Influence of BCG vaccine strain on the immune response and protection against tuberculosis

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Abstract
The Bacille Calmette–Guérin (BCG) vaccine has been used for more than 80 years to protect against tuberculosis. Worldwide, over 90% of children are immunized with BCG, making it the most commonly administered vaccine, with more than 120 million doses used each year. Although new tuberculosis vaccines are under investigation, BCG will remain the cornerstone of the strategy to fight the worsening tuberculosis pandemic for the foreseeable future. The recent delineation of genetic differences between BCG vaccine strains has renewed interest in the influence of the vaccine strain on the protective efficacy against tuberculosis. This review critically examines the data from animal and human studies comparing BCG vaccine strains. Although there is good evidence to support the notion that the induced immune response and protection afforded against tuberculosis differs between BCG vaccine strains, currently, there are insufficient data to favour or recommend one particular strain. Identifying BCG strains with superior protection would have a dramatic effect on tuberculosis control at a population level: a small increment in protection provided by BCG immunization will prevent large numbers of cases of severe tuberculosis and deaths, particularly in children.

Introduction
Bacille Calmette–Guérin (BCG) was one of the first live-attenuated vaccines to be used in humans. It remains the most commonly used vaccine worldwide, with over 120 million doses administered each year. BCG immunization has proven its efficacy in children, in whom it provides c. 80% protection against severe and disseminated tuberculosis, such as tuberculous meningitis and miliary disease (Trunz et al., 2006; Walker et al., 2006). BCG immunization also reduces the risk of tuberculosis in adults, by an average of 50% in a recent meta-analysis, although there is a wide range of efficacy in different populations (Colditz et al., 1994; Brewer, 2000). In addition, BCG may protect against latent tuberculosis infection in children (Soysal et al., 2005) and has important nonspecific beneficial effects on infant mortality (Shann, 2004; Roth et al., 2006). Although there are at least 16 new tuberculosis vaccines under development (Skeiky & Sadoff, 2006), novel, heterologous boost vaccines are likely to be used to enhance specific immunity primed by BCG (Kaufmann, 2005; Knezevic & Corbel, 2006). Therefore, BCG given at birth is likely to remain the key vaccine in future tuberculosis immunization strategies.

The development of the BCG vaccine
The BCG vaccine was developed between 1908 and 1921 by Albert Calmette and Camille Guérin in France by culturing Mycobacterium bovis on bile-containing medium. After only a few culture cycles (passages), the bacterium showed decreased virulence in a number of experimental animals. After 230 passages over a 13-year period, the BCG strain was further attenuated but still provided protection against a lethal challenge of Mycobacterium tuberculosis in calves and guinea-pigs. Subsequent to its first use in humans in France in 1921, daughter strains were distributed around the world for use in vaccine manufacture. As a result of repeated passage under different conditions in different laboratories
worldwide, BCG vaccine strains diverged genetically. There is evidence that single nucleotide polymorphisms, duplications and deletions continued to emerge until lyophilization was introduced in the 1960s to store freeze-dried seed lots for vaccine production.

**The genetic divergence of BCG vaccine strains**

Behr & Small (1997) first suggested a decade ago that the passage of BCG vaccine strains induced progressive loss of efficacy. This observation led to the first investigation of the genetic diversity of BCG vaccine strains (Behr & Small, 1999; Behr et al., 1999). The genomic sequences of different BCG vaccine strains were compared with *M. tuberculosis* as a common reference. Common to all BCG vaccine strains is the deletion of region of difference one (RD1) that is preserved in *M. bovis* and *M. tuberculosis*. The loss of this region is now known to have been the critical event in the attenuation of the initial *M. bovis* strain. Importantly, Behr and colleagues found significant genetic divergence among BCG vaccine strains (Behr & Small, 1999; Behr et al., 1999). For example, BCG-Moreau and BCG-Japan, two strains obtained from the Pasteur Institute before 1926, have two copies of the insertion region (IS) 6110. Strains obtained after 1931 from the Pasteur institute, such as BCG-Denmark, BCG-Tice and BCG-Glaxo, have only one copy of IS6110 and have lost RD2 in addition (Table 1). On the basis of the genomic sequence data and historical written records, Behr proposed a genealogy of BCG vaccine strains (Behr & Small, 1999; Behr et al., 1999). Further genetic differences between strains have been described since this seminal work (Mostowy et al., 2003; Charlet et al., 2005).

The potential influence of these genetic differences, through antigenic variation, on the protective efficacy of BCG immunization with different vaccine strains has generated considerable concern internationally (Corbel et al., 2003; Charlet et al., 2005). Some of the discrepancies in the protective efficacy of BCG observed in different trials might at least partially be explained by the genetic difference between BCG vaccine strains.

A number of recent studies suggest that the genetic differences between BCG vaccine strains are indeed important. A recent *in vitro* transcriptome analysis revealed significant differences in gene expression between different BCG vaccine strains (Brosch et al., 2007). This study also investigated differences in the tandem duplications DU1 and DU2. Four novel groups were identified within DU2, which endorsed the genealogy proposed by Behr and colleagues (Behr & Small, 1999; Behr et al., 1999). Another gene-expression study, significantly the first and only such study in BCG-immunized infants, identified by cluster analysis two distinct patterns of immune response induced by different BCG vaccine strains (discussed below) (Wu et al., 2007).

In addition to influencing gene expression (Brosch et al., 2007), the genetic diversity of different BCG vaccine strains has also been shown to have an effect on immunogenicity (Davids et al., 2006; Aguirre-Blanco et al., 2007), virulence (Hengster et al., 1992; Kroger et al., 1995) and viability (Gheorghiu & Lagrange, 1983), all of which are likely to affect the protection against tuberculosis induced by BCG immunization. Furthermore, the shorter survival of BCG vaccine within the host is associated with reduced immune stimulation, resulting in early waning of protective immunity when compared with a modified strain of *M. tuberculosis* (Pinto et al., 2004). However, to our knowledge, the duration of survival of different BCG vaccine strains within the host has not been compared.

**BCG vaccine strains used worldwide**

While it is recognized that existing BCG vaccine strains differ and are likely to induce different degrees of protective immunity, currently, there are no means to determine which strain is superior. There is therefore no agreement or recommendation on the optimal strain for general use. As a result, different strains are used worldwide (Fig. 1 and Table 1).

UNICEF is the largest supplier of BCG vaccines, distributing more than 120 million doses each year to more than 100 countries. Worldwide, the most commonly used strains are currently BCG-Denmark, BCG-Japan and BCG-Bulgaria (genetically identical to BCG-Russia [Stefanova et al., 2003]) simply because these are the strains supplied by UNICEF (Supply division of UNICEF, pers. commun.). Many developing countries have several BCG vaccine strains in use concomitantly. In contrast, in most developed countries usually only one strain is licensed for use. Most countries import BCG vaccine from one of the international manufacturers but a few countries produce their own BCG vaccine. Examples of nationally produced BCG vaccines are: BCG-Poland based on BCG-Moreau (National tuberculosis and Lung Institute in Warsaw, pers. commun.), BCG-Romania based on BCG-Pasteur (National Lung Institute, Bucharest, pers. commun.) and BCG-China based on BCG-Denmark (Director of the National Immunisation Program, pers. commun.).

**Objective of this review**

This paper reviews current knowledge about the influence of BCG vaccine strain on immune response and/or protective immunity against tuberculosis-induced immunization. It critically examines the animal and human studies that have addressed this issue and describes the *in vitro* and clinical correlates of protective immunity used in these studies. To provide a context to the studies, the general concepts of...
Table 1. Characteristics of currently available BCG strains and the countries in which they are used listed by year each strain was obtained by the host laboratory (data from Wang et al., 1988; Milstien & Gibson, 1990; Oettinger et al., 1999; Behr, 2002; Mostowy et al., 2003; Brosch et al., 2007 and vaccine manufacturers)

<table>
<thead>
<tr>
<th>Strain (synonym)</th>
<th>Passage no</th>
<th>Year obtained*</th>
<th>RD1</th>
<th>RD2</th>
<th>Other deletions</th>
<th>IS6610 copies</th>
<th>DU group</th>
<th>Particles per 0.1 mL (× 10^6)</th>
<th>Examples of countries in which strain is used</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moreau (Brazil, Rio)</td>
<td>1924</td>
<td>–</td>
<td>+</td>
<td>RD16</td>
<td>2</td>
<td>DU2 I</td>
<td>Brazil</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Russia (Moscow)</td>
<td>1924</td>
<td>–</td>
<td>+</td>
<td>RD Russia</td>
<td>2</td>
<td>DU2 I</td>
<td>Russia, Turkey</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bulgaria (Sophia)</td>
<td>1950s</td>
<td>–</td>
<td>+</td>
<td>RD Russia</td>
<td>2</td>
<td>DU2 I</td>
<td>Bulgaria, UNICEF</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Japan (Tokyo)</td>
<td>1925</td>
<td>–</td>
<td>+</td>
<td></td>
<td>2</td>
<td>DU2 I</td>
<td>3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Romania</td>
<td>1925</td>
<td>–</td>
<td>+</td>
<td></td>
<td>1</td>
<td>DU2 II</td>
<td>Romania</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sweden (Gothenburg)</td>
<td>1926</td>
<td>–</td>
<td>+</td>
<td></td>
<td>1</td>
<td>DU2 II</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Birkhaug</td>
<td>1927</td>
<td>–</td>
<td>+</td>
<td></td>
<td>1</td>
<td>DU2 II</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Denmark (Copenhagen, Danish)</td>
<td>1931</td>
<td>–</td>
<td>–</td>
<td>RD Denmark/Glaxo</td>
<td>1</td>
<td>DU2 III</td>
<td>0.15–0.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tice (Chicago)</td>
<td>1934</td>
<td>–</td>
<td>–</td>
<td>nRD18</td>
<td>1</td>
<td>DU2 II</td>
<td>USA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Frappier (Montreal)</td>
<td>1937</td>
<td>–</td>
<td>–</td>
<td>nRD18, RD8, RD Frappier</td>
<td>1</td>
<td>DU2 III</td>
<td>0.2–0.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phipps (New York, Park, Philadelphia)</td>
<td>1938</td>
<td>–</td>
<td>–</td>
<td>nRD18</td>
<td>1</td>
<td>DU2 III</td>
<td>0.2–3.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prague (Czechoslovakian)</td>
<td>1947</td>
<td>–</td>
<td>–</td>
<td></td>
<td>1</td>
<td>DU2 III</td>
<td>0.52–1.12</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Connaught (Toronto)</td>
<td>1948</td>
<td>–</td>
<td>–</td>
<td>RD8</td>
<td>1</td>
<td>DU2 III</td>
<td>0.8–3.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glaxo (London F10)</td>
<td>1954</td>
<td>–</td>
<td>–</td>
<td>RD Denmark/Glaxo</td>
<td>1</td>
<td>DU2 III</td>
<td>Australia, Canada, New Zealand</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mérieux</td>
<td>1970</td>
<td>–</td>
<td>–</td>
<td>RD Denmark/Glaxo</td>
<td>1</td>
<td>DU2 III</td>
<td>Chile, Peru</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mexico</td>
<td>1948</td>
<td>–</td>
<td>–</td>
<td></td>
<td>1</td>
<td>DU2 III</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Beijing</td>
<td>1948</td>
<td>–</td>
<td>–</td>
<td></td>
<td>1</td>
<td>DU2 III</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shanghai</td>
<td>1948</td>
<td>–</td>
<td>–</td>
<td></td>
<td>1</td>
<td>DU2 III</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lanzhou</td>
<td>1948</td>
<td>–</td>
<td>–</td>
<td></td>
<td>1</td>
<td>DU2 III</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chanchun</td>
<td>1950s</td>
<td>–</td>
<td>–</td>
<td>RD18, RD14</td>
<td>1</td>
<td>DU1</td>
<td>0.04–0.5</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*From Pasteur Institute unless mentioned.
†Genetically identical.
‡Strain has not been genotyped.
DU, duplication; RD, deleted region; IS, insertion.
correlates of protection and determinants of protective immunity to tuberculosis are discussed in the next section.

Correlates of protection against tuberculosis

The immunological mechanisms and interactions leading to protection after BCG immunization are poorly understood (Kaufmann, 2005; Fletcher, 2007). In humans, for many years, the only parameters that were used to assess protective immunity induced by BCG were the presence and size of a BCG scar, delayed-type hypersensitivity (DTH) and prospective case finding of pulmonary tuberculosis. Although studies continue to evaluate BCG scar size and/or DTH, including in the context of the BCG vaccine strain (Nyboe & Bunch-Christensen, 1966; Horwitz & Bunch-Christensen, 1972; Galbraith & Hall, 1974; Vallishayee et al., 1974; Bottiger et al., 1983; Guerin et al., 1999; Suciliene et al., 1999; Roth et al., 2005; Castro-Rodriguez et al., 2007), these will not be discussed further in this review, as there is robust evidence that these parameters do not predict protective efficacy in humans (Comstock, 1988; Fine et al., 1994; Sterne et al., 1996; Ota et al., 2006).

Recent advances in the understanding of the immunobiology of tuberculosis, together with the development of new tuberculosis vaccines, have brought to light a number of potential immunological correlates of protection and renewed interest in exploring the protective immune response induced by BCG.

CD4 T cells

Both animal and human data support the key role of CD4 T cells in the control of M. tuberculosis following infection. Greater morbidity and mortality in CD4 T cell-depleted mice confirm the critical role of this T cell subset (Kaufmann & Flesch, 1988; Caruso et al., 1999; Chackerian et al., 2001). The resurgence of tuberculosis associated with the HIV epidemic also demonstrates that loss of CD4 T cells increases susceptibility to tuberculosis (Selwyn et al., 1989; Swaminathan et al., 2000; Sonnenberg et al., 2001). The primary effector function of CD4 T cells is believed to be a T helper type 1 (Th1) response involving the production of IFN-γ and IL-2, which leads to the activation of macrophages. Several studies have found that IFN-γ production by CD4 cells is of major importance for protection against tuberculosis in mice (Cooper et al., 1993; Flynn et al., 1993; Lagrange et al., 1996; Schroder et al., 2004; Fernando & Britton, 2006; Rodgers et al., 2006). Similarly, in humans, IFN-γ receptor deficiency is associated with increased susceptibility to mycobacterial infection (Newport et al., 1996; Dorman et al., 2004; Fernando & Britton, 2006).
Accumulated evidence has led to the concept that a Th1-dominated immune response, reflected by IFN-γ release by CD4 T cells after stimulation with *M. tuberculosis* antigens, provides the best correlate of protective immunity induced by BCG immunization currently available. However, although IFN-γ production is essential for protection against tuberculosis, there is emerging evidence that other immunological and regulatory factors play a more critical role than thought previously (Olsen et al., 2000; Elias et al., 2005; Foulds et al., 2006). In addition, the correlation between IFN-γ production and protection may not be linear: potentially, once a certain threshold is reached, higher cytokine levels may be of no additional benefit.

**CD8 T cells**

A large body of evidence supports a role for CD8 T cells in protection against tuberculosis (Kauffman & Flesch, 1988; Grotzke & Lewinsohn, 2005). One of the more recently described pathways for CD8 T cell activation in *M. tuberculosis* infection is cross-presentation of antigens (Winau et al., 2005; Winau et al., 2006). *Mycobacterium tuberculosis* induces apoptosis in infected macrophages, which leads to the release of vesicles containing tuberculosis antigens. These vesicles are engulfed by dendritic cells, which then present antigen in conjunction with major histocompatibility complex (MHC) class I, leading to activation of CD8 T cells. The role of CD8 T cells is supported by experiments in MHC I-deficient mice that have shortened survival following infection with *M. tuberculosis* (Flynn et al., 1992). Previous immunization of these mice with BCG prolongs their survival but does not protect them from death, highlighting the requirement of CD8 cells in protective immunity (Flynn et al., 1992; Behar et al., 1999). The effector mechanism of CD8 T cells is incompletely understood but is not explained by cytolytic activity alone. The relative contribution of CD8 T cells to protection is difficult to assess as a result of the overlap of function and interaction with CD4 T cells. There is evidence, however, that CD4 T cells are more important in acute infection whereas CD8 T cells are responsible for the containment of latent infection (van Pijneteren et al., 2000). The specific response of CD8 T cells after BCG immunization in newborns has only recently been investigated and was found to be either cytotoxic, or cytokine-releasing or, less frequently, both (Murray et al., 2006).

**The balance of Th1- and Th2-type immune response**

Th2 immune responses, characterized by IL-4, IL-5 and IL-13 production, may modulate presumed protective Th1 immune responses against tuberculosis (Rook et al., 2004). It has been speculated that progressive tuberculosis disease might not be due to absence of a Th1 response but due to the subversive effect of IL-4 suppressing the Th1 response. IL-4-producing T cells and IL-4 mRNA are increased in pulmonary tuberculosis and correlate with the extent of lung cavitation (Rook et al., 2004). Further, tuberculosis-exposed health care workers who developed active disease within 2–4 years have a higher frequency of *M. tuberculosis*-specific IL-4-producing CD8 T cells than those who do not (Ordway et al., 2004). Similarly, preexisting Th2 activity in mice infected with *M. tuberculosis* leads to increased severity of infection and earlier death (Wangoo et al., 2001). This suggests that the balance of Th1/Th2 responses is an important factor in protective immunity (Lindblad et al., 1997; Rook et al., 2004).

**Other proposed effector molecules and cells**

There are several reports of other effector molecules and cells of the immune system that may play a role in protection against tuberculosis including TNF-α (Flynn et al., 1995; Saunders et al., 2005), IL-12 (Cooper et al., 1997), IL-17 (Cruz et al., 2006), IL-23 (Wozniak et al., 2006), γδ T cells (Hoft et al., 2002), NK T cells (Junqueira-Kipnis et al., 2003) and regulatory T cells (Hanekom, 2005; Quinn et al., 2006).

To date, no single immunological marker has been shown to predict the effectiveness of BCG vaccine (Viney et al., 2005), reflecting the complexity of the mechanisms and interactions underlying protection against tuberculosis. Most studies have investigated immune markers quantitatively with the assumption that larger immune responses are better. This is likely to be an oversimplification as illustrated by recent studies (Kamath et al., 1999; Triccas et al., 2002; Skinner et al., 2003). A potential new tuberculosis vaccine candidate induced increased production of IFN-γ in mice but this was not associated with better protection (Skinner et al., 2003). This finding is consistent with the theoretical concept mentioned above that potentially once a certain threshold of cytokine production is reached, further increase does not translate into greater protection. This result is also consistent with the concept that the role of any individual cytokine is dependent on the context provided by other cytokines and regulatory factors. A combination of immunological parameters or a certain pattern of cytokine release is most likely to provide a robust correlate of protective immunity.

**Animal studies**

Mice are the most frequently used animal in studies of protective immunity induced by BCG immunization (Martin, 2006). However, there are limitations when extrapolating findings from studies of the immune response in animal models to humans. For example, cytokines such as IFN-γ and TNF-α can have different effects on mononuclear
cells in different species (O’Garra & Britton, 2008). Similarly, reactive nitrogen intermediates are important in defence against tuberculosis in mouse monocytes but their role in humans infected with tuberculosis is controversial (Choi et al., 2002; Nathan, 2006). Moreover, mice infected with tuberculosis do not develop the same pathological changes observed in human lungs. Guinea-pigs have also been commonly used to investigate the biological activity of BCG vaccines, because their DTH response is similar to that of humans. They remain important in testing new tuberculosis vaccines because of their susceptibility to tuberculosis infection (Williams et al., 2005). Other animal models, including rabbits, ferrets, possums, cattle and deer, are of limited value for BCG vaccine studies because of differences in their cell-mediated immune function (Griffin et al., 1995; Dannenberg & Collins, 2001). Non-human primates are the best model as they are susceptible to infection, develop similar disease to humans and can be protected by BCG immunization (Gupta & Katoch, 2005; Kamath et al., 2005). Interestingly, however, even in two closely related species of macaque monkeys, the protection conferred by BCG-Denmark immunization differed. Protection was significantly higher in cynomolgus than in rhesus monkeys, even though in vitro immune response, measured by purified-protein derivative (PPD)-induced lymphoproliferative response and IFN-γ release, was higher in rhesus monkeys (Langermans et al., 2001). Despite these limitations, the immune response to BCG has been studied in many different animal models (Table 2) and a wide range of immunological and clinical parameters have been investigated (Table 3).

Studies in mice

In a recent study in mice, 10 different subcutaneously injected BCG vaccines were compared (Castillo-Rodal et al., 2006). Eight weeks after immunization, animals were challenged with intratracheal M. tuberculosis H37Rv and lung pathology was measured 2 and 4 months later. The least damage, and presumed best protection, was observed in mice immunized with BCG-Phipps, followed in order by BCG-Connaught, BCG-Sweden, BCG-Moreau, BCG-Mexico, BCG-Birkhaug and BCG-Frappler. Mice immunized with BCG-Denmark, BCG-Pasteur and BCG-Tice had significantly greater lung damage. Correspondingly, the lungs with the highest CD4 T cell counts were associated with the least damage. BCG-Mexico, BCG-Tice and BCG-Sweden had the highest proportion of cells expressing IL-2 and IFN-γ. IL-10 expression was the lowest with BCG-Phipps and BCG-Pasteur. Each BCG strain showed a different cytokine profile, but this was not helpful in predicting which strain was most protective.

Contrasting results were found in a mouse study that used five different BCG strains for subcutaneous immunization (Lagranderie et al., 1996). Four months after immunization, the mice were challenged with a recombinant BCG (rBCG) strain prepared from either BCG-Japan or BCG-Pasteur. Lack of protection against the challenge strain was assessed by measuring rBCG CFU in the spleen. BCG-Pasteur, BCG-Glaxo and BCG-Russia were associated with the strongest growth inhibition compared with unvaccinated control mice, whereas BCG-Japan and BCG-Prague did not inhibit growth of the challenge rBCG. There was no significant difference in cytokine production by spleen cells between the different groups. However, the in vitro cytotoxic capacity of spleen effector cells to macrophages sampled by bronchoalveolar lavage in the same mice was the highest in mice immunized with BCG-Pasteur and BCG-Russia and absent in mice immunized with BCG-Japan. The use of rBCG rather than virulent M. tuberculosis in the challenge experiments is an important factor that offers one explanation for the different order of efficacy of BCG vaccine strains determined in this study.

In another study, a difference was observed between BCG-Glaxo and BCG-Pasteur immunized mice depending on the strain of aerosolized M. tuberculosis used for challenge (Collins, 1985). The growth of M. tuberculosis in the lungs was lower in the BCG-Pasteur immunized mice at 14 and 21 days after challenge with the Erdman strain of M. tuberculosis. In contrast, when challenged with an Indian M. tuberculosis strain, both BCG vaccine strains showed equal protection, suggesting the intriguing notion that certain BCG vaccine strains protect against certain strains of M. tuberculosis.

A study by Gheorghiu & Lagrange (1983) challenged mice with intravenous M. tuberculosis 7 weeks after subcutaneous immunization with BCG. All immunized mice survived, whereas all nonimmunized mice died within 90 days of challenge. The survival of M. tuberculosis, assessed by measuring CFU, in the spleen, liver and lungs was significantly lower in BCG-Glaxo and BCG-Pasteur immunized mice than in those immunized with BCG-Denmark and BCG-Japan. In the same study, lymphoproliferation 14 days after challenge with M. tuberculosis was the highest in mice immunized with BCG-Denmark, intermediate in BCG-Glaxo and BCG-Pasteur and lowest in those immunized with BCG-Japan.

In another study, mice were immunized with BCG-Glaxo, BCG-Moreau, BCG-Prague or BCG-Russia and challenged with the homologous strain either intravenously or subcutaneously (Junior & Gontijo Filho, 1979). The immune response, assessed by measuring change in lung weight, was the highest with BCG-Moreau. Spleen and hepatic weights were the highest with BCG-Prague. This study is limited by the use of BCG rather than virulent M. tuberculosis as the challenge agent. Moreover, the validity of using the weight of organs as surrogate markers of immune response is uncertain.
Table 2. Summary of animal studies comparing different BCG vaccine strains listed in reverse chronological order

<table>
<thead>
<tr>
<th>Author, reference, publication year, animal model</th>
<th>BCG strains</th>
<th>No of animals, route of BCG immunization</th>
<th>Immunization to challenge interval (weeks)</th>
<th>In vivo challenge agent and route</th>
<th>Challenge to outcome interval (weeks)</th>
<th>Parameters measured</th>
<th>Main findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Castillo-Rodal et al. (2006) Mouse</td>
<td>Birkaug (1)</td>
<td>10 per strain s.c.</td>
<td>8</td>
<td>Aerosolized H37Rv</td>
<td>9, 18</td>
<td>Tissue damage lungs, MTB CFU in lungs, T cell subpopulations in lungs, Intracellular cytokines, DTH, Survival</td>
<td>Tissue damage smaller in 1, 2, 4, 5, 6, 8, 9, than in 3, 7, 10, Greatest decrease in CFU with 1, 2, 7 and 8, Highest CD4 cells in lungs in 9, followed by 2 and 5, Every strain show a particular cytokine profile (IL-2, IFN-γ, IL-10 and IL-4)</td>
</tr>
<tr>
<td>Lagranderie et al. (1996) Mouse</td>
<td>Glaxo (1)</td>
<td>Unspecified s.c. oral i.v.</td>
<td>18</td>
<td>rBCG s.c.</td>
<td>2, 4, 8, 12</td>
<td>rBCG CFU in spleen, T cell proliferation, Cytokine production after stimulation with PPD, Cytotoxic T cell responses, IgG, IgM, IgA anti-PPD in i.v. immunized mice, DTH</td>
<td>Reduced rBCG CFU in 1, 3 and 5, T cell proliferation and production of IFN-γ and IL-2 not different in all 5 strains, Cytotoxicity high in 3 and 5, not detectable with 2</td>
</tr>
<tr>
<td>Collins (1985) Mouse</td>
<td>Glaxo Pasteur</td>
<td>5 per strain and time-point s.c.</td>
<td>4, 14</td>
<td>Aerosolized Erdman and Indian MTB strain</td>
<td>1, 2, 3</td>
<td>Growth of MTB in lungs, Growth of MTB in spleen, DTH, Survival</td>
<td>Lower growth of MTB in the lungs in the Pasteur group if challenged with MTB Erdman, No difference of MTB growth in spleen between the two strains, No difference in survival</td>
</tr>
<tr>
<td>Gheorghiu &amp; Lagrange (1983) Mouse</td>
<td>Denmark (1)</td>
<td>10 per strain s.c.</td>
<td>7</td>
<td>H37RV i.v.</td>
<td>7</td>
<td>In vivo cell proliferation in popliteal draining lymph nodes, MTB CFU in spleen, liver and lung, DTH, Survival</td>
<td>Highest lympho-proliferation in 1 and lowest in 3, MTB CFU in spleen and liver lower in 2 and 4, Lowest CFU in lungs in 4 highest in 3</td>
</tr>
<tr>
<td>Junior &amp; Gontijo Filho (1979) Mouse</td>
<td>Glaxo (1)</td>
<td>30 per strain i.v.</td>
<td>4</td>
<td>Homologous BCG strain i.v. and s.c.</td>
<td>0.5</td>
<td>Pulmonary density, Weight of lungs, spleen, liver, DTH</td>
<td>Highest pulmonary density in 2, Highest lung weight in 2, Splenic and hepatic weight highest in 3 and lowest in 1</td>
</tr>
<tr>
<td>Buraczewska et al. (1969) Mouse</td>
<td>Moreau Pasteur</td>
<td>10 per strain i.p.</td>
<td>18</td>
<td>None</td>
<td></td>
<td>Histology of lymph nodes, liver, spleen, lung</td>
<td>Pasteur and Russia induced greater changes in all organs, Moreau showed least changes</td>
</tr>
</tbody>
</table>

(Continued)
<table>
<thead>
<tr>
<th>Author, reference, publication year, animal model</th>
<th>BCG strains</th>
<th>No of animals, route of BCG immunization</th>
<th>Immunization to challenge interval (weeks)</th>
<th>Challenge to outcome interval (weeks)</th>
<th>Parameters measured</th>
<th>Main findings</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Guinea pig</strong>&lt;br&gt;Wang et al. (1988)<strong>&lt;br&gt;北京 (1) Chanchung (2) Denmark (3) Lanzhou (4) Shanghai (5)</strong>&lt;br&gt;i.d. 6</td>
<td>Aerosolized or s.c. H37Rv</td>
<td>● Number of MTB in spleen &amp; DTH ● No difference in outcome between different challenge methods</td>
<td>● Lowest number of MTB in 3, followed by 5, 1, 4, and 2</td>
<td>Number of MTB in spleen/C15</td>
<td>DTH</td>
<td>● Lowest number of MTB in 3, followed by 5, 1, 4, and 2 ● No difference in outcome between different challenge methods</td>
</tr>
<tr>
<td><strong>Smerak et al. (1982)</strong>&lt;br&gt;Denmark (1) Japan (2) Pasteur (3) Prague (4) Russia (5)&lt;br&gt;30 per strain i.p. 4</td>
<td>H37Rv s.c. 5</td>
<td>● Spleen weight ● Cytotoxic index (migration inhibition) ● DTH</td>
<td>● Lowest spleen weight and highest cytotoxic index in 4 1, 2, 3 and 5 similar in spleen weight and cytotoxic index</td>
<td>Spleen weight/C15</td>
<td>Cytotoxic index migration inhibition</td>
<td>● Lowest spleen weight and highest cytotoxic index in 4 1, 2, 3 and 5 similar in spleen weight and cytotoxic index</td>
</tr>
<tr>
<td><strong>Hank et al. (1981)</strong>&lt;br&gt;Denmark Prague</td>
<td>Aerosolized H37Rv and clinical MTB strain Various intervals 13</td>
<td>● Culture of primary lesions in the lungs</td>
<td>● Number of organisms in lungs lowest in Denmark group regardless of strain of challenge</td>
<td>Weight of spleen/C15</td>
<td>Spleen weight</td>
<td>● Number of organisms in lungs lowest in Denmark group regardless of strain of challenge</td>
</tr>
<tr>
<td><strong>Ladefoged et al. (1976)</strong>&lt;br&gt;Former Denmark (1) Denmark (2) Glaxo (3) Japan (4) Madras (5) Moreau (6) Prague (7) Pasteur (8) Russia (9) Sweden (10) R (11) Y (12)&lt;br&gt;5–10 per strain i.d. 13</td>
<td>Virulent MTB strain i.p. 7–12</td>
<td>● Weight of spleen, omentum, macroscopic ● Changes in organs ● DTH</td>
<td>● None</td>
<td>Weight of spleen/C15</td>
<td>Weight of spleen</td>
<td>● None</td>
</tr>
<tr>
<td><strong>Pruchova &amp; Sir (1972)</strong>&lt;br&gt;Japan Denmark Prague</td>
<td>H37Rv s.c. 5</td>
<td>● Spleen weight</td>
<td>● Spleen weight highest in Prague lowest in Denmark</td>
<td>Spleen weight/C15</td>
<td>Spleen weight</td>
<td>● Spleen weight highest in Prague lowest in Denmark</td>
</tr>
<tr>
<td><strong>Yakuwa (1953)</strong>&lt;br&gt;Japan Pasteur (2)</td>
<td>Kikuchi MTB strain 6</td>
<td>● Cultivation of MTB in inguinal lymph node, spleen, lung, liver and kidney ● DTH</td>
<td>● With higher dose of BCG, MTB more frequently cultured in 1 ● With lower dose of BCG, equal frequency of positive cultures</td>
<td>Kikuchi MTB strain/C15</td>
<td>Cultivation of MTB in inguinal lymph node, spleen, lung, liver and kidney</td>
<td>● With higher dose of BCG, MTB more frequently cultured in 1 ● With lower dose of BCG, equal frequency of positive cultures</td>
</tr>
<tr>
<td>Author, reference, publication year, animal model</td>
<td>BCG strains</td>
<td>No of animals</td>
<td>Immunization to challenge interval (weeks)</td>
<td>In vivo challenge agent and route</td>
<td>Challenge to outcome interval (weeks)</td>
<td>Parameters measured</td>
</tr>
<tr>
<td>-----------------------------------------------</td>
<td>-------------</td>
<td>---------------</td>
<td>------------------------------------------</td>
<td>---------------------------------</td>
<td>-------------------------------------</td>
<td>---------------------</td>
</tr>
<tr>
<td>Bank vole</td>
<td>Ladefoged et al. (1970)</td>
<td>Denmark (1) Glaxo (2) Japan (3) Madras (4) Moreau (5) Pasteur (6) Prague (7) Russia (8) Sweden (9) R (9) Y (10)</td>
<td>12 per strain i.p.</td>
<td>8</td>
<td>Virulent M. bovis i.v.</td>
<td>8</td>
</tr>
<tr>
<td>Hamster</td>
<td>Bunch-Christensen et al. (1970)</td>
<td>Denmark (1) Glaxo (2) Moreau (3) Sweden (4) R (5)</td>
<td>52 per strain i.p.</td>
<td>• Survival</td>
<td>• 3 most virulent</td>
<td>2 and 5 least virulent</td>
</tr>
<tr>
<td>Baboon</td>
<td>Chege et al. (2005)</td>
<td>Japan Pasteur</td>
<td>8 i.d.</td>
<td>4, 8, 14, 22, 26, 108</td>
<td>• PBMC proliferation after stimulation with PPD</td>
<td>• Proliferation index not different between groups</td>
</tr>
</tbody>
</table>

The number in parentheses after each vaccine strain in the second column is used to identify strains in the final column. ELISA, enzyme linked immunosorbent assay; ELISPOT, enzyme-linked immunospot assay; H37Rv, M. tuberculosis laboratory strain; i.v., intravenous; i.d., intradermal; i.p., intraperitoneal; MTB, M. tuberculosis; R, experimental strain derived from BCG-Japan; Y, experimental strain derived from BCG-Phipps.
Studies in guinea-pigs and hamsters

In a study comparing four different Chinese BCG vaccine strains (three derived from BCG-Denmark) with BCG-Denmark, guinea-pigs were challenged with aerosolized or subcutaneously inoculated \textit{M. tuberculosis} 6 weeks after intradermal immunization (Wang \textit{et al.}, 1988). Animals immunized with BCG-Denmark had the lowest CFU recovered from the spleen but this was only significantly different from one of the four Chinese BCG strains.

In another study, BCG-Denmark also induced better protection than BCG-Prague against an aerosol challenge in previously intradermally immunized guinea-pigs (Hank \textit{et al.}, 1981).

A series of studies aiming to rank the protective efficacy of BCG vaccine strains were initiated by the WHO in the 1970s. The largest of these studies compared 12 different BCG vaccine strains (Ladefoged \textit{et al.}, 1976). After intradermal immunization, guinea-pigs were challenged with \textit{M. tuberculosis} intraperitoneally. At autopsy 7–12 weeks later, the weights of the spleen and omentum in addition to macroscopic changes in different organs were analysed. Unfortunately, the \textit{M. tuberculosis} challenge strain had apparently been inadvertently attenuated, the challenge dose was dissimilar in the test animals and not all the animals were killed at the same time after challenge. Because of this variability, the authors did not analyse this part of the study.

Focusing on the results of DTH instead, and including results from their previous studies, a ranking of presumed best protection was provided but is of little value as it is now well established that DTH is not related to protective immunity.

In a Japanese study, guinea-pigs were immunized either intradermally or subcutaneously with BCG-Japan or BCG-Pasteur (Yakuwa, 1953). The animals were challenged subcutaneously 6 weeks later with \textit{M. tuberculosis} and after 12 weeks the lung, liver, spleen and lymph nodes were cultivated and macroscopic changes were assessed. The lymph nodes of animals immunized with BCG-Pasteur showed lower CFU, suggesting better protection compared with those vaccinated with BCG-Japan.

Intraperitoneal injection of different BCG strains in guinea-pigs with or without later subcutaneous challenge with \textit{M. tuberculosis} has been performed by a number of different groups (Bunch-Christensen \textit{et al.}, 1968, 1970; Buraczewska \textit{et al.}, 1969; Ladefoged \textit{et al.}, 1970; Pruchova & Sir, 1972; Smerak \textit{et al.}, 1982). In one study, unimmunized animals survived longer than those that received intraperitoneal BCG (Bunch-Christensen \textit{et al.}, 1968), suggesting better protection compared with those vaccinated with BCG-Japan.

**Studies in other animals**

In a study, unique in using bank voles as a model, 11 different BCG vaccine strains were compared in six different experiments, each involving five strains (Ladefoged \textit{et al.}, 1970). Eight weeks after immunization (the route was not

<table>
<thead>
<tr>
<th>Parameter measured</th>
<th>Mouse</th>
<th>Guinea pig</th>
<th>Bank vole</th>
<th>Hamster</th>
<th>Baboon</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>in vivo</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DTH</td>
<td>A–E</td>
<td>F–H</td>
<td>O</td>
<td>O</td>
<td>O</td>
</tr>
<tr>
<td>BCG scar</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cell proliferation in lymph nodes</td>
<td>B</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Survival of animal</td>
<td>C, E</td>
<td>M, N</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>in vitro</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Subcutaneous challenge with subsequent cultivation of challenge agent in spleen and/or lymph nodes</td>
<td>I–K</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Subcutaneous challenge with subsequent measurement of spleen weight</td>
<td>G, K</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aerosolised MTB challenge with subsequent cultivation of challenge agent in lung or spleen</td>
<td>C, E</td>
<td>C, E, K, L</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intravenous challenge with subsequent cultivation of challenge agent in liver, spleen, lungs and measurement of survival</td>
<td>B</td>
<td>C, E</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weight of spleen, liver and lung after immunisation</td>
<td>A, B</td>
<td>D</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BCG growth in lymphoid tissue</td>
<td>D</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MTB/BCG specific lympho-proliferation and immuno-phenotyping</td>
<td>D, E</td>
<td>O</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Th1/Th2 cytokine response in T cells</td>
<td>D, E</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IFN-γ in supernatant after stimulation</td>
<td>D</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cytotoxic T-cell response</td>
<td>D</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Migration inhibition stimulation of spleen effector cells</td>
<td>G</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PPD-specific IgA, IgG, IgM</td>
<td>D</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\textsuperscript{a}Junior & Gontijo Filho (1979), \textsuperscript{b}Gheorghiu & Lagrange (1983), \textsuperscript{c}Collins (1985), \textsuperscript{d}Lagranderie \textit{et al.} (1996), \textsuperscript{e}Castillo-Rodal \textit{et al.} (2006), \textsuperscript{f}Ladefoged \textit{et al.} (1976), \textsuperscript{g}Smerak \textit{et al.} (1982), \textsuperscript{h}Wang \textit{et al.} (1988), \textsuperscript{i}Yakuwa (1953), \textsuperscript{j}Pruchova & Sir (1972), \textsuperscript{k}Wang \textit{et al.} (1988), \textsuperscript{l}Hank \textit{et al.} (1981), \textsuperscript{m}Ladefoged \textit{et al.} (1970), \textsuperscript{n}Bunch-Christensen \textit{et al.} (1968), \textsuperscript{o}Chege \textit{et al.} (2005).

DTA, delayed type hypersensitivity; MTB, \textit{M. tuberculosis}.
### Table 4. Summary of human studies in reverse chronological order comparing different BCG strains (Findings relating to scar size and DTH are in brackets because of their limited value, see text)

<table>
<thead>
<tr>
<th>Author, reference year of publication</th>
<th>Country</th>
<th>Study period</th>
<th>BCG strains (route i.d. unless indicated)</th>
<th>No. of individuals studied (age BCG given at)</th>
<th>Interval between immunization and investigation / Duration of follow-up</th>
<th>Cytokine response</th>
<th>Lympho proliferation</th>
<th>Cytotoxicity</th>
<th>Regulatory T cells</th>
<th>Development of TB disease</th>
<th>HU/DTH scar</th>
<th>Main findings (Findings relating to BCG scar and DTH do not correlate to protection, see text)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wu et al. (2007)</td>
<td>Mexico</td>
<td>2003–2004</td>
<td>Denmark Japan Moreau</td>
<td>107 (at birth)</td>
<td>12 months X X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>Higher RNA levels for IFN-γ, IL-12, IL-27 with Moreau and Denmark</td>
</tr>
<tr>
<td>Castro-Rodriguez et al. (2007)</td>
<td>Chile</td>
<td></td>
<td>Japan Glaxo</td>
<td>341 (at birth)</td>
<td>5 months X X</td>
<td>X</td>
<td></td>
<td></td>
<td>X</td>
<td>X</td>
<td></td>
<td>(Mean size of DTH larger with Japan)</td>
</tr>
<tr>
<td>Davids et al. (2006)</td>
<td>South Africa</td>
<td>2002–2004</td>
<td>Denmark (DID) Japan (IID) Japan (IPC)</td>
<td>89 (at birth)</td>
<td>10 weeks X X</td>
<td>X</td>
<td>X</td>
<td></td>
<td>X</td>
<td>X</td>
<td></td>
<td>IFN-γ producing CD4 and CD8 cells more frequent in JPC than in DID; no difference between other groups</td>
</tr>
<tr>
<td>Gorak-Stolinska et al. (2006)</td>
<td>UK</td>
<td>1999–2004</td>
<td>Denmark Glaxo</td>
<td>230 (school-children)</td>
<td>12 months X</td>
<td>X</td>
<td></td>
<td></td>
<td>X</td>
<td>X</td>
<td></td>
<td>Rise in IFN-γ production after 12 month (&gt; 62 pg mL−1) in both groups</td>
</tr>
<tr>
<td>Roth et al. (2005)</td>
<td>Guinea-Bissau</td>
<td></td>
<td>Bulgaria Denmark Glaxo</td>
<td>2689 (at birth)</td>
<td>2, 6 and 12 months X X X</td>
<td>X</td>
<td></td>
<td></td>
<td>X</td>
<td>X</td>
<td></td>
<td>(BCG scar and DTH more often positive with Glaxo)</td>
</tr>
<tr>
<td>Aronson et al. (2004)</td>
<td>US</td>
<td>1935–1938</td>
<td>Phipps 317 Pasteur 575</td>
<td>3025 (1 month – 20 years)</td>
<td>Follow up 60 years X X</td>
<td>X</td>
<td></td>
<td></td>
<td>X</td>
<td>X</td>
<td></td>
<td>Phipps 317 lower protective efficacy than Pasteur 575</td>
</tr>
<tr>
<td>Hussey et al. (2002)</td>
<td>South Africa</td>
<td></td>
<td>Denmark (DID) Japan (IID) Japan (IPC)</td>
<td>51 (at birth)</td>
<td>10 weeks X X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
<td>No difference between strains in lymphocyte proliferation</td>
</tr>
<tr>
<td>Hoft et al. (1999)</td>
<td>US</td>
<td></td>
<td>Connaught Tice Placebo</td>
<td>54 (18–45 years)</td>
<td>0, 1, 5 and 7 weeks X X X X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
<td>T cell proliferation higher with Connaught than with Tice</td>
</tr>
<tr>
<td>Guerin et al. (1999)</td>
<td>Senegal</td>
<td>1990</td>
<td>Japan Glaxo</td>
<td>548 (8–10 years)</td>
<td>10–14 weeks X X</td>
<td>X</td>
<td></td>
<td></td>
<td>X</td>
<td>X</td>
<td></td>
<td>(No difference in frequency of BCG scar or scar size significantly larger with Glaxo)</td>
</tr>
</tbody>
</table>

(Continued)
### Table 4. Continued.

<table>
<thead>
<tr>
<th>Author, reference year of publication</th>
<th>Country</th>
<th>Study period</th>
<th>BCG strains (route i.d. unless indicated)</th>
<th>No. of individuals studied (age BCG given at)</th>
<th>Interval between immunization and investigation / Duration of follow-up</th>
<th>Cytokine response</th>
<th>Lympho. proliferation</th>
<th>Cytotoxicity</th>
<th>Regulatory T cells</th>
<th>Development of TB disease</th>
<th>BCG scar (DTH)</th>
<th>Main findings (findings relating to BCG scar and DTH do not correlate to protection, see text)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sucliene et al. (1999)</td>
<td>Lithuania</td>
<td></td>
<td>Denmark WHO ref. strain</td>
<td>525 (1 week or 3 months)</td>
<td>3 and 12 months</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td><em>(Larger BCG scars and DTH in the group vaccinated at 3 months)</em></td>
</tr>
<tr>
<td>Tuberculosis Research Centre (ICMR) (1999), Narayanan (2006)</td>
<td>India</td>
<td>1968–1971</td>
<td>Denmark Pasteur Placebo</td>
<td>117 718 (1 month – &gt; 45 years)</td>
<td>Active surveillance every 30 months Follow-up for 15 years</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td><em>(No difference in the levels of protection between Denmark and Pasteur)</em></td>
</tr>
<tr>
<td>Vijayalakshmi et al. (1995)</td>
<td>India</td>
<td>1990–1992</td>
<td>Glaxo Madras Japan</td>
<td>300 (infants)</td>
<td>6 weeks and 6 months</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td><em>(Lymphocyte migration inhibition (cytotoxicity) higher with Glaxo but not statistically significant)</em></td>
</tr>
<tr>
<td>Bottiger et al. (1983)</td>
<td>Sweden</td>
<td></td>
<td>Behringwerke Denmark Glaxo</td>
<td>2997 (14–15 years)</td>
<td>6 weeks</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td><em>(Mean size of DTH largest with Glaxo, followed by Behringwerke and Denmark)</em></td>
</tr>
<tr>
<td>Galbraith &amp; Hall (1974)</td>
<td>UK</td>
<td>1971–1974</td>
<td>Denmark Glaxo</td>
<td>240 (12–14 years)</td>
<td>9 weeks</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td><em>(Larger scars with Denmark)</em></td>
</tr>
<tr>
<td>Vallishayee et al. (1974)</td>
<td>Denmark</td>
<td>1966–1968</td>
<td>Former Denmark Glaxo Japan Glaxo Madras 809 Moreau Paraguay Russia Sweden Y</td>
<td>2512 (in India 1–15 years, in Denmark 7 years)</td>
<td>8–11 weeks</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td><em>(Association between BCG scar and DTH)</em></td>
</tr>
<tr>
<td>Horwitz &amp; Bunch-Christensen (1972)</td>
<td>Denmark</td>
<td>1964–1970</td>
<td>Connaught Glaxo Denmark Japan Pasteur Prague Russia Ref. strain</td>
<td>955 (7 years)</td>
<td>8–10 weeks and 5 years</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td><em>(Largest DTH with Denmark and Japan)</em></td>
</tr>
</tbody>
</table>

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described), study animals were challenged intravenously with *M. bovis*. The duration of survival of the voles was dependent on the dose and generally increased with a higher dose of vaccine. The survival of animals immunized with BCG-Denmark was longer at all doses than those immunized with BCG-Glaxo and BCG-Prague but shorter than those immunized with BCG-Moreau. BCG-Denmark was the only strain used in all experiments, limiting comparisons of results.

The only study comparing BCG vaccine strains in non-human primates evaluated BCG-Japan and BCG-Pasteur in a small number of baboons 10–14 weeks after immunization (Chege *et al.*, 2005). The authors found no difference in peripheral blood mononuclear proliferative responses to BCG or PPD in two baboons immunized with BCG-Japan compared with two immunized with BCG-Pasteur.

**Summary of animal studies**

In summary, BCG-Pasteur, BCG-Denmark and BCG-Glaxo are the strains most commonly associated with better protection in mice and BCG-Denmark is the most effective strain in guinea-pigs. However, any firm conclusion is seriously limited by inconsistencies between study designs, including the animal model, numbers of BCG vaccine strains included (Table 5), timing and mode of challenge and parameters measured (Table 3). Another important potential source of variability among animal studies is the strain of *M. tuberculosis* used as a challenge (Table 2). Preliminary data point to the importance of differences between laboratory and clinical strains, or even the specific strains used as a challenge agent (Collins, 1985). Further animal studies comparing different BCG vaccines would be most useful if there was standardization of study design including factors such as the dose and route of immunization, interval between immunization and challenge and the type and route of administration of the challenge agent.

**Studies in humans**

To date, there are at least 18 published abstracts and studies comparing different BCG vaccine strains in humans. The details of all the studies are summarized in Tables 4 and 5.

The first study to investigate gene expression after immunization with different BCG vaccine strains involved 107 children given either BCG-Moreau, BCG-Denmark or BCG-Japan at birth (Wu *et al.*, 2007). One year after immunization, peripheral blood mononuclear cells were stimulated with *M. tuberculosis* antigens and the expression of 17 genes was investigated using quantitative PCR. BCG-Moreau and BCG-Denmark induced significantly higher levels of IFN-γ, IL-12β and IL-27 mRNA. BCG-Japan-immunized newborns expressed higher levels of IL-1, IL-6 and IL-24 mRNA, suggesting a higher proinflammatory
Table 5. Summary of animal and human studies showing BCG vaccine strains investigated in each study

<table>
<thead>
<tr>
<th>BCG vaccine strain</th>
<th>Total number of strains in study</th>
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<tbody>
<tr>
<td>Beijing</td>
<td>1</td>
</tr>
<tr>
<td>Behringwerke</td>
<td>1</td>
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<tr>
<td>Birkhaus</td>
<td>1</td>
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<td>Chanchun</td>
<td>1</td>
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<tr>
<td>Connaught</td>
<td>1</td>
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<tr>
<td>Denmark</td>
<td>22</td>
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<tr>
<td>Former</td>
<td>3</td>
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<tr>
<td>Denmark</td>
<td>1</td>
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<tr>
<td>Frappier</td>
<td>18</td>
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<tr>
<td>Glaxo</td>
<td>1</td>
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<tr>
<td>Glaxo 626–629</td>
<td>1</td>
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<tr>
<td>Lanzhou</td>
<td>1</td>
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<tr>
<td>Japan</td>
<td>18</td>
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<tr>
<td>Madras</td>
<td>5</td>
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<tr>
<td>Mexico</td>
<td>8</td>
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<tr>
<td>Moreau</td>
<td>1</td>
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<tr>
<td>Norway</td>
<td>14</td>
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<tr>
<td>Pasteur 575</td>
<td>1</td>
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<tr>
<td>Pasteur</td>
<td>1</td>
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<tr>
<td>Poland</td>
<td>11</td>
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<td>Prague</td>
<td>2</td>
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<tr>
<td>Phipps</td>
<td>1</td>
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<tr>
<td>Phipps 317</td>
<td>2</td>
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<tr>
<td>Russia/Bulgaria</td>
<td>9</td>
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<td>R</td>
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<tr>
<td>Sweden</td>
<td>5</td>
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<tr>
<td>Shanghai</td>
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<td>Tice</td>
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<td>WHO ref strain Y</td>
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<thead>
<tr>
<th>BCG vaccine strain</th>
<th>Mouse</th>
<th>Guinea-pig</th>
<th>Hamster</th>
<th>Bank vole</th>
<th>Baloon</th>
<th>Human</th>
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<tr>
<td>Castillo-Rodal (2006)</td>
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<td>Collins (1985)</td>
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<td>Gheorghiu (1959)</td>
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<td>Wang (1982)</td>
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<td>Smerak (1981)</td>
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<td>Wu (2007)</td>
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<td>Aronson (2004)</td>
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<td>Davids (2006)</td>
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<td>Valimay (1974)</td>
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<td>Chan (1966)</td>
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<td>Nyboe (1966)</td>
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response. In this study, no difference was detected in gene expression of regulatory markers such as FoxP3, IL-10 and TGFβ1 between the three groups.

A study evaluating immune response to BCG (other than BCG scar and/or DTH) took advantage of a change in vaccine policy in the United Kingdom in August 2002. The immune response was assessed in schoolchildren before and after BCG-Glaxo was replaced by BCG-Denmark (Gorak-Stolinska et al., 2006). Blood was taken before and 12 months after immunization and stimulated for 6 days with *M. tuberculosis* or PPD. Compared with preimmunization, there was a statistically significant increase in the IFN-γ response 1 year after immunization but no difference was found between the two vaccine strains. However, the two strains in this study are very closely related, if not genetically identical (Mostowy et al., 2003; Brosch et al., 2007), and therefore a significant difference in induced immune response would not be expected.

A study from South Africa compared the immune response of newborns 10 weeks after immunization soon after birth with either BCG-Denmark intradermally (DID), BCG-Japan intradermally (JID) or BCG-Japan percutaneously (JPC) (Davids et al., 2006). Despite the relatively small size of this study, plasma levels for IFN-γ, after 7 h of incubation of whole blood with BCG, were significantly different between all three groups, with the highest levels in the JPC and the lowest levels in the DID group. TNF-α levels were the highest in the JPC group and the lowest in the DID group. IL-4 levels were inversely highest in the JPC group and the lowest in the IDC group. Differences in TNF-α and IL-4 levels reached statistical significance only between the JPC and DID groups. However, the ratio of IFN-γ/IL-4 was significantly different in all three groups. Interestingly, the number of IFN-γ-producing CD4 and CD8 cells, assessed by a whole-blood intracellular cytokine assay, was the highest in the JPC group, but did not differ between subjects who received the two vaccine strains intradermally.

A preliminary study by the same investigators used a similar design but included additional groups to test two different devices for administering percutaneous BCG (Hussey et al., 2002). Proliferative and cytokine responses differed depending on the mycobacterial stimulant (BCG, H37Rv or PPD) used. Proliferative responses were significantly greater in BCG-immunized compared with unimmunized infants. Differences between BCG vaccine strains or route of administration were not detected in this small study. IFN-γ and IL-10 production were also not significantly different between BCG vaccine strains or routes but a trend for higher levels of these cytokines was seen in infants who received BCG-Denmark. The small number of infants in each group limited further analysis and conclusions.

The only randomized-controlled trial using BCG vaccine strains still in use today included 54 TST-negative adult volunteers and measured the immune response at 0, 1, 5 and 7 weeks following immunization with BCG-Connaught or BCG-Tice (Hoft et al., 1999). *Mycobacterium tuberculosis* whole lysate, *M. tuberculosis* culture filtrate, BCG-Connaught (live and heat killed) and PPD stimulation of peripheral blood mononuclear cell (PBMC) were compared. A higher frequency of proliferating cells at 5 and 7 weeks were found in individuals immunized with BCG-Connaught. Higher levels of IL-4 and IFN-γ were found 5 weeks after immunization with BCG-Tice. A direct comparison with the two strains involved was unfortunately not performed.

A randomized-controlled trial of BCG vaccine efficacy by Aronson et al. (2004) is the longest follow-up study to date and provides interesting information about the effect of vaccine strain. This study randomized 3025 native children and adults in Alaska in the 1930s to receive either BCG-Phipps 317, BCG-Pasteur 575 or placebo. BCG-Phipps 317 had a lower (44%) protective efficacy than BCG-Pasteur 575 (59%) for any form of tuberculosis during a follow-up period of 50 years. Although both the BCG strains used in this trial are no longer in use, this unique study suggests a difference in protective efficacy between BCG vaccine strains even after 50 years.

A 15-year follow-up of the well-known, very large, randomized-controlled study in Chingleput did not find an overall difference in the tuberculosis incidence in children over 1 month of age immunized with either BCG-Pasteur or BCG-Denmark [Tuberculosis Research Centre (ICMR), 1999; Narayan, 2006]. However, only 25% of the children in this study were under 10 years of age. Moreover, half the children were given a 0.01-mg BCG dose, which may have been too low. A moderate protective effect was seen among children aged 1 month to 9 years. In this subgroup, BCG-Pasteur and BCG-Denmark were equally protective using a high (0.1 mg) dose and the difference between the protective efficacy of BCG-Denmark (25%) and BCG-Pasteur (17%) using a low (0.01 mg) dose was not statistically significant.

A later study in India compared BCG-Madras, BCG-Glaxo and BCG-Japan in infants (Vijayalakshmi et al., 1995). Three and six months after immunization, an in vitro assay investigating the PPD-induced capacity of lymphocytes to inhibit migration of leucocytes (LMI1) showed that BCG-Glaxo was consistently more likely to produce an LMI1. However, the significance of this finding is uncertain.

In the 1960s, a large study involving 162.953 newborns compared the protective efficacy of BCG-Pasteur or BCG-Glaxo in Hong Kong (Chan et al., 1966). Within a 4-year period, newborns immunized with BCG-Pasteur were significantly better protected against tuberculosis. Unfortunately, the findings from this study are limited because they have only been published as an abstract.

In a small study investigating the immune response to BCG-Denmark, BCG-Glaxo or BCG-Pasteur in adult
better or worse within the same study depending on whether
immunogenic when compared with BCG-Japan in another
et al. in one study (Gorak-Stolinska et al., 2002; Davids
et al., 2006; Gorak-Stolinska et al., 2006; Hussey et al., 2002;
Hoft et al. (1999); Vijayalakshmi et al. (1995)).

Summary of studies in humans

In summary, of the 18 studies in humans, only nine have
used measures of protective immunity or efficacy other than
BCG scar and/or DTH, which does not provide relevant
information about protective efficacy as discussed earlier.

Six studies investigated immunological parameters (Fig. 2)
and reported contradictory results (Vijayalakshmi et al., 1995;
Hoft et al., 1999; Dannenberg & Collins, 2001; Hussey et al.,
2002; Davids et al., 2006; Gorak-Stolinska et al., 2006; Wu
et al., 2007). Whereas one study suggests that BCG-Denmark
is more immunogenic than BCG-Japan (Wu et al., 2007), two
other studies suggest the contrary (Hussey et al., 2002; Davids
et al., 2006). BCG-Glaxo was comparable to BCG-Denmark
in one study (Gorak-Stolinska et al., 2006) but was more
immunogenic when compared with BCG-Japan in another
(Vijayalakshmi et al., 1995). BCG-Connaught performed
better or worse within the same study depending on whether
cytokine or cytotoxic T cells were measured (Hoft et al.,
1999). As in animal studies, conclusions are limited by
inconsistencies in study design such as the choice of BCG
vaccine strains, age at immunization and immune correlates
investigated. Furthermore, the number of individuals in all
these studies is small. Until accurate biomarkers of protection
against tuberculosis are defined, the clinical significance of
these findings remains unclear. However, these studies suggest
that the immune response induced by BCG is affected by
genetic differences among BCG strains and supports the
notion that there may be significant differences in protection
against tuberculosis afforded by different vaccine stains.

Three studies have evaluated clinical outcomes with
follow-up periods ranging from 4 to 60 years. Unfortunately,
of these, one study used BCG vaccine strains that are no
longer in use (Aronson et al., 2004), another was published
only as an abstract with limited information (Chan et al.,
1966) and the Chingleput study used two different doses of
BCG and a wide age range [Tuberculosis Research Centre
(ICMR), 1999; Narayanan, 2006]. Therefore, these studies
do not help determine which of the currently available BCG
vaccine strains are most likely to offer superior protection.

Conclusions

Evidence from animal and human studies shows that there
are significant differences in the immune response induced
by different BCG vaccine strains. The key question remains
whether these differences in vitro translate to differences in
protective efficacy against tuberculosis in humans. This
review highlights the current lack of data on which to determine the superiority of individual BCG vaccine strains. However, the evidence that BCG vaccines strains are genetically divergent and perform differently in almost all studies is of potential great importance.

The superior efficacy of one strain over another would have a direct bearing on BCG vaccine strain choice and global tuberculosis control. Although differences in protective efficacy between different BCG strains might be small, a change in the use of the BCG vaccine strain associated with greatest protection would have a dramatic impact on a population basis. For example, based on the WHO surveillance data (Corbett et al., 2003), a 1% improvement in protective efficacy would save 18 000 lives (particularly in children) and prevent 83 000 from tuberculosis disease each year.

Well-designed, adequately powered studies are therefore urgently needed to compare the immune response induced by different BCG vaccine strains in humans. In particular, comparison between genetically distant strains would be most helpful. This will also require a better understanding of the in vitro correlates of protective immunity against tuberculosis. Better use of existing BCG vaccines could play an important part in the WHO Global Plan and strategy to fight the worsening tuberculosis pandemic.

Search strategy and selection criteria
Publications for this review were identified by a search of the Cochrane Library, Medline (1950 to December 2007), Embase (1980 to December 2007) and Web of Science (1990 to December 2007). The search terms ‘Bacille Calmette–Guérin’, ‘BCG’ and each of the names of the BCG vaccine strains listed in Table 1 (including all their synonyms) were individually combined with the search terms: ‘immun*’, ‘protect*’ or ‘strain’. To exclude articles about the use of BCG in the treatment of bladder cancer, the Boolean operator ‘not’ was used to exclude the terms ‘bladder’ and ‘cancer’. Papers comparing BCG vaccine strains were identified by selecting only those describing the BCG- induced human T-cell activation and gamma interferon production in vitro. Infect Immun 75: 3197–3201.

Conflict of interest
The authors certify that they have no affiliations with or involvement in any company or organization with a direct financial interest in the subject matter of this review.

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