ISSN 1669-9106

SPECIAL ARTICLE - REVISION

MEDICINA (Buenos Aires) 2021; 81: 1007-1014

ONE HUNDRED YEARS OF BCG VACCINE

JUAN ANTONIO BARCAT*, ISABEL N. KANTOR*, VIVIANA RITACCO*†

Editorial Board, Medicina (B Aires)
**Authors are listed in alphabetical order*
†Dr. Viviana Ritacco passed away on November 14, 2021

Abstract The BCG vaccine was given for the first time in 1921, in Paris, to a newborn of a mother with tuberculosis. Between 1924 and 1960, the Pasteur Institute delivered BCG cultures to more than 50 laboratories around the world. In 1925, Dr Andrés Arena introduced the BCG seed to Argentina, where the vaccine began to be produced and applied orally to newborns. The original strain underwent diverse genetic changes in different parts of the world, which did not seem to affect its protective efficacy. In Argentina, a study (1978-1985) showed that BCG prevents primary TB in general, and has 100% efficacy in meningitis and other extra-pulmonary TB locations. BCG effect is independent of TB control measures (case detection and treatment). Furthermore, BCG provides nonspecific protection from various infections and is used in the treatment of bladder cancer. By 2020, at least five technologies had already been established for the future development of anti-TB vaccines: cellular vaccines, protein subunits, nucleic acids, with adenovirus vector, and with recombinant influenza virus as a vector. There are currently more than 20 TB vaccine candidates under evaluation. History teaches, and the COVID-19 pandemic has confirmed, that vaccination is a fundamental instrument for the control of infectious diseases. Until a more effective vaccine becomes available, BCG will continue to be included in the Argentine National Vaccination Calendar for application to newborns.

Key words: BCG vaccine, non-specific immunity, adaptive immunity, tuberculosis vaccines, Argentina

Resumen ***Cien años de vacuna BCG.*** La vacuna BCG fue administrada por primera vez en 1921, en París, a un recién nacido de madre tuberculosa. Entre 1924 y 1960, el Instituto Pasteur entregó cultivos de BCG a más de 50 laboratorios de todo el mundo. En 1925, el Dr. Andrés Arena lo introdujo en Argentina, donde se comenzó a producir y aplicar la vacuna a recién nacidos por vía oral. La cepa original sufrió múltiples cambios genéticos que, sin embargo, no parecen haber afectado su eficacia protectora, establecida aun sin que se conociera el mecanismo de acción. En Argentina, un estudio (1978-1985) demostró que la BCG previene la TB primaria en general, y en un 100% la meningitis y otras localizaciones extrapulmonares. Su efecto es independiente de las medidas de control de la TB (detección de casos y tratamiento). Además, se la usa en el tratamiento del cáncer de vejiga y provee protección inespecífica contra diversas enfermedades infecciosas. En 2020 ya se habían establecido por lo menos 5 tecnologías para el futuro desarrollo de vacunas anti-TB: vacunas celulares, de subunidades proteicas, de ácidos nucleicos, con vector adenovirus, y con virus influenza recombinante como vector. Actualmente hay más de 20 vacunas candidatas anti-TB. La historia enseña, y la pandemia de COVID-19 ha contribuido a revalorizar, que la vacunación es un instrumento fundamental para el control y la erradicación de las enfermedades infecciosas. Y hasta que haya disponible otra más eficaz, BCG seguirá figurando en el Calendario de Vacunación Nacional, para ser aplicada al recién nacido.

Palabras clave: vacuna BCG, respuesta inmune inespecífica, inmunidad adaptativa, vacunas anti-tuberculosis, Argentina

Received: 24-IX-2021 Accepted: 6-X-2021

Postal address: Isabel N. Kantor, Revista Medicina (Buenos Aires), Combatientes de Malvinas 3150, 1427 Buenos Aires, Argentina
e-mail: isabel.kantor1@gmail.com

KEY POINTS

- BCG, an attenuated *Mycobacterium bovis* strain, has been used in vaccination against tuberculosis for 100 years and has been applied to more than 4 billion children in over 180 countries. More than 120 million doses are administered annually, which has made it – until 2021 – the most widely used vaccine in the world.
- BCG protects against the disease that follows primary tuberculosis infection. It is also associated with nonspecific protection against various other infectious diseases and cancer treatment.
- The COVID-19 pandemic has confirmed that vaccination is a fundamental instrument for the control of infectious diseases.
- Although there are more than 20 anti-tuberculosis vaccine candidates in various formats (cellular, protein subunits, nucleic acids, with adenovirus vectors), BCG will continue being included in the National Vaccination Calendar for application to newborns until a more effective one is available.

BCG turns 100 and it is the only tuberculosis vaccine in use. On July 18, 1921, in the maternity nursery of the Hôpital de la Charité in Paris, the paediatrician Dr Benjamin Weill-Hallé and Dr Raymond Turpin administered 6 mg of BCG to a newborn, by mouth, in three doses. His mother, who suffered from tuberculosis, died soon after delivery and the baby was going to be in the care of his grandmother, who also suffered from tuberculosis and lived in an unhealthy setting. Days before, on July 1, Weill-Hallé had consulted Calmette (Fig. 1) about the case and the possibility of vaccinating the newborn with BCG. And, aware of their "scruples as experimenters", they decided that it was "their duty to try"[1, 2].

Albert Calmette (1863-1933) and Camille Guérin (1872-1961) had proven that their bacillus was "harmless" to bovines, laboratory animals, and even to man. A year earlier they wrote in an article: "From other experiences that do not fall within the scope of this work, we have acquired the certainty that our live bovine bacillus is harmless to man, even by intravenous inoculation at the dose of, at least, 44 000 bacilli"[3]. Who has been inoculated? It is no longer mentioned in their publications.

Six months later, the first vaccinated person was in good health, with normal development, and for this reason, Calmette, Guérin, Weill-Hallé, Turpin, and Miss Coloni (mentioned only in reference 2) decided to apply BCG to other children. This time to "([…] young children whose parents have been kind enough to allow us"). They did so from 1922 to 1924. The results were presented by Calmette, Guérin, Weill-Hallé, and their collaborators, at the session of the *Académie Nationale de Médicine* on June 24, 1924[1].

After 20 years of studies, Calmette and Guérin presented there the foundations and results of attenuating

Fig. 1.– Le Docteur Albert Calmette. Chanteclaire, 1906. B. Moloch (Alphonse H. Colomb, 1849-1909). Welcome Collection. In: *https://wellcomecollection.org/works/zt2ka9gx; 9/11/2021*

Mycobacterium bovis tuberculogenesis. They had cultured the bacillus in a medium with ox bile to hereditarily modify its physicochemical properties. They managed to attenuate it after an uninterrupted series of 230 passages that took them 13 years. These attenuated bacilli, the Bacilli Calmette Guérin, became harmless but capable of immunizing against the virulent forms of *M. bovis* and *M. tuberculosis*, in all the host species they studied, guinea pigs, rabbits, cattle, and apes.

Between 1921 and 1924, they report the results of inoculating BCG orally, in three doses, 217 infants born of mothers with tuberculosis. They lost the follow-up of 39 who came from families in the suburbs, without a fixed address. Of the remaining 178, 9 died in the 18 months following inoculation, they established the cause of death in only three, two due to bronchopneumonia and one to digestive disorders. They controlled the tuberculin skin reaction three months after inoculation in 53; negative in 47, five in contact with smear-positive patients; positive

in six, three in contact with acid-fast bacilli smear-positive tuberculosis cases. To compare results, the authors announced a second series of inoculations limited to a central and elegant district of Paris (*VI*e).

They say that they do not pretend that their BCG, a modified, avirulent and non-tuberculogenic bacillus, although "still toxic and producer of tuberculin", solves the problem. They only show their experimental results and believe that rational use of BCG can reduce mortality and morbidity of infants and subjects never exposed, in an old civilization that has coexisted with the bacillus for centuries. They are convinced that tuberculosis in adolescents and adults is mostly a late manifestation of a cradle-acquired infection. We add BCG is a controlled primary infection.

In 1931 at the Royal Society of Medicine, Calmette presents a historical and technical review of the subject coming to the centre of controversy: the possibility that BCG loses stability, mutates its virulence, and becomes pathogenic. Calmette maintains the opposite based on his own and other's experience and refutes each of the objections in a detailed analysis. He and Guérin always took into account this risk, shared with other vaccines. The so-called "Lübeck disaster" and the possibility of BCG mutation are mentioned: "Does this give us the right to conclude that no laboratory device will ever succeed in transforming BCG into a virulent bacillus, in the same way that Guérin and I managed to transform a virulent bacillus into BCG? I would be the last to sustain it!". It hadn't happened until then. He ends by saying: "What doctor, what health authority knowing these facts and with all the information now available would deliberately refuse to apply this simple method against the most virulent of human diseases?"[2]. We will see later that BCG evolves and changes its genetic, but remains immunogenic without becoming tuberculogenic.

The Lübeck disaster occurred in 1929-33. Of 251 newborns inoculated orally, 173 became ill with tuberculosis, 72 of them died. Cause: BCG contamination with an *M. tuberculosis* strain; contamination: a human error; consequence: a mistrust that persisted for many years[4]. Despite the mistrust, BCG continued to be used in almost all European countries and cities in the USA, Canada and the rest of America[5].

The BCG arrived in Argentina in 1925. Dr Andrés R. Arena (1887-1971), director of the Anti-Rabies Laboratory of the Bacteriological Institute of La Plata, brought it. In 1924, the provincial government granted him financial aid to study a vaccine produced by Dr J. Ferrán in Barcelona. After a few months of unsuccessful trials with this vaccine, Arena moved to the Pasteur Institute in Paris, where he spent just eight days before embarking back to Argentina. Calmette himself gave him a group of BCG cultures on the condition "that the vaccine should be given free of charge and the cultures should be also provided for those who requested them to carry out research experiments". Arena placed the cultures in the ship's engine room to keep them at the proper temperature. In 1925, the Bacteriological Institute in La Plata city prepared the BCG and delivered the first doses to Dr Antonio Igartua, who inoculated thirty children in the Maternity of the Midwives School in that city. We will not dwell on the bureaucratic and political vicissitudes that Arena endured throughout his career[5]. They do not surprise us. Now BCG is among the vaccines on the National Vaccination Calendar, it can be read printed on retail milk packages.

The genetic analysis of the BCG vaccine and its immunizing capacity

From 1924 to the 1960s, the Pasteur Institute delivered BCG cultures to more than 50 laboratories around the world[6-8]. There is evidence that during this period the (disappeared) original strain underwent multiple genetic changes that gave rise to various substrains. Growth in medium with glycerol (GG+) added to resistance to cycloserine (RC+) and pyrazinamide (RPZ+) are phenotypic characteristics common to all BCG substrains that allow a simple differentiation from *M. tuberculosis* (GG+, RC-, RPZ-) and *M. bovis* (GG-, RC- RPZ+). Additionally, BCG is always susceptible to fluoroquinolones, clarithromycin, doxycycline, and gentamicin.

The genomic variations observed in certain BCG substrains consist of deletions, duplications, and point mutations. Position variations in DNA sequences, single nucleotide polymorphisms (SNPs) and also duplications and deletions all occurred before 1960 when lyophilization was introduced and vaccine production was standardized. The availability of complete *M. tuberculosis* H37Rv, *M. bovis* and BCG genome sequences, allowed comparative analysis[9]. A series of chromosomal deletions common to virulent and avirulent species were identified, but a single locus, RD1 (Chromosomal Region of Difference 1), is deleted in all BCG substrains. This genetic event undoubtedly contributed to its attenuation and would explain why that condition has been maintained. After the experimental reintroduction of RD1 into the BCG chromosome, certain changes in the colonies morphology that resembled the virulent bacillus were observed, and an increase, albeit partial, in virulence for the mouse. This would indicate that the attenuation of virulence in BCG is a poly-mutational process[10].

BCG-Moreau (Brazil), BCG-Russia and BCG-Japan, obtained from the Pasteur Institute before 1926, have two copies of the *IS*6110 insertion region, while those obtained after 1931, such as BCG-Denmark, BCG-Tice and BCG-Glaxo, have a single copy of *IS*6110 and also lost the RD2 region. Furthermore, only the "early" strains have methoxy mycolic acids in the cell wall. These mycolic

acids are considered virulence factors, but it is not clear whether their loss reduces protective efficacy in "late" BCG strains[11].

In short, BCG is not a single vaccine; there are different substrains, with different properties, and even different genotypes within each substrain, which could be partly responsible for variations in immunogenicity and residual virulence[12]. There are published meta-analyses on the protection conferred by BCG in various countries and regions, as well as clinical trials comparing the efficacy of different substrains[13-16]. However, these differences have not been reliably related to protective capacity[11].

Although WHO experts recommend conducting new comparative effectiveness studies of the different substrains, they affirm that, among the validated BCG products, there is no one preferred for use in any age or risk group[13]. Global demand for BCG is estimated at ~350 million doses. Of the 19 BCG producing institutions in the world, only 4 had, in 2017, sufficient production capacity, and had passed the international quality controls (with WHO qualification) required to supply more than 169 countries, through UNICEF and WHO. The BCG strains most used today are BCG-Denmark, BCG-Japan and BCG-Bulgaria (genetically identical to BCG-Russia)[13, 14].

Mechanism of action and predictors of immunity

In BCG, as in many other vaccines, efficacy was demonstrated without understanding the mechanism of action. Later, the relationship between post-vaccination tuberculin conversion and protection was observed. Therefore, the leading role of cell-mediated immunity in the protection conferred was deduced.

Both the classic intradermal tuberculin test with PPD and the more modern interferon-gamma blood release tests are an indirect measure of infection because they detect the response of memory T cells to *M. tuberculosis* antigens. These tests do not distinguish between latent and active TB infection. In addition, they lose diagnostic capacity in subjects with a low population of CD4+ T cells. Even though the tuberculin test is imperfect, it can serve as a measure of response to vaccination, because PPD contains antigens common to members of the *M. tuberculosis* Complex. It was, and still is, widely used to measure, not only infection rates in a population but also BCG vaccination coverage. The interferon-gamma tests use purified antigens present in RD1 (ESAT-6 and CFP-10) are not useful for measuring response to vaccination because BCG lacks these antigens[17].

In most infectious diseases, the detection of antibodies in the blood is a good marker of response to vaccines, but in TB the antibodies have not been shown to provide valid information on protection. A validated indicator of BCG protection against TB is still missing.

The efficacy of BCG vaccination in Argentina

In a retrospective case-control study including 1050 children under 6 years of age, carried out in three hospitals in the Buenos Aires suburbs in 1978-1985, it was shown that BCG vaccination exerts effective prevention of child primary and disseminated TB [73% (62-82%)][18]. The proven protection was 100% for meningitis and other extrapulmonary sites, 88% for miliary TB, and 65% for pulmonary TB. When the analysis was limited to cases with bacteriological confirmation, that is, with diagnostic certainty, the percentage of protection amounted to 96%. In studies carried out in Bahia and Sao Paulo (Brazil) with BCG produced there (Moreau strain), 98 and 92% protection against TB meningitis, respectively, was found[15, 16]. In those years, in Argentina, vaccination coverage among newborns was, approximately, 77%, and freeze-dried BCGs of different origins and substrains were used (Paris 1978, Glaxo 1979-81, Japan 1984, and Laboratorio Central de Salud Pública de La Plata, Paris strain 1984-88). BCG vaccination coverage in children under 4 years of age continued to increase, reaching 97% in 2017[19-21].

Figure 2 compares BCG vaccination coverage and TB notification (total TB cases, pulmonary TB cases, and TB meningitis in children under 4 years of age) between 1980 and 2000. The decrease in child TB meningitis was markedly greater than that of total TB. Table 1 compares the decrease in the incidence of total TB cases between 2004 and 2019, which was 19%, with that of TB meningitis in children under 4 years of age, which reached 65%. Taken together, these data coincide in showing that vaccination in the first years of life exerts an effect that is independent of other TB control measures (case detection and treatment).

BCG, infection, disease and anti-TB treatment

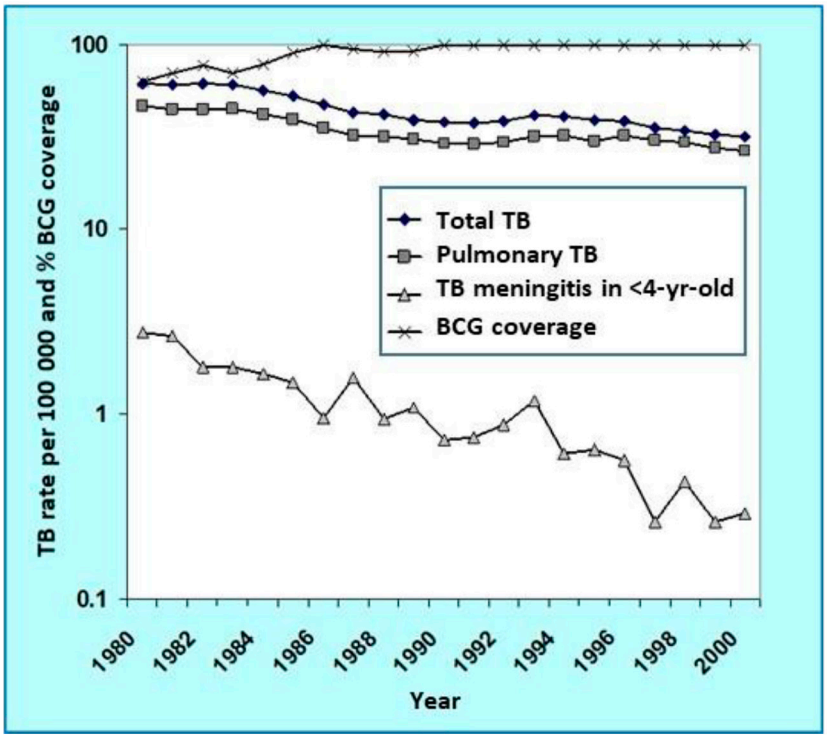
The fundamental value of BCG vaccination is to prevent hematogenous spread once *M. tuberculosis* infection is established. This is the basis for the recommendation to vaccinate the newborn, currently followed in 141 of the 194 countries that use it[13].

BCG vaccination does not prevent adult pulmonary TB, which may be due to reinfection (in highly endemic areas), reactivation and, very rarely, primary infection, but it is capable of preventing both drug-susceptible and drug-resistant or multi-resistant TB.

Would BCG vaccination also protect against *M. tuberculosis* infection?

The paucity of elements to answer this question is due to the fact that the tuberculin skin test does not distinguish

Fig. 2.– Semi-logarithmic scale comparison of rates per 100 000 inhabitants of total TB, pulmonary TB, TB meningitis in children younger than 4 years of age, and BCG coverage, Argentina, 1980 to 2000



Source: Miceli I. Tuberculosis Confederal Meeting, National Epidemiological Surveillance System (SINAVE), Epidemiology Direction, INER Coni, ANLIS Malbrán, 2006

TABLE 1.– *Incidence per 100 000 inhabitants of total TB and TB meningitis in children under 4 years of age, Argentina, 2004 vs. 2019, and percentage decrease of both rates in that period*[19, 21]

TB cases	2004 (n/100 000)	2019 (n/100 000)	Decrease (%)
All forms	32.0	26.0	19
Meningitis in children <4-yr-old	1.7	0.6	65

TB: tuberculosis

whether a positive response is due to *M. tuberculosis* infection, BCG vaccination, or non-tuberculous mycobacterial infection. Reactions due to vaccination or sensitization to other mycobacteria are, in general, smaller than those due to recent and progressive TB infection. If BCG vaccination was received 15 or more years before, it can be ignored as a cause of a positive response to PPD, especially when indurations is 15 mm wide or more[22].

In contrast, T-cell-based interferon gamma release tests (IGRA) could differentiate TB infection (IGRA+) from previous BCG vaccination and most non-tuberculous mycobacterial infections (IGRA-)[17, 22]. This allows investigating whether BCG protects not only against TB disease but also against TB infection. In a meta-analysis published in 2014, with information on the progression to active TB in child contacts of TB cases, which included 14 studies carried out in 9 countries, it was possible to establish, in 6 of them, the relationship between children vaccinated IGRA+ or IGRA-, and TB cases. Thus, BCG vaccination was associated with a reduction of latent *M. tuberculosis*

infection by 27% (RR 0.73), and TB disease by 71% (RR 0.29)[23]. The authors pointed out some limitations of the study, among them: the degree of exposure to the TB index case could not be determined, and the use of IGRA as a specific indicator of TB infection would still be subject to debate.

Nonspecific or heterologous immunity. Other uses of BCG

The innate immune system can develop immune memory and provide lasting protection against foreign invaders. BCG vaccination has been associated with protection against leprosy, Buruli ulcer, various parasites, and decrease of all-cause mortality in childhood[24, 25]. Immunotherapy with concentrated BCG in situ is effective in bladder cancer. It is also used in other types of cancer, multiple sclerosis and type 1 diabetes, among other non-infectious diseases[26]. The BCG vaccine could boost natural immunity against SARS-CoV-2 and other pathogens[27]. In particular, it is associated with protection against respiratory infections and sepsis[28]. These heterologous effects of BCG would be due to its ability to alter immune set points through T cells, as well as epigenetic changes in cells of innate immunity, a process known as "trained immunity".

The COVID-19 pandemic and BCG vaccination

The COVID-19 pandemic has drastically affected health care. Priorities were modified, both the demand for and the supply of medical consultations fell. Child vaccination coverage, including BCG, was also affected for operational reasons, which fell, in the Americas, from 97% in 2017 to 85% in 2020[19-21]. On the other hand, the COVID-19 pandemic has contributed to valuing vaccination as a basic infection control strategy. Between 2020 and 2021, more vaccines have been developed, tested and applied than ever before in history. And this has a positive effect on the development of new vaccines against TB and other endemic diseases.

New TB vaccines?

The general objective of any vaccination is not to prevent infection but to achieve lasting protection against it, and also to reduce morbidity and mortality associated with the development of the disease. BCG vaccine especially protects against the disease that follows primary TB infection. The average duration of this protection is estimated at 10 to 15 years. In addition, it is one of the safest vaccines, in terms of post-vaccination accidents or side effects[13].

So, what is expected of a new TB vaccine? There are several demands. Among them: that it can prevent TB by reinfection, and therefore be used in adolescents and young adults, who are the most frequent sources of TB transmission; that it can replace BCG in newborn vaccination, and/or be administered as a booster; that acts as an adjuvant in the treatment of multidrug-resistant TB and the prevention of recurrence after the end of anti-TB treatment, and as a post-exposure vaccine in case-contacts[29].

In 2012, some basic criteria were established for the development of anti-TB vaccines and in 2021, they were completed with a guideline, which ranges from early clinical trials on safety and immunogenicity, to implementation and efficacy trials[13, 29, 30]. At the beginning of 2020, several technologies were already established for the future development of anti-TB vaccines: inactivated and attenuated cellular vaccines, protein subunits, nucleic acids, with adenovirus vector, and with recombinant influenza virus as vector[30-32].

There are currently more than 20 proposed TB vaccines. Two of them, VPM1002, and MTBVAC, in advanced development, are cellular vaccines. There are several examples of cellular vaccines, inactivated or attenuated, against viral diseases: MMR (measles, mumps, rubella, measles, mumps and rubella, attenuated), IPV (inactivated polio vaccine), and the anti-COVID-19 Sinopharm and Sinovac (inactivated), but there are fewer aimed at preventing bacterial pathogens (*Bordetella pertussis*, in DPT).

VPM1002 is a live attenuated recombinant BCG, originating from the Max Planck Institute for Infection Biology. Results from pre-clinical trials confirmed that it is at least as safe and immunogenic as BCG. MTBVAC, developed by the University of Zaragoza, the Pasteur Institute and Biofabri, is a live attenuated *M. tuberculosis* strain. Safety and immunogenicity trials in infants and newborns have been successful.

MIP is another cellular vaccine based on the immunotherapeutic potential of inactivated *M. indicus pranii*, which is proposed as an adjuvant to treatment. On the other hand, ChAdOx1.PPE15 is a recombinant vaccine in a replication-deficient adenovirus, which expresses the mycobacterial protein PPE15. It is part of the UOXF TB Program (University of Oxford) for a BCG booster vaccination in adolescents. The inhalation route of application is tested.

Recombinant vaccines express antigens and, as they cannot replicate, they are therefore considered completely safe. Nucleic acid vaccines use genetic material (DNA or RNA) to induce the manufacture of antigenic specific proteins. In our cells, the DNA code is translated into messenger RNA (mRNA) that is used as a template to make specific antigenic proteins. mRNA vaccines can be produced rapidly in large volumes. In the case of COVID-19, this type of vaccine was developed, passed phase III tests, and has already been applied to millions

of people around the world. They also constitute a promising field of application against various endemic diseases, including TB. DNA vaccines are already used in veterinary medicine, they are simple to produce and more stable than mRNA vaccines, but they must penetrate the cell nucleus, a problem that can be solved with the use of appropriate adjuvants. Instead, mRNA vaccines only need to reach the cytoplasm[31-33].

Among the main barriers to the development of vaccines against TB are the complexity of the immune response to TB infection, and the lack of markers correlating with the protection achieved.

Animal models show evidence that the direct application of a vaccine to the respiratory mucosa, where there are different subtypes of T cells, could improve the protective immune response[13, 34, 35].

It was shown that mycobacteria release extracellular vesicles containing lipoglycans and lipoproteins that play an important role in anti-TB immunity, and could be applied in vaccine production technology as well as in diagnostic methods[36, 37]. Exosomes of macrophages infected by *M. tuberculosis* have also been found to contain proteins, such as antigen 85-C and ESAT-6, of importance in the activation of T cells against TB infection[38-40].

Conclusions

BCG, an attenuated *M. bovis* strain, has been the only anti-TB vaccine in use for 100 years, applied to more than 4 billion children in more than 180 countries. More than 120 million doses are administered annually, which made it - until 2021 - the most widely used vaccine in the world.

This year when BCG turns 100, TB will kill more than one million people, which is what it does every year. However, history teaches us, and the progress made against COVID-19 confirms it, that vaccination is a fundamental instrument for the control and eradication of infectious diseases, including TB, one that has been among us for more than 3 million years.

Until there is another more effective, widely available, and inexpensive vaccine, BCG should continue to appear in the National Vaccination Calendar and be applied to newborns before discharge from maternity[41].

Conflict of interest: None to declare

References

1. Calmette A, Guérin C, Weill-Hallé B, et al. Essais d'immunisation contre l'infection tuberculeuse. *Bull Acad Méd* 1924; 91: 787-96.
2. Calmette A. Preventive vaccination against tuberculosis with BCG. *Proc R Soc Med* 1931; 24-1481-90.
3. Calmette A, Guerin C. Nouvelles recherches expérimentales sur la vaccination des bovidés contre la tuberculose. *Ann Inst Pasteur* 1920; 34: 553-60.
4. Fox GJ, Orlova M, Schurr E. Tuberculosis in newborns: The lessons of the "Lübeck Disaster" (1929-1933). *PLoS Pathog* 2016; 12: e1005271.
5. Baudou AC. A cincuenta años de la primera aplicación de la primera vacuna antituberculosa en el hombre. Homenaje a la memoria del ex-académico de número Doctor Andrés R. Arena. *Anales de la ANAV* 1970-71; Tomo XXIV. In*: http://sedici.unlp.edu.ar/handle/10915/33037*; accessed September 2021.
6. Oettinger T, Jørgensen M, Ladefoged A, Hasløv K, Andersen P. Development of the *Mycobacterium bovis* BCG vaccine: review of the historical and biochemical evidence for a genealogical tree. *Tuber Lung Dis* 1999; 79: 243-50.
7. Abdallah MA, Behr MA. Evolution and strain variation in BCG. *Adv Exp Med Biol* 2017; 1019: 155-69.
8. Ritz N, Hanekom WA, Robins-Browne R, et al. Influence of BCG vaccine strain on the immune response and protection against tuberculosis. *FEMS Microbiol Rev* 2008; 32: 821-41.
9. Brosch R, Gordon SV, Garnier T, et al. Genome plasticity of BCG and impact on vaccine efficacy. *Proc Natl Acad Sci U S A* 2007; 104: 5596-601.
10. Pym AS, Brodin P, Brosch R, Huerre M, Cole ST. Loss of RD1 contributed to the attenuation of the live tuberculosis vaccines *Mycobacterium bovis* BCG and *Mycobacterium microti. Molecular Microb* 2002; 46: 709-17.
11. Belley A, Alexander D, Di Pietrantonio T, et al. Impact of methoxymycolic acid production by *Mycobacterium bovis* BCG vaccines. *Infect Immun* 2004; 72: 2803-9.
12. Shann F. Editorial Commentary: Different strains of Bacillus Calmette-Guérin vaccine have very different effects on tuberculosis and on unrelated infections. *Clin Infect Dis* 2015; 61: 960-2.
13. WHO. Report on BCG vaccine use for protection against mycobacterial infections including tuberculosis, leprosy, and other nontuberculous mycobacteria (NTM) infections. In: *https://www.who.int/immunization/sage/meetings/2017/october/1_BCG_report_revised_version_online.pdf?ua=1*; accessed September 2021.
14. Lange C, Aaby P, Behr MA, et al. 100 years of *Mycobacterium bovis* bacille Calmette-Guérin *Lancet Infect Dis* 2021; Published online Sept 7, 2021. In: *https://doi.org/10.1016/S1473-3099(21)00403-5*; accessed September 2021.
15. Colditz GA, Berkey CS, Mosteller F, et al. The efficacy of bacillus Calmette-Guerin vaccination of newborns and infants in the prevention of tuberculosis: metaanalysis of the published literature. *Pediatrics* 1995; 96: 29-35.
16. Trunz BB, Fine P, Dye C. Effect of BCG vaccination on childhood tuberculous meningitis and miliary tuberculosis worldwide: a metaanalysis and assessment of cost-effectiveness. *Lancet* 2006; 367: 1173-80.
17. Lalvani A, Pareek M. Interferon gamma release assays: principles and practice. *Enferm Infecc Microbiol Clin* 2010; 28: 245-52.
18. Miceli I, Kantor IN, Colaiácovo D, et al. Evaluation of the effectiveness of BCG vaccination using the case-control method in Buenos Aires, Argentina. *Int J Epidemiol* 1988, 17: 629-34.
19. Ministerio de Salud. Dirección de Estadísticas e Información de la Salud. Indicadores Básicos. In: *https://www.argentina.gob.ar/salud/deis/indicadores*; accessed September 2021.
20. OPS/OMS. Inmunización en las Américas. In: *www.paho.org/inmunizacion/boletin*; accessed September 2021.
21. Ministerio de Salud. Dirección de Estadísticas e Información de la Salud. Indicadores Básicos. In: *https://www.argentina.gob.ar/sites/default/files/indicadores_basicos_2020.pdf*; accessed September 2021.

22. Dinnes J, Deeks J, Kunst H, et al. A systematic review of rapid diagnostic tests for the detection of tuberculosis infection. *Health Technol Assess* 2007; 11: 121-7.
23. Roy A, Eisenhut M, Harris RJ, et al. Effect of BCG vaccination against Mycobacterium tuberculosis infection in children: Systematic review and meta-analysis. *BMJ* 2014; 349: g4643.
24. Schaltz-Buchholzer F, Berendsen M, Roth A, et al. BCG skin reactions by 2 months of age are associated with better survival in infancy: a prospective observational study from Guinea-Bissau. *BMJ Glob Health* 2020; 5: e002993.
25. Benn CS, Roth A, Garly ML, et al. BCG scarring and improved child survival: a combined analysis of studies of BCG scarring. *J Intern Med* 2020; 288: 614-24.
26. Singh AK, Netea MG, Bishai WR. BCG turns 100: its nontraditional uses against viruses, cancer, and immunologic diseases. *J Clin Invest* 2021; 131: e148291.
27. Kantor IN. ¿BCG *versus* COVID-19? *Medicina (B Aires)* 2020; 80: 292-4.
28. Brook B, Harbeson DJ, Shannon CP, et al. BCG vaccination-induced emergency granulopoiesis provides rapid protection from neonatal sepsis. *Sci Transl Med* 2020; 12: eaax4517.
29. Kantor IN. BCG, nuevas vacunas y la eliminación de la tuberculosis. *Medicina (B Aires)* 2019; 79: 71-3.
30. The Global TB Vaccine Partnership (GTBVP). TB Vaccine Development Pathway 2021. In: *https://www.tbvi.eu/wp-content/uploads/2021/06/Updated-TB-Development-Pathway-2021.pdf*; accessed September 2021.
31. Roorkink D, Williams A, Fritzell B. The TB vaccine development pathway – An innovative approach to accelerating global TB vaccine development. *Tuberculosis (Edinb)* 2021; 126: 102040.
32. Brazier B, McShane H. Towards new TB vaccines. *Semin Immunopathol* 2020; 42: 315-31.
33. Fan XY, Lowrie DW. Where are the RNA vaccines for TB? *Emerg Microbes Infect* 2021; 10: 1217-8.
34. Ahmed M, Jiao H, Domingo-Gonzalez R, et al. Rationalized design of a mucosal vaccine protects against *Mycobacterium tuberculosis* challenge in mice. *J Leukoc Biol* 2017; 101: 1373-81.
35. Dijkman K, Aguilo N, Boot C, et al. Pulmonary MTBVAC vaccination induces immune signatures previously correlated with prevention of tuberculosis infection. *Cell Rep Med* 2021; 2: 100187.
36. Khurana A, Allawadhie P, Khurana I, et al. Role of nanotechnology behind the success of mRNA vaccines for COVID-19. *Nano Today* 2021; 38: 101142.
37. Penn-Nicholson A, Hraha T, Thompson EG, et al. Discovery and validation of a prognostic proteomic signature for tuberculosis progression: a prospective cohort study. *PLoS Med* 2019; 16: e1002781.
38. Sun Y-F, Pi J, Xu JF. Emerging role of exosomes in tuberculosis: from immunity regulations to vaccine and immunotherapy. *Front Immunol* 2021; 12: 628973.
39. Mohammadzadeh R, Ghazvini K, Farsiani H, Soleimanpour S. *Mycobacterium tuberculosis* extracellular vesicles: exploitation for vaccine technology and diagnostic methods. *Crit Rev Microbiol* 2021; 47: 13-33.
40. Chaudhary N, Weissman D, Whitehead KA. mRNA vaccines for infectious diseases: principles, delivery and clinical translation. *Na*t Rev Drug Discov (2021). Online ahead of print. *https://doi.org/10.1038/s41573-021-00283-5*
41. Calendario Nacional de Vacunación. República Argentina. In: *https://bancos.salud.gob.ar/sites/default/files/2020-10/calendario-nacional-vacunacion-2020.pdf*; accessed September 2021.