Review

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A second-generation anti TB vaccine is long overdue
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Summary
Mycobacterium bovis BCG vaccine significantly reduces the risk of tuberculosis by 50% and continues to be used to prevent tuberculosis around the world. However, it has been shown to be ineffective in some geographical regions. The existence of different BCG strains was described more than 60 years ago, these vary in their antigenic content but the genetic mutations in BCG strains have yet been shown to affect their protection. After the declaration of tuberculosis as a global emergency in 1993, current research attempts to develop a novel more-effective vaccine. Using new technologies, recombinant, auxotroph, DNA, subunit and phylogenetically closely related mycobacteria, naturally or genetically attenuated, have been used as vaccines in animal models, but their protective efficacy, is less than that offered by the current BCG vaccine. Today it is mandatory that a major effort be made to understand how different BCG vaccine strains influence immune response and why in some cases vaccines have failed, so we can rationally develop the next generation of tuberculosis vaccines to reduce the prevalence from 10% to less than 2 % for developed countries.

Introduction
Tuberculosis (TB) was the first disease to be declared a global emergency by the World Health Organization (WHO). It is an infectious disease caused by 3 closely related *Mycobacterium* strains (*M. tuberculosis*, *M. africanum and M. bovis*), which are commonly known as the TB-complex. Human tuberculosis continues to be a major worldwide health problem and is the leading killer of youths and adults in developing countries, being responsible for approximately 3 million deaths each year. The HIV epidemic and the appearance of multidrug-resistant strains of *M. tuberculosis* (MDRTB) have contributed to the resurgence of TB. The risk of developing TB has increased and for HIV positive patients, TB enhances the progression of HIV infection to AIDS [1].

The most well known form of disease prevention is BCG vaccination, for which a variable efficacy rate from 0% to 80% has been reported [2,3,5]. In regions of the world where the disease is most widespread, BCG vaccination is ineffective and therefore, the search for novel, more effective vaccines is paramount. There are a number of possible explanations for the discrepancies in BCG protector efficacy: (i) genetic host susceptibility [5], (ii) a wide range of virulence among *M. tuberculosis* strains, (iii) progressive loss of BCG capacity to stimulate a durable immune response (iv) prevalence of other mycobacterial infections in the study population, (v) variations in protection against different forms of tuberculosis, and (vi) the level of exposure to environmental mycobacteria [6,2], however, none of these have strong clinical and research support [3].

BCG development

Albert Calmette and Camille Guèrin developed BCG between 1908 and 1921 by successfully passing an isolated strain of *M. bovis in vitro*. In the first half of the 20th Century, BCG vaccines were prepared and preserved by different manufacturing laboratories. This resulted in genotypic and phenotypic differences in the daughter strains with variations in tuberculin conversion and the frequency of adverse reactions. During this time, BCG showed a progressive decrease in virulence, the most important during the first 15 passes [7,8]. Over the next 40 years, until the freeze-dried process of the Pasteur strain in 1961 and after more than 1,000 passes, it was clear that the standardization and stability of vaccine strains needed to be enforced. This led to the adoption of the seed-lot system by the International BCG Technical Conference in 1956 [8]. Furthermore, Colditz, *et al* [3] independently quantified the detrimental or protective benefit of the vaccine in several prospective trials from case control studies and concluded that BCG significantly reduced the risk of active TB development by an average of 50%. The different criteria applied to interpret the confounding variables in the human trials make it difficult to identify the impact of BCG vaccines on protective efficacy [4].

In 1999, Behr *et al* [9] elucidate some of the molecular events that occur during BCG attenuation. Under laboratory conditions used for bacterial passes, and using microarray technology and genome sequence of *M. tuberculosis* H37Rv as a framework, they inferred the genealogy of BCG strains in an evolutionary approach, which was supported by BCG historical records [10]. These studies only led to the understanding of the genes missing from *M. bovis* leaving deeper research to be carried out to explore the association between the 61 ORF genes deleted from BCG during evolution and virulence attenuation, and may be more importantly, research into the loss in protective efficacy over the last eight decades [11]. What remains unknown is the irrefutable role that the smallest deletions, duplications and polymorphisms in the nucleotide sequence play to induce protective efficacy, as well as the production of antigens in the overall attenuation of BCG [9,12].

### Polymorphism in BCG daughter strains

Following adoption of the seed-lot system, several BCG sub-strains of the original vaccine strain are now in circulation. It is known that these sub-strains vary in certain characteristics (Table 1), such as their antigenic structure, secreted protein profiles [13-15], IS6110 copy number [11], mycolic acid pattern [16], and the characteristics and quantity of exoquelins [17]. Alterations in the mycolic acid subclass directly affect not only cell wall fluidity and permeability to hydrophobic agents, but also the ability of strains to grow within macrophages. In addition, strains with an altered cord factor have altered virulence [16,18].

Today, the most commonly used BCG sub-strains are the Pasteur, Japanese and the Glaxo (Figure 1) but other daughter strains are/or were in use (Moreau, Montreal, Russian, Prague, Danish, Birkhaugh, Australian) [12].

Table 1: Listing of gene and antigen differences presented in *Mycobacterium bovis* BCG daughter strains. The data was taken from research and historical reports. Genetic and phenotypic differences of *M. bovis* BCG daughter strains.

STRAIN	SYNONIMOUS	DELETIONS (ORF's)	MPB64	MPB70	Methoxymycolic acid's	EXOCHELIN'S μg/ml
PASTEUR		DR1, DR2, DR14 (28)	P	LOW	N	345
PHIPPS	PHILADELPHIA	DR1, DR2 (20)	P	LOW	N	ND
FRAPPIER	MONTREAL	DR1, DR2, DR8 (24)	P	LOW	N	ND
CONNAUGHT	TORONTO	DR1, DR2, DR8 (24)	P	LOW	N	ND
TICE	CHICAGO	DR1, DR2 (20)	P	LOW	N	390
DENMARK	DANISH 1331	DR1, DR2 (20)	P	LOW	N	140
GLAXO		DR1, DR2 (20)	P	LOW	N	75
PRAGUE		DR1, DR2 (20)	P	LOW	N	ND
BIRKHAUG		DR1, DR2 (20)	P	LOW	N	ND
SWEDEN	GHOTHENBURG	DR1 (9)	N	HIGH	P	ND
JAPAN	TOKYO	DR1 (9)	N	HIGH	P	150
MOREAU	BRAZIL	DR1, DR 16 (15)	N	HIGH	P	ND
RUSSIA	MOSCOW	DR1 (9)	N	HIGH	P	ND
MEXICO		DR1, DR2 (20)	P	LOW	N	ND

DR: Deleted Regions, **ORF:** Open Reading Frame, **P:** Protein or antigen expression, **N:** Non protein or antigen expression, **ND:** Non determinate.
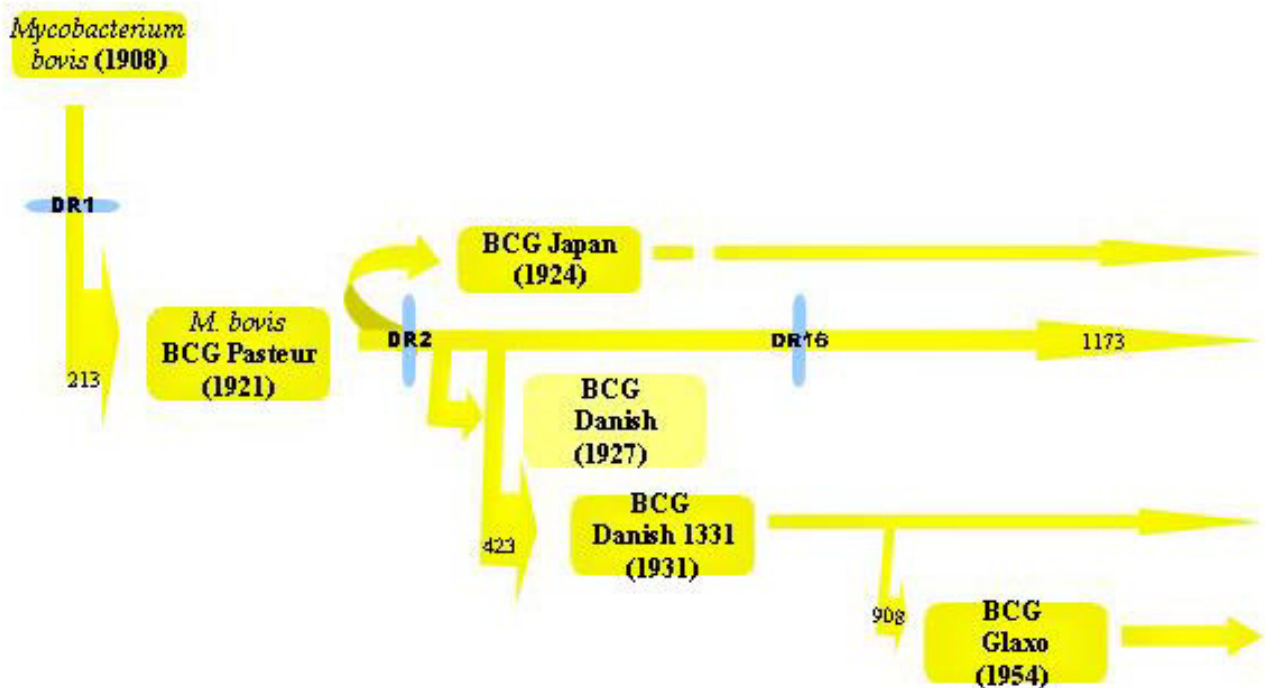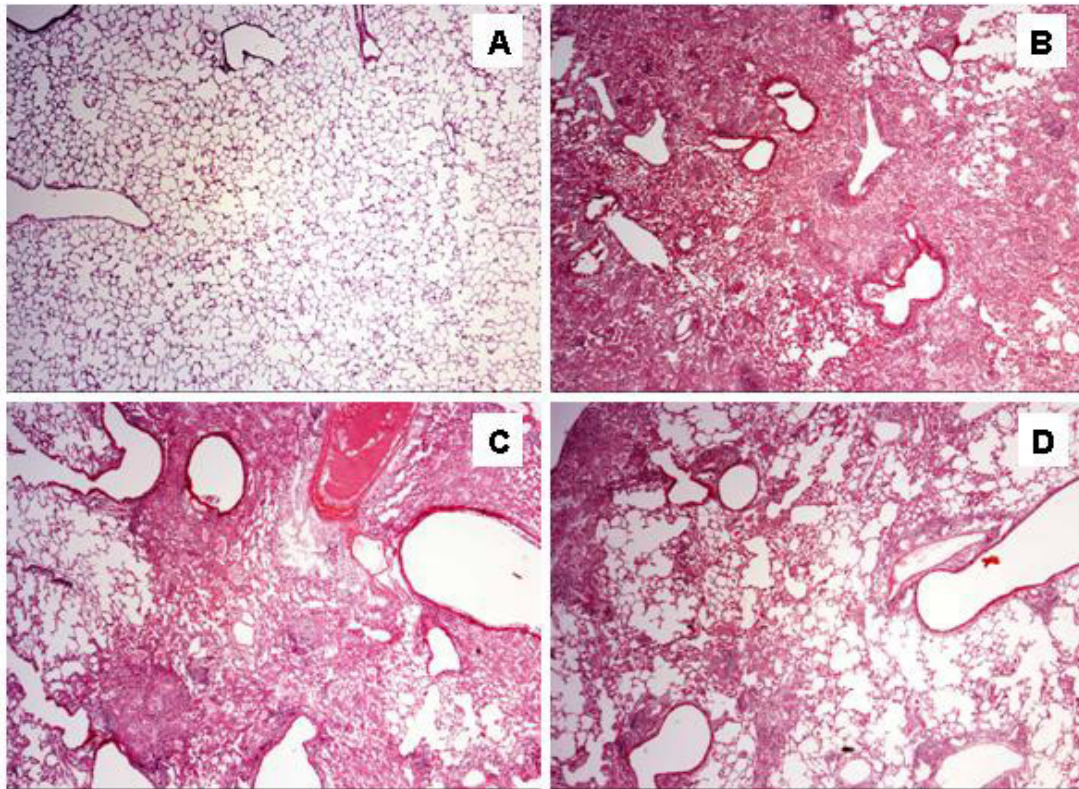
Figure 1
Documented evolution of the four *M. bovis* BCG strains currently licensed to produce for use in humans by the World Health Organization. The arrows indicate the chronologic evolution of Japanese and Danish strains from the original BCG Pasteur 1921 and Glaxo strain from Danish 1331. Boxes indicate occurrence of deletions of the genome during derivation of BCG. The year of derivation are indicated by (). The number in the arrows is the in vitro passages documented to have occurred during the evolution of BCG Pasteur and daughter strains since 1921 to the freeze dried of BCG in 60th.

Recently, Lagranderie *et al* [20] evaluated the capacity of five BCG sub-strains to trigger and maintain the immune response in mice and found statistically significant differences in their protective efficacy. However, the data are subject to the particular experimental conditions used in their mice model. In reality, vaccine candidates are tested in several models and under different evaluation criteria. It is clear that the strategies for the development and evaluation of the new vaccine candidates need to be standardized under shared criteria in order to understand their ability to induce protection [21] in animal models that mimic the key aspects of naturally occurring human tuberculosis [22]. Currently, work is being carried out to understand the differences in the major BCG vaccines being used in human trials. In these studies, BCG vaccines are being studied in a well-characterized mouse model of pulmonary tuberculosis, which tries to mimic natural human infection as much as possible [23]. Under these conditions 10 different daughter strains shown to induce a wide range of protection against *M. tuberculosis* intratracheal infection displaying differences in terms of colony

forming units (CFU's) reduction, delayed type hypersensitivity response and proportion lung surface affected by pneumonia going to be difficult correlate the protective response offered by the current disposable vaccines with the protective efficacy determined in previous human clinical trials (Figure 2 shown representative lung section of mice unvaccinated or vaccinated with these daughter strains) [unpublished data].

The availability of the complete genome sequence of two *M. tuberculosis* strains and the partial sequence of the BCG Pasteur sub-strain, may lead to the understanding of the principal source of antigenic variations, which would be significant for vaccine design and for inducing protective immunity in tuberculosis [24-26]. As seen in BCG substrains, variation in colony morphology among isolates of *M. tuberculosis* from patients is extremely common and multiple phenotypes are often apparent even from a microbial culture from a single sputum sample. However, attempts to correlate such differences with pathogenic potential cannot be sustained.

Figure 2
Representative cross-sections from lungs of BALB/c mice vaccinated and infected with virulent *Mycobacterium tuberculosis* H37Rv. **A**. Typical lung section from health non infected mice, no pneumonic or infiltrate were detected. **B**. Lung from mice non vaccinated and challenge with *M. tuberculosis*. **C**. Lung from mice vaccinated with heat inactivated *Mycobacterium vaccae* a closely related Mycobacterial strains. **D**. Lung from mice vaccinated with BCG Phipps strain. Significant reduction in the tissue damage was seen only in the mice vaccinated with BCG, the use of saprophytic bacilli as vaccine no induce immune response capable to control infection and pneumonic development. Vaccines were evaluated under a mice model of pulmonary tuberculosis (unpublished data) (H:E stain 50×).

Immunological considerations for new tuberculosis vaccine development

The development of a new vaccine with improved protective immunity against *M. tuberculosis* depends on the efficient recruitment of antigen-specific T-cells principally CD4+ in the lungs, as well as on the cytokines that are released particularly IFN-γ, which is important for inducing the macrophage killing activation mechanism [27].

Cell-mediated immunity plays the principal role in containing infection, and the routes of vaccine administration and immunization influences immune response development. In infants, BCG vaccination generally induces a Th1 cytokine response and stimulates cytotoxic T-lymphocyte activity in neonates [28]. An alternative route of BCG administration, which does not induce the side effects associated with subcutaneous immunization, is via rectal delivery. This method induces a similar immune response and protection in several animal models [29,30] without altering the recruitment patterns of activated T-cells [31]. Intranasal immunization induces higher protection by rapid induction of IFN-γ and T-cell response in the lung

tissue but there are some who have serious misgivings in using live bacilli. However, nasal administration of recombinant BCG as a means to deliver immune dominant antigens to the mucosa shows some promise for therapeutic use in HIV infection [32,33].

M. tuberculosis has a complex multiplicity of antigens with a diverse chemical and immune reactive nature as has been demonstrated in lipids, polysaccharides, and proteins. Some of these induce the granuloma formation, macrophage activation, and adjuvant activity while others are immunosuppressive and enhance host toxicity [27,34]. It should be remembered that the T-cell mediated immune response can result in protective immunity but it may also lead to pathological immunity that is detrimental to the host. The understanding of specific antigens expressed during early infection, disease, latency or reactivation and their immunological characterization are key for the development of new vaccines.

Looking back in the history of tuberculosis research, during the second half of 20th Century, various types of vaccines have been developed against mycobacterial infection to control bacilli replication and dissemination in order to stop tuberculosis transmission and future eradication. This vaccine development has been included the use of secreted proteins and surface exposed proteins, nucleic acid vaccination, rational attenuation of *Mycobacterium sp* strains, and recombinant BCG vaccines, all of which are currently being tested in a wide variety of animal models before future use in human clinical trials [25,35-37].

Now a day *M. bovis* BCG vaccine is the only live bacterial vaccine in use, which shows no major side effects. This is the most used vaccine and has been administered to more than two billon people worldwide and has showed long-lasting immunity.

Until now, medical staffs and health care workers accepted that BCG strains have been able to induce protection in the first year of life as well in all animal models that have been used; something that other vaccine candidates have still failed to achieve. Recent data of protective efficacy coming from more than 180 vaccine candidates put in perspective the evaluation of existing BCG vaccines in a way to identify potential candidate for a rationally designed recombinant BCG vaccine or an auxotroph vaccine by comparing protective efficacy [21]. These new designed recombinant vaccines have been shown to induce Th1 and long-lasting protective response with a smaller number and size of granulomas and a reduced load of colony forming units.

The development of new acellular vaccines composed of one or more antigens may offer a faster route for creating an alternative to BCG vaccines. However, this approach needs a distasting research to identify and defining those potential proteins recognized by human T-cells which are capable of inducing cell-mediated and long-lasting immunity. On last ten years attention has mainly been focused on the group of proteins secreted by the bacteria in culture media during the active replication of *M. tuberculosis* in early growth phase. These have been recognized in the early stages of tuberculosis infection in several animal models and may be the reason that viable mycobacteria are needed for effective vaccination against *M. tuberculosis* infection [25,35].

Various approaches are currently being analyzed using sub-unit vaccines based on culture filtrate proteins of *M. tuberculosis*, which when are administrated with an adjuvant have been shown to induce protective immunity in mice, guinea pigs and non-human primate models [40]. Antigen isolation has depended on the use of monoclonal antibodies [39] or by direct chromatography of culture filtrates. Such isolation procedures are characterized by biochemical and immunological techniques, with the corresponding genes being sequenced and cloned for further use as a vaccine. Several antigens have been cloned and purified as recombinant proteins, due to the large-doses required for immunization. The choice of an antigen or several antigens to formulate optimal immunological cocktails for vaccine use needs to be tested through *in vitro* and *in vivo* studies. In *in vitro* human studies, ESAT-6, CFP-10, MPT64, MPB70 and fusion proteins of the ESAT-6 and antigen 85B have been tested to show their ability to induce protective T-cell response. The most used criteria for tuberculosis vaccine designs are antigen-specific proliferation and IFN-γ secretion assays [35,40-42]. ESAT-6 was a promises in this area because is an immune dominant antigens most frequently recognized by TB patients, which contains a great number of T-cell epitopes. In animal models, ESAT-6 has been recognized in the first phase of infection and has been demonstrated to be a strong T-cell immunogen inducing prime memory immunity, which persisted in individuals who had recovered from disease [25,43]. The 30/32-kDa complex is among the most important secretor proteins of *M. tuberculosis* and has been shown to be protective in the guinea pig model of pulmonary tuberculosis. Their abundant production either extracellulary in broth culture or intracellulary in human monocytes, suggests a vital role in the physiology of the bacterium.

The recent elucidation of complete genome sequence of two *M. tuberculosis* strains and accelerated development of genetic tools for the study of *M. tuberculosis* has enabled the development of DNA vaccination, which has been

tested in several animal models [44,45]. Over the last few years, genetic immunization has become the most popular strategy in vaccine development and may be a successful alternative for the delivery of *M. tuberculosis* antigens that drive the cellular immune response. The DNA vaccines that expressed the 85B, MPT64 and ESAT-6 antigens has been demonstrated that they reduced the level of pulmonary infection following *M. tuberculosis* challenge, by the specific CD4+ and CD8+ T-cell response [44,46,47]. It was also shown that combined vaccination with three antigens was more effective than vaccination with a singe vector. The use of cytokines to increase vaccine protection enhanced the specificity of the immune response triggered against these antigens, thereby potentially maintaining the TH1 response [46]. These results concur with previous studies concerning sub-unit vaccines that have shown them to be only equal at best to BCG vaccines. In these way, immunization using a DNA vaccine of the 32-kDa protein stimulated protective immunity against BCG and *M. tuberculosis* infection [47,48]. Plasmid vectors expressing the 38-kDa glycoprotein induce a comparable level of protection with other DNA vaccines and develop a strong antigens-specific Th1 response in human and murine lymphocytes, characterized by IFN-γ secretion [45]. However, genetic vaccination with the 19-kDa lipoprotein resulted in a non-protective antibody response [49]. Even though 38 kDA and 19 kDa are important antigens that induce a Th1 type immune response, they do not protect mice from *M. tuberculosis* infection. Similarly, no protection was observed when immunization used chaperon-like proteins, in contrast with early works in which the heat-shock proteins like HSP60 and HSP70 resulted in a protective effect. Similar results to those using 38 kDa and 19 kDa have been obtained by immunizing with MPB83 DNA vaccines [50,51].

The major advance in DNA immunization is the notion that intramuscular vaccination is better for inducing a protective Th 1 type immune response against tuberculosis without sensitizing the animal to tuberculin testing. An alternative to DNA immunization is the use of recombinant vaccine viruses that express immune dominant antigens [52].

Although DNA vaccines are an attractive alternative for the development of a new vaccine, they do not surpass the protection conferred by BCG vaccination [53] and need further safety evaluation prior to testing in human populations.

The stable expression of foreign DNA in BCG on a plasmid vector established a basis for the construction of polyvalent recombinant BCG vaccine but the antibiotic resistance markers are not appropriate for the selection or maintenance of recombinant plasmid containing anti-

gens. An alternative would be to develop auxotroph mutants that would maintain stability, as they offer a genetic function required for the survival of the mycobacteria in their host. For this purpose defining mycobacterial promoter sequences is critical for expressing high levels of selected antigens responsible for cell-mediated immunity.

Recombinant BCG vaccines will have the excellent adjuvant activity of BCG and thus, significant levels of T-cell reactivity but it is uncertain whether they will be capable of generating high levels of cytotoxic T-cell response. The use of BCG as a live vaccine vector [60] for the presentation of heterologous and homologous antigens can be a powerful method for driving the immune response to the Th1 phenotype or for replacement of BCG target genes by homologous recombination [55].

Auxotroph and avirulent mutants of *M. tuberculosis* and *M. bovis* [56-59] have been developed showing wide variability in their avirulent character giving equivalent protection between them and comparable to that of BCG, which have also proved safe in individuals with immunodeficiency disease.

Post-genomic approach
With the complete genomic sequence and database comparisons, it is now possible to attribute tentative functions to roughly 40% of the 3924 protein coding genes for the genome of *M. tuberculosis* [24]. In addition, this information reveals new members of family proteins and potential variation between *M. tuberculosis*, *M. bovis* and *M. bovis* BCG [60]. Until recently, there have been two principal ways to learn more about the role of new antigens in the immune pathology of tuberculosis. All primary results come from some biochemical or immune proliferative assays induced by individual proteins with further characterization under specific conditions in animal models. Studies using such tools have resulted in the identification of immune dominant antigens, which are strongly recognized in humans and with the potential for developing of a novel TB vaccine. An applied approach allowed the identification of deleted genes and the development of antigens that can distinguish between *M. tuberculosis* infection and BCG vaccination [61]. This offers the prospect of starting vaccine design using the genetic information by reverse vaccinology [26].

Comparative analysis of the complete genome sequence of *M. tuberculosis*, *M. bovis*, BCG strains and *M. leprae* and other mycobacterial species may allow the identification and development of subunit vaccines with an exceptional specificity, however the high similarity of these strains limit this potential window of antigens.

The recent development of recombinant BCG vaccines that secrete cytokines or specific antigens is one of the most secure routes in the development of improved vaccines against tuberculosis [36,62]. However, as seen in the sub-unit vaccine approach, adverse results from antigens which are capable of enhancing protection efficacy of the current BCG vaccine could be found. Using recombinant BCG that secretes, IFN-γ or IL-2, which have been shown to reduce disease in a pre-infection vaccine model, as well as TNF-alpha which is indispensable for the formation of tuberculous granulomas, leading to an increase in lung pathology without reducing bacillary loads [48,63].

Other recombinant live vaccines that produce pore-forming cytolysin [64], Interferon alpha 2B [62] demonstrate enhanced immune genecity but not improved protection. Recently, Horwitz [65] reported enhanced protective response to challenges with virulent *M. tuberculosis* following vaccination with recombinant BCG that secreted 30-Kda antigen of *M. tuberculosis*.

The use of closely related species of *Mycobacterium* may be the second most interesting route to follow for vaccine improvement. *Mycobacterium microti* is a naturally attenuated strain with a narrow host range. In mice and rabbit models, vaccination with this strain resulted in significant reduction in the *M. tuberculosis* load and histopathology lesions [66]. Another approach is the use of different host vectors that express shared antigens [64,67] such as *Salmonella thyphimurium* that secretes ESAT-6, which is used as a vaccine and reduces the load of mycobacteria throughout the course of infection. *M. vaccae* is a saprophytic bacillus which, as other members of the *Mycobacterium* genera, share the major antigens and this was used recently as alternative immunotherapy in addition to chemotherapy in MDRTB cases [68].

Finally, little is known about the effect of vaccine boosters using some antigens and how this strategy may be able to drive the response to maintain a prorogued Th1-type memory T-cell response].

The use of atypical mycobacteria as hosts for the production of *M. tuberculosis* antigens needs to be designed with the same precautions as for BCG. Post *et al* [69], demonstrated the inefficacy of recombinant strains of *M. vaccae* and *M. smegmatis* that expressed the 19-kDa lipoprotein, and the abrogation of the protection conferred by host strains alone [49]. On the other hand, the deletion of these genes in *M. bovis* has a significant effect on the ability of BCG to protect against *M. tuberculosis* challenge [70].

The rational mutation of virulence genes or putative virulence factors is being evaluated for their biological function [71]. In order to develop better vaccines, several

mutants have been produced by transposon mutagenesis or illegitimate recombination. The current strategy is to use antibiotics to select mutant strains that better express antigens than induce protection. However, the use of antibiotics in this way and the possibility to revert their virulence or recombination events between these and the wild type strains means that this alternative is a long way from human trials [37]. The exciting aspect of this early research is the specificity of the response elicited by each one of these strains, which plays a key role in protection [59] through homologous or analogous challenge with the virulent strain.

Discussion

BCG is an attenuated derivative of virulent bacilli generated by 230 serial passes of the parent strain through laboratory media. It is believed that attenuation is caused by chromosomal rearrangements such as small duplications or inversions. Such variations would have been difficult to detect by the genetic and molecular approaches that currently exist.

The mechanism by which BCG induces protective immunity has not been established. The mutation that affects BCG provides potential answers as to why there have been differences in protective efficacy during various human trials.

The development of new vaccines requires a clear understanding of the nature of both innate and acquired immunity in the lung and the role that these two sets of mechanisms play in the protective immune response against *M. tuberculosis*, as well as the construction of delivery vectors with the ability to elicit an optimal protective response that controls the pathogen. Despite the rapid development of molecular biology techniques, DNA vaccines have not proved to be better than live attenuated vaccines, such as BCG, for inducing protective immunity against tuberculosis. *M. tuberculosis* has evolved many interrelated processes that favour the particular ecological niche, which the organism occupies; the resistance of the bacilli to chemical injury, dehydration and certain antibiotics is directly related to the low permeability of its unique cell wall envelope, which also is a rich source of immunogenic antigens.

Some unresolved issues regarding BCG efficacy are the duration of protective immunity, the efficacy of the BCG daughter strains, the phenotypic differences among BCG vaccines, and their overall protection. In bovine studies, it has been demonstrated that BCG protection would be enhanced with repetitive immunization at short intervals [72]. Also, the prevalence of BCG infection is unknown because many laboratories cannot quickly differentiate between BCG and other members of the *M. tuberculosis*

complex. The development of a low-cost vaccine that immunizes efficiently with only one dose would be important for some developed countries, which do not offer a free national immunisation programme for BCG, especially considering that most (69.5%) of the TB cases in 1990 occurred among racial and ethnic minorities.

An improved BCG vaccine would help in fulfilling a number of established criteria: (i) the World Health Organization recommends that BCG should be administrated at birth; (ii) one dose should be sufficient to confer long lasting immunity; (iii) it is the most effective known adjuvant in animals and man; (iv) the production cost is low (~$0.55 per dose) (v) BCG is offered as an integral compound of the World Health Organization Expanded Program on Immunization (EPI).

It is necessary to identify the efficacy, safety and biochemical differences between the BCG sub-strains in use today. Understanding these characteristics involves much more than simply testing for growth in the spleen or lung of animal models used for this purpose. In addition, care must be taken when considering the very artificially high-dose intravenous infection models of the disease, which involves significant dissemination and in which it is impossible to evaluate factors that influence their efficiency, nature of the granulomatous response or cessation. BCG is the only TB vaccine currently licensed and administered to children. The development and eventual acceptance of a new TB vaccine is several years away with safety, protection and long-term efficacy still needing to be proved. The use of recombinant BCG vaccines that over produce secreted antigens may be of particular significance in the induction of a protective immune response but in the same way, they may produce increased tissue damage as they may enhance delayed-type hypersensitivity reaction following infection. Other mycobacterial genes still need to be defined in terms of their capacity to polarize Th1 type immune response and protect against tuberculosis. A careful integration of the biochemistry and pathogenesis of *M. tuberculosis*, in parallel with a complete study of the immune response and resistance to infection compiled during the last Century, may hold the key to the development of a really effective vaccine against tuberculosis.

Conclusions

The development of a new attenuated vaccine that is more effective than BCG depends not only on the identification of genes and products that contribute to pathogenesis in order to drive the attenuating mutations, but also a precise definition of the antigenic machinery used by the immune system to develop a protective response. Another issue requiring consideration is the potential that a new vaccine has for clearing the infection because many cases

of clinically active tuberculosis arise from reactivation of an infection acquired years before and reflect the re-emergence of the actively growing organism from an apparently non-replicative state. In addition, there is no evidence that current TB vaccine protection lasts for more than 15 years in any population [15,73]. The number of patients developing tuberculosis through reactivation versus re-infection is a topic currently being investigated in developing countries.

Finally, we can conclude that developing of new vaccines would be directed in two ways, in one hand we need a vaccine that induce protective immunity in infants and control and clearance the infection, and in the other hand a vaccine to treat the high proportion of latent infected people. These approaches in the optimal scenery can be present in a new based BCG vaccine that could be administrated at any time of life, as single dose infancy in high incidence countries and at the time of exposition in the developed countries when the rates of infected people are lesser than 10/ 100 000 people. Until development of this vaccine (s) the reduction of latent infection, and proportion of infected subjects (10%) whose develop tuberculosis In meanwhile, the actual incidence rate will no decreased promptly.

Before field introduction of any new vaccine, several researches should be focused to support the enhanced efficacy of NEW vaccines against tuberculosis, mainly in their capability to clearance initial tuberculosis infection named granuloma, due to many of the inefficacy of contemporary BCG vaccine to control tuberculosis infection where innate and acquired immune response play an important role in the develop of a vaccine success.

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