovis* and absent from all BCG strains.[19] Gene complementation and gene deletion studies have confirmed the importance of the RD1 locus for full virulence of the bacterium.[19–21] In addition to being of reduced virulence, the bacterium—when injected intravenously—protected cattle against challenge infection for approximately 1·5 years, especially newborn calves.[2] Even high bacillary doses were safe in guinea pigs, rabbits, and non-human primates. Although tuberculosis foci developed in the lungs, lymph nodes, liver, and spleen, caseation was never observed and pathological alterations soon resolved. Ultimately, efficacy trials in animals revealed that multidose oral administration held the greatest promise.[2]

On the basis of these observations, on July 18, 1921, Calmette, Guérin, and Benjamin Weill-Hallé performed the first vaccination of a neonate.[2] The neonate received three 2 mg BCG doses suspended in milk; neither disease nor adverse events developed during the subsequent 5·5 years. Between July, 1921, and July, 1922, 120 additional infants were vaccinated and the vaccination schedule evolved to three 1 mg applications every second day within 10 days post-birth (total $1·2\times10^9$ bacilli). BCG vaccination initially targeted newborn babies living in tuberculosis-affected families. By Feb 1, 1927, 21 200 infants had received BCG vaccination.[2] 1 year later, the intradermal route, still used today, was found to be more reliable because it allowed precise and reproducible dosing[22] (of note, oral delivery of BCG is still used to control tuberculosis in both domestic livestock and wildlife[23]). Moreover, intradermal administration was found to induce 100% tuberculin skin test conversion with a reduction of 10–15% over 5 years. By contrast, oral application induced 40–85% conversion with a loss of 40–60% over 1 year.[24]

Calmette observed that among 8075 vaccinated children mortality was only 4·6%, whereas among non-vaccinated children it was at least 16%; this finding suggested that BCG not only reduced tuberculosis-specific mortality but also substantially reduced all-cause infant mortality. In a 1931 lecture, Calmette provided two speculative explanations for the higher general mortality in non-vaccinated versus vaccinated neonates: first, "Can it be that tuberculous infection plays a more important part in infant mortality than we have supposed?" Or second, "…does the harbouring of BCG, followed by its digestion and elimination, confer on the organism a special aptitude to resist those other infections which are so frequent in young children?"[25]

Although it is possible that tuberculous infections might have been underdiagnosed, the second alternative describing protection against other infections appears more intriguing and foreshadowed later observations of BCG's heterologous or non-specific protection.

The Lübeck disaster

By 1929, in France alone, approximately 250 000 neonates had received oral BCG without noticeable adverse events. However, strong concerns remained that reversion to pathogenicity of any live vaccine was an ever-present threat.[26] During the second half of 1929, George Deycke and Ernst Altstaedt, the medical personnel responsible for tuberculosis control in Lübeck, Germany, requested a supply of BCG from Calmette for an immunisation programme targeting infants born into tuberculosis-endemic environments.

After delivery, the vaccine was unfortunately stored and processed in a hospital laboratory where virulent *M tuberculosis* was also stored. During the spring of 1930, BCG vaccination was offered to all 412 newborn babies in Lübeck. Tragically, 251 of these newborn babies received oral BCG that had been inadvertently contaminated by virulent *M tuberculosis*. Subsequently, the majority of them developed tuberculosis and 72 infants died from this (figure 2).[27]

An in-depth assessment of the disaster, coordinated by Albert Moegling,[27] found no evidence that BCG had reverted to pathogenicity; the disaster was rather the consequence of negligent contamination of BCG.[28]

Different BCG strains: current vaccination policies

Following demonstration of BCG safety and efficacy in France in the 1920s, there was global interest in introducing BCG to countries with a high prevalence of tuberculosis. The introduction of BCG faced challenges such as identifying the target population and ensuring consistent manufacturing.[29] From a production

standpoint, one key challenge was that BCG was not a defined biochemical entity; it was a living, mutating microbe propagated in the laboratory by transferring clumps of bacteria at regular intervals into fresh media.[30]

The donor laboratory (Institut Pasteur) propagated BCG until 1961 and provided unique passage numbers with each BCG lot sent to requesting laboratories, where it was grown on non-standardised culture media. Hence, BCG was mutating both at the Institut Pasteur and at other BCG production laboratories worldwide for 40–50 years[31] (depending on when laboratories created lyophilised seed lots), resulting in genetically distinct BCG strains.[32,33] This early history of BCG propagation leads to three pertinent questions: (1) are BCG strains phenotypically different in the laboratory; (2) do different BCG strains have similar rates of adverse effects; and (3) do different BCG strains confer differing levels of protection?

A number of phenotypic differences have been noted between BCG strains, when conducting in-vitro biochemical analyses.[34,35] For some strains, the responsible mutation has been causally identified.[36,37] For others, an in-vitro phenotype has been noted but not confidently assigned to one specific mutation. As an example of the latter, a higher concentration of isoniazid is required to inhibit BCG-Denmark growth in the laboratory; the clinical significance of this remains undetermined.[38] Some of the in-vitro variants have been subject to in-vivo study in experimental infection models. While it is clear that licensed BCG vaccines differ markedly in their content of viable mycobacteria, possibly contributing to formulation-dependent activation of innate and adaptive immunity and distinct protective effects,[39] it has not been shown that this affects protection afforded against an *M tuberculosis* challenge.[40]

Although genetic sequencing has conclusively documented that BCG strains are different[33] and distinct levels of virulence might be explained by strain-specific duplications and deletions of genomic DNA,[41] a more pressing concern is whether BCG strains behave differently in the more than 100 million infants vaccinated each year. Natural experiments in strain-change have been associated with higher or lower rates of adverse events,[42] but changes have also been seen when the same strain is made by a different producer.[43] Likewise, natural experiments of BCG strain-change have been associated with fluctuations in the rate of childhood tuberculosis, but clinical trials looking at protection against tuberculosis that used two strains are few, and none have directly compared early BCG strains that produce antigenic proteins, such as MPT64, MPT70, and MPT83, with late strains that fail to make these proteins (table 1). Currently, India uses both an early strain (BCG-Russia) and a late strain (BCG-Denmark); pharmacosurveillance in this large country with a high tuberculosis burden might provide data on relative rates of adverse effects and disease.

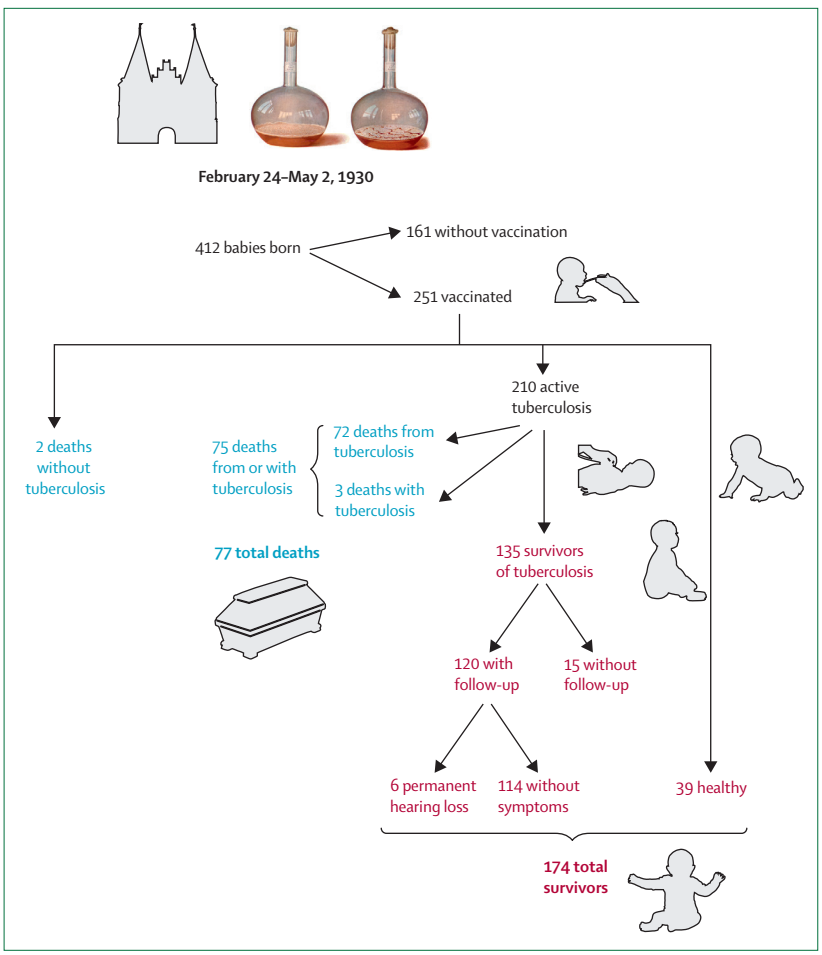

Figure 2: **The unfolding of the BCG disaster in Lübeck in 1930**
Note that three of the 251 infants were vaccinated before the start of the BCG campaign on Feb 24, 1930.

National BCG vaccination policies vary broadly with respect to dosing and strains[47] and are catalogued in the BCG World Atlas.[48] Because studies have shown minimal or no evidence of additional benefit in the protection against tuberculosis or for overall survival from repeat BCG vaccination,[49] WHO does not recommend revaccination even if the tuberculin skin test is negative. Countries with a low tuberculosis prevalence might selectively vaccinate high-risk neonates, such as those born to mothers with pulmonary tuberculosis or into families originating from countries with high tuberculosis prevalence.

For **BCG World Atlas** see http://www.bcgatlas.org

Prevention and reduction of morbidity and mortality

For the prevention of pulmonary tuberculosis, clinical trials have estimated BCG vaccine efficacy to range broadly from 0% in the Chingleput trial in south India (rate ratio 1·05, 95% CI 0·88–1·25)[4,50] to 80% in the UK Medical Research Council trial (0·22, 0·16–0·31).[4,51] There are many hypothesised reasons for this wide

	Current manufacturer(s)[44]	Produces antigenic proteins MPT64, MPT70, and MPT83[45]	Produces the virulence lipids PDIM and PGL[46]	Efficacy in randomised trial[4]
BCG-Russia	Microgen; Serum Institute of India	Yes	Yes	Not tested
BCG-Sofia	BulBio	Yes	Not reported	Not tested
BCG-Tokyo	Japan BCG laboratory	Yes	No	Not tested
BCG-Moreau	Fundação Ataulpho de Paiva	Yes	No	Not tested
BCG-Denmark	AJ Biologics; Green Signal Pharma	No	Yes	Variable (0–80%)
BCG-China	China National Biotec	Yes	Not reported	Not tested
BCG-Tice	Merck	No	Yes	Variable (0–90%)
BCG-Glaxo	Aventis Pasteur	No	No	~30%
BCG-Pasteur	ANLIS, Argentina; BioFarma, Indonesia; Pasteur Institute of Iran, Iran; Institute of Virology, Vaccines and Sera (Torlak), Serbia; Institut de Pasteur Tunis, Tunisia; Institute of Vaccines and Medical Biologicals, Vietnam	No	Yes	Variable (0–75%)

PDIM=phthiocerol dimycocerosates. PGL=phenolic glycolipid.

Table 1: **Summary of currently produced BCG strains**

	Age at vaccination	Study excluded tuberculin-reactive individuals	Efficacy* RR (95% CI)	Between-study heterogeneity (τ^2)
Infants	Neonatal	Assumed non-reactive	0·41 (0·29–0·58)	0·0065
Children	School age	Yes	0·26 (0·18–0·37)	0·048
Children	School age	No	0·59 (0·35–1·01)	0·095
Mixed community and institutional	Older than school age	Yes	0·88 (0·5–1·31)	0
Mixed community and occupational	Older than school age	No	0·81 (0·55–1·22)	0·091

Data are from Mangtani and colleagues' sytematic review on protection against tuberculosis by BCG.[4] RR=rate ratio.
*Random effects within strata summary effect estimate.

Table 2: **BCG efficacy against pulmonary tuberculosis stratified by age at vaccination and study design**

variation, including age at and time since vaccination,[52–55] sex,[56] risk of tuberculosis in the study population,[57] geographical latitude of the study setting,[58,59] and study design.[55] The protective effect of BCG is inversely associated with age at vaccination, with neonatal vaccination affording greater protection than vaccination of older children and adults (table 2).[4] Furthermore, BCG efficacy appears to be higher in studies that use tuberculin skin testing to stringently limit eligibility to participants with no evidence of previous *M tuberculosis* infection compared with studies that include participants with reactive tuberculin skin tests.[4] There is a direct linear relationship between the protective effect of BCG and the distance of the study setting from the equator.[4,59,60] The possible causes for this variation (eg, a higher prevalence of non-tuberculous mycobacteria in equatorial regions) remain speculative, and the explanation is likely to be multifactorial.[58]

There is evidence indicating that BCG vaccination affords protection in high-risk populations in settings with a low burden of tuberculosis, while being a cost-effective strategy.[61,62] Similarly, computer-simulated modelling suggests that, within the US population, tuberculosis elimination can be achieved through the use of targeted BCG vaccination combined with targeted preventive treatment.[63] Key assumptions in this model include low risks of *M tuberculosis* infection, multidrug-resistant tuberculosis, and HIV infection.

The greatest benefit of BCG immunisation, which drives its high cost-effectiveness, is prevention of paediatric tuberculosis, particularly extrapulmonary tuberculosis in young children.[64] In this age group, the potential impact of BCG is amplified due to children's high risk of progression to severe forms of tuberculosis.[65] The protective effect of vaccination against all forms of paediatric tuberculosis has been estimated at 0·74 (95% CI 0·62–0·83) in randomised controlled trials (RCTs), and 0·52 (0·38–0·64) in case-control studies.[66] When analysis is limited to laboratory-confirmed tuberculosis reported in case-control studies, estimated effectiveness against tuberculosis is 0·83 (0·58–0·93).[66] Among children without evidence of previous *M tuberculosis* infection, the protective effect of BCG increases to 0·92 (rate ratio 0·08 [0·03–0·25]).[4] The greatest impact of BCG has been shown in infants, in whom the protective effect is 0·90 (rate ratio 0·10 [0·01–0·77])[4,67,68] for the prevention of meningeal and miliary tuberculosis.

BCG also prevents primary infection with *M tuberculosis*. Among European children completing testing for latent infection with *M tuberculosis*, previous BCG vaccination was associated with a negative result (QuantiFERON-TB Gold In-Tube: odds ratio [OR] 0·41, p<0·001; T-SPOT.TB: OR 0·41, p<0·001).[69] Among adults with recent household tuberculosis exposure, there was also a negative association between BCG vaccination and latent tuberculosis infection (adjusted OR 0·70, 95% CI 0·56–0·87; p=0·0017) yielding a vaccine efficacy of 0·30.[70] Although vaccine efficacy declined with time since vaccination, the prevalence of latent tuberculosis infection was lower among vaccinated than non-vaccinated participants, even 20 years or more after vaccination. Similarly, among adolescents in a tuberculosis high-risk setting, BCG revaccination reduced the rate of sustained QuantiFERON-TB Gold In-Tube conversion with an efficacy of 0·45 (p=0·03).[71]

Complications of BCG applications

As a live vaccine, BCG can cause localised adverse reactions including hypersensitivity, abscess formation, and regional lymphadenitis when applied by intradermal injection.[72–75] These complications are mostly self-limiting and rarely require surgical or medical interventions. The risk of regional suppurative lymphadenitis following intradermal vaccination of children younger than 1 year is less than five per 1000 in most countries (range 0·0006–36 cases per 1000).[76]

Children who develop disseminated BCG infection usually have an underlying genetic or acquired immunodeficiency. The risk of disseminated BCG disease was 0·06–1·56 per million vaccinated children in the pre-HIV era.[77] Today, HIV infection is the most common cause for disseminated BCG disease in neonates. In a South African multicentre surveillance study, the pooled incidence for disseminated BCG disease in vaccinated children living with HIV was 992 (95% CI 567–1495) per 100 000.[78] After a decade of debate regarding the risk–benefit ratio of BCG in people living with HIV, WHO guidelines recommend that adults, children, and neonates living with HIV should receive BCG vaccination if they are stable on antiretroviral therapy.[79] Furthermore, the revised WHO guidelines recommend that in settings with high HIV and tuberculosis burden, all healthy neonates should receive a single dose of neonatal BCG.[79]

BCG vaccination is one of the most common causes of death in children with primary immunodeficiencies worldwide,[74] with an overall lethality of 50% and higher.[80] Genetic risk factors include severe combined immune deficiency, chronic granulomatous disease, combined immunodeficiency, leukocyte adhesion deficiency type 1, and mendelian susceptibility to mycobacterial disease.[81–85]

Intravesical BCG application is the preferred treatment for high-risk non-muscle-invasive bladder cancer (NMBIC) and an optional treatment for intermediate-risk NMIBC.[86,87] Intravesical BCG therapy of NMIBC can be complicated by local adverse events such as cystitis, bladder contracture, bladder ulceration, granulomatous balanitis, epididymo-orchitis, prostatitis, urethritis, and kidney infections, or systemic complications including mycotic aneurysms, granulomatous hepatitis, reactive arthritis, spondylitis, miliary tuberculosis (appendix p 1), and rarely (1 in 15 000 patients) sepsis.[88–90] Treatment requires anti-tuberculosis therapy over 9 months or longer. Like other strains of *M bovis*, BCG is intrinsically resistant to pyrazinamide.[91]

Non-specific benefits of BCG vaccination

BCG immunotherapy induces initial complete response rates of 55–65% for high-risk NMIBCs and 70–75% for carcinoma in situ; however, 40% of patients will eventually relapse despite initially successful BCG immunotherapy.[92] BCG is administered once a week for 6 weeks intravesically, followed by maintenance therapy for 1–3 years, depending on the risk of tumour progression. BCG induces a complex immunological cascade of innate and adaptive immune responses resulting in cell-mediated tumour-specific killing of urothelial carcinoma cells.[92] Compared with BCG vaccination, the number of bacteria given for intravesical therapy is orders of magnitude greater (~10^6 bacilli for vaccination; ~10^9 bacilli for bladder cancer).

In the 1970s to 1980s, the measles vaccine was found to have major beneficial effect for non-measles-specific child survival.[93] Observational studies examining other childhood vaccines suggested that BCG also had major non-specific benefits.[94,95] Because neonatal BCG was recommended by WHO, it was impossible to test these benefits in RCTs. To circumvent observational bias, studies compared BCG-vaccinated children with and without a BCG scar[6] and interpreted differences in mortality to reflect the non-specific benefits of effective versus ineffective BCG vaccination. BCG scarring was linked to the quality of vaccination (supervision, quantity, depth of vaccination, and BCG strain). In the most recent meta-analysis, BCG scarring was associated with a 52% (95% CI 38–63) reduction in infant mortality.[5] It is, however, important to underline that the presence of the BCG scar could alternatively signify a stronger immune response itself, which could subsequently explain the association with improved outcome.

In Guinea-Bissau, recommendations against BCG vaccination of low-birthweight children created an opportunity for randomised trials. In three RCTs, random assignment to receive BCG-Denmark at hospital discharge was associated with a 38% (95% CI 17–54) reduction in neonatal mortality.[96] Similar effects were found for physician-diagnosed non-tuberculous disease in an RCT of BCG-Denmark in Uganda.[97] RCTs from intensive care units have not found similar non-specific effects,[98] possibly because of differences in BCG strain or cause-specific mortality. BCG's non-specific benefits primarily result from reduction in neonatal sepsis and respiratory infections.[96,99] BCG also provides protection against leprosy (overall protective effect of 26% [95% CI 14–37] with an enhanced effect after revaccination),[100] non-tuberculous mycobacteria lymphadenitis (relative risk 0·04 [95% CI 0·01–0·21]),[101] and Buruli ulcer (0·50 [0·37–0·69]).[101–103]

Child survival is correlated with the quality of BCG vaccination, which is assessed by the size of early reactions, scarring, and tuberculin skin test responses.[104] BCG vaccination enhances non-specific effects for child survival.[105] Notably, non-specific effects are much stronger if the child's mother has a BCG scar.[106,107]

See Online for appendix

BCG has the strongest effect on survival in the first months of life, when infants have received only two live vaccines—BCG and oral polio. Following vaccination with non-live vaccines, such as diphtheria-tetanus-pertussis (DTP), it becomes difficult to measure non-specific effects because live and non-live vaccines interact immunologically. WHO recommends neonatal BCG and the first of three doses of DTP at the age of 6 weeks. Administration of DTP after BCG reduces the non-specific benefits of BCG, and mortality is increased particularly for girls.[108] However, co-administration of BCG and DTP can partly rectify the interaction.[109] In four studies examining vaccine sequence (appendix p 2), simultaneous administration of BCG and DTP was associated with a 43% (95% CI 27–56) reduction in mortality compared with administration of DTP after BCG in accordance with WHO guidelines. Given the strong effects of BCG on child survival, vaccination coverage, age, and quality should be monitored. The

findings from these studies of BCG and DTP suggest that BCG might modify the immunological effects of other sequences of vaccines.

Research on non-specific effects has focused on childhood BCG, but there is growing interest in whether BCG administered in childhood affects adults and whether BCG's immune training used among adults can modify disease patterns. The largest study showed that BCG (together with vaccinia) was associated with 46% (95% CI 19–64) lower mortality from natural causes between 7 years and 45 years of age in Denmark.[110] BCG has been shown to modify the course of diabetes and multiple sclerosis.[111–113] Observations further suggest that BCG therapy lowers the incidence of Alzheimer's disease in patients with bladder cancer.[114] BCG given to older adults has also been associated with major reductions in respiratory infections.[115]

Given the observed non-specific effects of BCG, discontinuation of BCG vaccination programmes in countries of low tuberculosis prevalence might substantially reduce these beneficial BCG effects as well. Furthermore, BCG has also been tested extensively against COVID-19 infection in more than 20 RCTs, the largest being the BRACE trial (NCT04327206) which aims to recruit more than 10 000 health-care workers; in an RCT among older adults in Greece, BCG revaccination reduced the risk and severity of COVID-19, according to one manuscript that has not yet been peer reviewed.[116]

BCG vaccination and induction of heterologous T-cell and trained immunity

Although epidemiological evidence for non-specific beneficial effects of BCG has accumulated over the past century,[117] the lack of a biological explanation hampers recognition of the importance of these effects. One suggested mechanism through which BCG vaccination might induce beneficial non-specific effects is activation of heterologous lymphocyte responses. Studies during the 1960s demonstrated cross-protection between unrelated bacterial pathogens,[118] mediated by lymphocytes with an increased capacity for production of IFN-γ after BCG vaccination. Subsequently, macrophage activation induced by IFN-γ promoted innate immunity against a secondary infection.[119] These experimental animal studies were later complemented by human studies reporting that BCG increases IFN-γ production by peripheral blood mononuclear cells upon stimulation with unrelated microorganisms.[120] However, these data cannot explain all these effects, and increasing evidence showed that innate immune cells are also able to build memory characteristics, termed trained immunity,[121] which partially drive the heterologous protection induced by BCG.

Trained immunity induced by BCG results in improved cytokine (TNF, IL-1, IL-6) responses by monocytes and macrophages,[120] and more effective release of reactive oxygen species, antimicrobial proteases, and enhanced pathogen killing by neutrophils.[122] To achieve long-term effects, functional changes are also induced by BCG at the level of bone marrow progenitors of myeloid cells biasing towards myelopoiesis.[123,124] The memory characteristics of innate immune cells can last for months and even years.[125] The molecular processes responsible for these effects are represented by changes in chromatin accessibility due to chemical processes at the DNA (methylation) and histone (methylation, acetylation) level, leading to more effective transcription of genes important for host defense[126] (figure 3).

Next-generation vaccines

Tuberculosis vaccine research and development has produced a number of novel vaccine candidates in the clinical trial pipeline with some encouraging results.[24,128] Principally, vaccines target prevention of disease. This can be achieved in three ways, either by (1) preventing infection; (2) containing infection with dormant *M tuberculosis*; or (3) preventing recurrence in patients who have been cured of tuberculosis but who are at risk of reinfection or relapse. Vaccine strategies include inactivated or viable whole-cell vaccine approaches and subunit approaches comprising fusion proteins of selected antigens in combination with a strong T-cell stimulating adjuvant. All vaccines are considered as boost vaccines on top of BCG prime given to different age groups either before or after exposure to *M tuberculosis*; in addition, viable vaccines are also considered as BCG replacement vaccines given pre-exposure with *M tuberculosis* to neonates. Improved application regimes for BCG are being considered as well. A study in non-human primates revealed that intravenous administration of BCG induced profound, and in some cases sterilising, immunity against *M tuberculosis* challenge.[129] Moreover, a BCG revaccination trial showed approximately 50% prevention of infection as measured by sustained IFN-γ release assay conversion, which measures antigen-induced IFN-γ secretion.[71] By contrast, immediate infection was not inhibited, indicating short-term survival of *M tuberculosis* which was subsequently terminated by host mechanisms—either innate immunity, or perhaps involving training or rapid mobilisation of acquired immune memory.

Major vaccine candidates are listed in the appendix (pp 3–4). Two adjuvanted subunit vaccines have reached an advanced stage of clinical assessment. Approximately 50% of active tuberculosis was prevented by the protein-adjuvant vaccine M72:AS01$_E$ over a 3-year clinical trial period.[130] This trial comprised HIV-uninfected individuals with latent infection with *M tuberculosis*, and the result might not apply to interferon-γ release assay-negative, non-BCG-vaccinated individuals. The H56:IC31 vaccine candidate is another protein adjuvant formulation undergoing phase 2b testing of prevention of tuberculosis recurrence (NCT03512249).

RUTI is composed of detoxified fragments of *M tuberculosis* and is administered as a liposomal preparation for therapy in adjunct to canonical drug

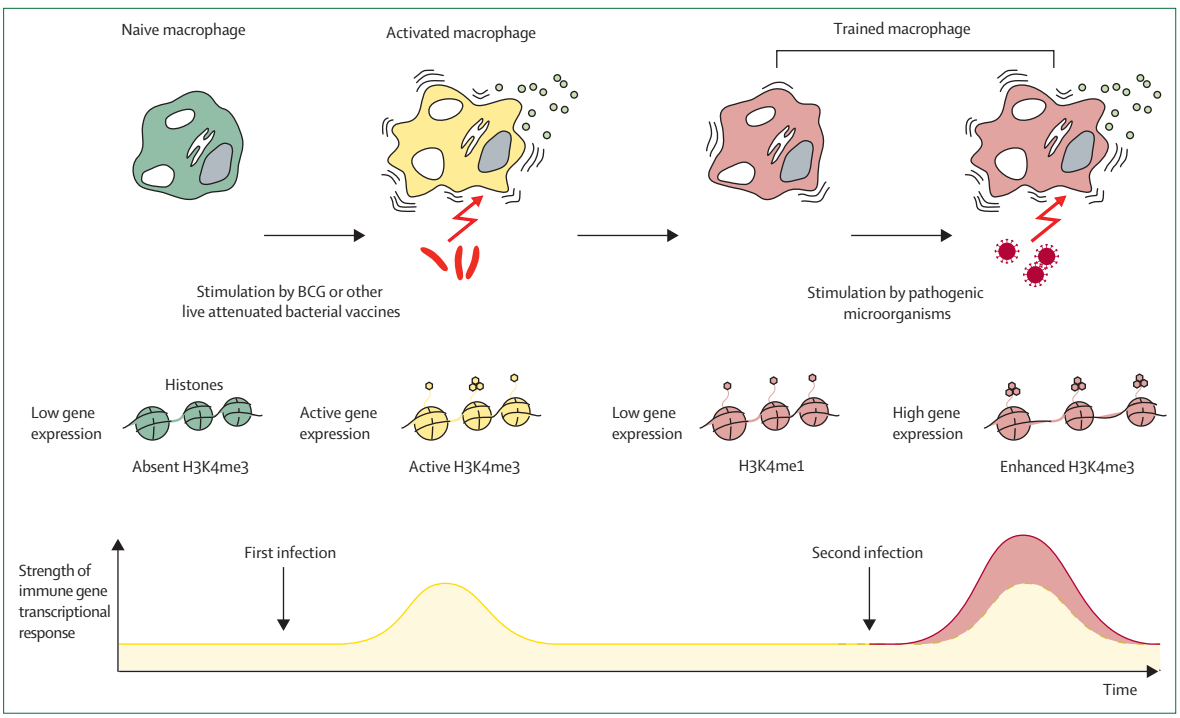

Figure 3: **Trained immunity induced by BCG**
The accessibility of chromatin, in particular of promoters and enhancers, is modulated by BCG vaccination. Upon cellular activation of innate immune cells, histone chemical modifications (eg, methylation, acetylation) result in an increased accessibility for transcription factor binding and initiation of gene transcription. Upon elimination of the stimulus, many of these changes are lost, but some histone marks (such as methylation) are preserved, bookmarking the genes important for host defense. Upon reinfection, chromatin architecture changes and induction of gene transcription can take place more quickly and strongly in an antigen-independent manner. The enhanced chromatin accessibility and gene transcription result in an improved innate immune response after BCG vaccination. Adapted from Chumakov and colleagues.[127]

treatment of tuberculosis; it is currently being evaluated in a phase 2a trial (NCT02711735).[128] DAR-901 is composed of inactivated *Mycobacterium obuense* and was found to be safe and well tolerated, but failed to prevent infection as determined by both immediate and sustained IFN-γ release in a phase 2b trial.[131] The third inactivated vaccine is based on *Mycobacterium indicus pranii* and has already been licensed as a leprosy vaccine. It demonstrated safety in a subgroup of patients with tuberculosis and provided evidence for improved bacillary clearance as determined by sputum culture conversion.[132] This candidate is currently being tested head-to-head with VPM1002 in household contacts of patients with newly diagnosed tuberculosis in India (Clinical Trials Registry India CTRI/2019/01/017026). VPM1002 is one of the two advanced viable vaccines. It is the only vaccine among all candidates in clinical evaluation that is based on BCG. It has been generated by exchanging the gene encoding urease C with the listeriolysin gene.[133] This genetic modification leads to several intracellular modifications in macrophages harbouring the vaccine, which increases immunogenicity of VPM1002 over BCG. After successful completion of phase 1 and 2 safety trials in adults and neonates[134,135] and in addition to the above-mentioned head-to-head trial, VPM1002 is also undergoing phase 3 trials in previously drug-treated patients in India to assess for prevention of recurrence (NCT03152903) and in neonates at different clinical trial sites in sub-Saharan Africa to assess for prevention of infection (NCT043451685). The other viable vaccine candidate is MTBVAC, a genetically attenuated *M tuberculosis* strain with two independent gene deletions that affect expression of a whole variety of virulence factors, but leave proteins of *M tuberculosis* (which are absent from *M bovis* BCG) unaffected. Following phase 1 and 2 trials that showed safety and immunogenicity in adults and neonates, MTBVAC is currently prepared for phase 3 efficacy trials in both age groups.[136,137] Not only are different vaccine candidates being tested, but there are also novel regimes being considered, notably aerosol vaccination to activate mucosal immunity in the lung, the major portal of entry and site of residence of *M tuberculosis*.[138–140]

Conclusion

During the past century, millions of lives have been saved by BCG vaccination due to the induction of anti-mycobacterial immunity and the prevention of tuberculosis, particularly its most severe forms. Lives have also potentially been saved by the non-specific effects of BCG vaccination on other infectious and non-communicable diseases. During the past decade, we have begun to unravel the molecular mechanisms of BCG's beneficial non-specific effects. 100 years after the

Search strategy and selection criteria

In April, 2021, a trained medical librarian did eight separate systematic literature searches, primarily using Ovid MEDLINE (MEDLINE on OvidSP) without the use of language or publication year filters. The concepts were developed using both controlled and natural languages. MeSH terms were used and keywords were gathered along with various synonyms. Search terms included "adverse reactions", "Bacille Calmette Guérin", "BCG", "BCG-itis", "bovine", "Calmette", "cattle", "collateral benefit", "coronavirus", "COVID19", "cow", "cross protection", "effectiveness", "efficacy", "drug-related side effects", "Guérin", "heterologous effect", "immunogenicity", "Lubeck disaster", "Mycobacterium bovis", "Mycobacterium tuberculosis", "nonspecific effect", "PPD", "purified protein derivative", "recombinant BCG vaccine", "Robert Koch", "SARS-CoV-2", "strains", "tuberculosis", "tubercle bacillus", "VPM1002", "vaccine potency", and "trained immunity". The keywords were searched using the title, abstract, and keyword fields within the Ovid MEDLINE database. A "Humans" filter was used on all but the Lübeck disaster search. Searches deemed low in numbers were also run in the Embase database. For continuity, after translation, the searches were all run again in each database on May 6, 2021.
Within EndNote citation manager, search results underwent automated and manual de-duplication. Thereafter, two authors (CL and AMM) manually reviewed titles to exclude literature not relevant to the topic. In addition, publications in the German, French, and English literature known to the authors were included and their references searched for relevant additional literature.

first human inoculation with BCG, we are close to improving tuberculosis vaccine effectiveness. The revitalisation of tuberculosis vaccine research and development programmes promises to propel our strides towards the WHO 2030 goal of reducing tuberculosis morbidity by 90% and mortality by 95%.[141]

Contributors

CL developed the concept and, together with AMM, did the systematic literature reviews. All authors contributed equally in drafting and revising the manuscript.

Declaration of interests

SHEK is coinventor of the tuberculosis vaccine VPM1002 and coholder of a patent licensed to Serum Institute of India, Pune, India. MGN is coholder of patents on trained immunity licensed to Trained Therapeutix Discovery. CL reports personal fees from Chiesi, Gilead, Janssen, Novartis, Oxford Immunotec, and Insmed, outside of the submitted work. All other authors declare no competing interests.

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