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BCG: the Past, Present and Future of a Tuberculosis Vaccine

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From a pharmaceutical perspective, Bacillus Calmette–Guérin (BCG) vaccine is a suspension of living attenuated *Mycobacterium bovis* cells, together with fragments of cells and aggregates of cells in various salts, lactose or glycerol, depending on the commercial origin of the vaccine. Commercially it is usually supplied as a lyophilized cake in sealed glass ampoules and is reconstituted with sterile distilled water before use. Pharmaceutical characterization of BCG was recently reviewed by Groves (1993).

Tuberculosis

Although tuberculosis (TB) is currently estimated to infect at least a third of the world's population, killing an estimated 3·1 million in 1995 (Bloom & Murray 1992; Collins 1993), it is not a new disease and may well have been a cause of death since humans first settled from a wandering existence to communities in neolithic times. TB probably occurred as a sporadic and unimportant disease during this early period, but by the eighteenth century it was well established in Europe and had spread to Africa, Asia, South America and Eastern Europe by the end of the nineteenth century. A general pattern has been recognized in that, once introduced into a new community, the disease peaks within 50–75 years and then slowly declines as resistant hosts emerge. It is likely that TB was introduced into America by European immigrants and mortality rates as high as 650 in 100 000 were seen in Boston by 1800. By 1904 this had decreased to 188 and by 1969 had dropped to 4 in 100 000. However, bone deformities associated with TB have been noted in prehistoric native populations of Illinois and Ohio, but by the time the Europeans had arrived, such cases were rare. This was to change rapidly as the North American Indians were forced into confinement with death rates in excess of 9000 per 100 000 being recorded. TB is still a significant disease amongst native Americans today in both Canada and the USA. Overall, by about 1980, the medical community, especially in the USA, had become somewhat complacent since the incidence of TB had continued to drop from its most recent peak associated with the Second World War. There was even talk of TB being vanquished by the end of the century, but the human immunodeficiency virus (HIV) epidemic seems to have triggered another sudden increase in tuberculosis in the USA (Fig. 1). The association between the two diseases is certainly evident in Africa today and it is anticipated that, as HIV spreads into South East Asia, this area will be significantly affected also (Table 1) (McKenna et al 1995). In addition, approximately 30% of TB patients in the USA have been born in another country and there have been outbreaks of the disease in hospitals, prisons, homeless shelters, hospices for AIDS patients, nursing homes and crack houses where crowding and occasionally hygiene are less than optimal. Unfortunately, although TB can be cured chemotherapeutically, there have also been occasional outbreaks of multi-drug-resistant TB (MDR-TB) which have been associated with very high mortality rates (Collins 1993; Lagrandderie et al 1993). The American experience has been repeated to some extent in most other industrialized nations, with the majority of new cases of TB being associated with immigrants. Two thirds of cases in Australia, for example, are in new arrivals, and 55% of cases in Israel are associated with the recently arrived Ethiopian community. However, in countries

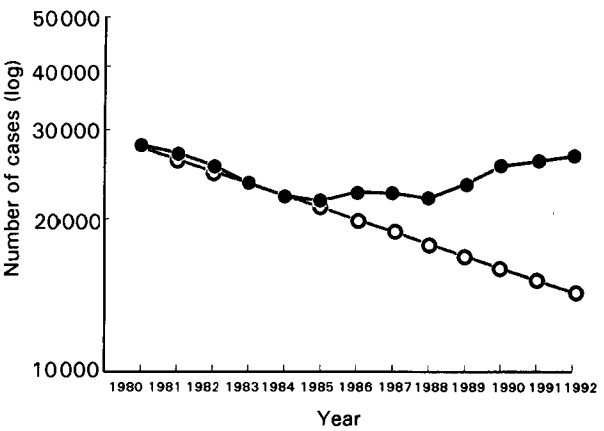

FIG. 1. The unexpected rise in the incidence of tuberculosis in the United States after the start of the HIV epidemic (~1983). ○ Expected cases; ● observed cases. Data from the Center for Disease Control.

Table 1. Estimated global distribution of adults who have been infected with HIV and TB, mid-1993.

Region	Infected adults
North America	110 000+
South America	450 000
Europe	60 000
North Africa	17 000+
Southern Africa (below Sahara)	3 760 000+
Russian Republics	11 000
China	9 000
East Indies	660 000+
Australia	5 000+
Total	~5 100 000

Data from WHO Tuberculosis Programme.

where HIV infection is not yet an issue, such as Japan and Australia, the overall incidence of TB has been steadily declining (Snider et al 1994). The medical community is perhaps less complacent than it was 15 years ago and there have been a number of appeals to the pharmaceutical industry for new and improved chemotherapies and vaccines (Script 1996).

Mycobacterium tuberculosis

Although TB (phthisis—Greek for wasting away) was known to Hippocrates, it was an Englishman, Benjamin Marten of London, who basically described the transmission of the disease from a consumptive to a healthy person in 1722 (Daniel et al 1994). Laennec, who himself died of TB in 1826, published an account of TB in his book on diseases of the chest in 1821. Jean Antoine Villemin transferred fluids from humans and cattle lesions to rabbits who then developed the disease (Villemin 1868). It easy to see that TB was of concern to the pioneers of biomedical science. However, it was Robert Koch who, on March 24, 1882, made a presentation to the Berlin Physiological Society which effectively established the science of microbiology. He demonstrated, convincingly, that the cause of TB was *Mycobacterium tuberculosis* (although other mycobacteria were later shown to be associated with the disease). However, he was unable to attenuate his isolates because they tended to grow in strings or clumps and sampling was difficult. Unfortunately, he also claimed that a filtrate of an *M. tuberculosis* culture was itself a cure for the disease. This proved controversial and was later discredited. However, purified filtrates, "Old Tuberculin" and the more stable "Purified Protein Derivative (PPD) of Tuberculin" are still used as diagnostic tests for tuberculosis.

Calmette and Guérin

Acquired immunity as a concept was only vaguely understood in the nineteenth century. Koch, for example, noted that infected animals who were subsequently exposed to a disease often survived the second exposure. Albert Calmette, a physician, was sent in 1894 to direct the newly founded Pasteur Institute in the northern French industrial city of Lille, where tuberculosis had reached epidemic proportions amongst factory workers. Three years later he was joined by Camille Guérin, a veterinarian, who had worked previously in Germany and was aware of Koch's experiments and the pioneering work of von Pirquet on tuberculin hypersensitivity. Having attempted grinding cultures in an agate mortar and pestle to produce more uniform dispersions, by accident they discovered that tuberculosis cultures could be dispersed by the addition of sterile beef bile. With modern hindsight we now recognize that they had added a natural wetting and dispersing agent to the culture. The failure by Koch to disperse these stringy, hydrophobic cultures, and therefore his inability to take samples from a growing culture, may have been the reason he failed to attenuate isolates and make a vaccine. In 1908, Calmette and Guérin began a series of serial passages into fresh medium every three weeks for a total of 231 passages, over a period of 13 years. Starting with a virulent bovine substrain previously isolated by Nocard from a heifer with tubercular mastitis, they found that the organism slowly lost its virulence to guinea-pigs, rabbit, rhesus monkeys, chimpanzees, cattle and horses. Transplanted to fresh medium, the organism rapidly reverted from having a rough, dry and granular surface growth to a smooth, viscous and moist colony, often with a greenish yellow colour. Smooth and rough cultures still appear but apparently this change in appearance has little to do with virulence, as was first supposed. Guérin (1980) has described the attenuation process, which was even carried out under the noses of the German forces occupying Lille for much of World War One.

Without knowing exactly what was happening genetically (we still do not know), they observed a change in colony morphology after only 39 passages, but, as they continued passage, the virulence was lost and ability to protect against TB infection was retained. By 1921 the first human was given the attenuated culture, orally. This was a newborn child in Paris who was considered to be at high risk because of TB in the immediate family and who, it should be noted, was free of the disease for his entire life. Calmette later reported in 1927 that, out of nearly 1000 children given the vaccine, only 3·9% died of TB (or unspecified causes) while the comparable mortality for unvaccinated children was 32·6%.

It is interesting to note that the original Nocard isolate has now disappeared and, of the three attenuated cultures maintained at the Institut Pasteur by 1930, only one has survived. This is not attributable to carelessness, but rather to the fact that freezers were not available in laboratories until around 1940. From a genetic point of view we cannot even say whether the original Nocard isolate was indeed *M. bovis* since the strain itself was only defined in the 1950s. Thus, although the genetic make up of what is now described as Bacillus Calmette–Guérin (BCG) has been described, we cannot determine how the attenuation process changed the factors originally responsible for virulence. Indeed, this is also true for the H37Ra and H37Rv avirulent and virulent substrains of a TB organism originally isolated by Steinken in 1934. It worth noting that the virulence factors of tuberculosis organisms have not been determined with any certainty even today. All we can say is that some genes have mutated, but what significance this has at a cellular level has yet to be determined.

The Lübeck Incident and Public Confidence in BCG

The reputation of BCG still seems to be tainted after the so-called Lübeck incident in 1929 in which 251 children were vaccinated orally with BCG accidentally contaminated with a highly virulent TB culture. Some 72 children died due to what was eventually proved to be poor laboratory practices where vaccine and a fresh isolate were stored side-by-side in the same refrigerator. BCG was completely vindicated in 1948, after a 15 year hiatus, during an international conference set up to discuss the issue (Rosenthal 1980). Since then the vaccine has been used extensively without any additional evidence of a lack of safety.

Two points arising from this unfortunate incident need to be observed. Firstly, although 72 children died, 179 did not. Secondly, the vaccine was given orally. The significance of these two observations remains unclear 67 years later.

Dissemination of BCG Around the World

As noted earlier, before the invention of the freezer, the preservation of cultures could only be achieved by continuous

passage, and by 1930 the Institut Pasteur had encouraged distribution of the original cultures around the world. As reviewed by Crispen (1989), by 1980 there was widespread international distribution (Table 2), with laboratories in over 50 countries apparently making their own unique contribution to the bacterial growth conditions and environment. By now it has to be admitted that by continuous passage under very different conditions, even the original BCG culture has itself most likely been subject to further mutation, fortunately without reverting to original virulence.

A typical case-history has been well documented for the development of the original Pasteur culture in the USA. In 1933 a young, medically qualified researcher by the name of Sol Roy Rosenthal was sent to the Institut Pasteur from the Chicago Municipal Tuberculosis Sanitarium (associated with Cook County Hospital, then, as now, the main hospital for the poor of Chicago) by the Director, Dr Frederick Tice. Rosenthal returned to Chicago with cultures from the Institut Pasteur, working initially at the University of Illinois, College of Medicine, and later in Tice's laboratory at Cook County. By 1950 he had modified cultural conditions to obtain what appeared to be a new substrain. This was dubbed the "Tice" (registered trademark) substrain in honour of his mentor, and was claimed to be a superior form of BCG (Rosenthal 1980). This claim has since been disputed although we have recently found differences between this and other commercial BCG substrains that may substantiate Rosenthal's claim (unpublished data). Incidentally, because of his connections with influential (i.e. wealthy) people in Chicago, Rosenthal was able to create the Institute for Tuberculosis Research under the State of Illinois statute in 1947. This Institute is still functioning today, although in a slightly different form.

Table 2. Some existing substrains of *Mycobacterium bovis* strain BCG (Crispen 1989).

Year	Location
1921	France (Pasteur Institute)
1924	USSR
1924	Brazil
1925	Japan
1926	Romania
1926	Sweden
	Equador (1954)
	Norway (1966)
1930	Belgium
1931	Denmark (State Serum Institute)
	Czechoslovakia (1947)
	Egypt (1954)
	UK (1952)
	Switzerland (1959)
	Japan (1965)
	India (1967)
1934	USA (Chicago, Tice)
1937	Canada (Quebec)
	Canada (Toronto, 1949)
1948	USA (New York)
	Australia (1951)
1957	Tunisia
1963	Senegal
1965	Netherlands
1965	People's Republic of China
1966	South Korea

A number of substrains are not listed, for example, Argentina (Buenos Aires), Germany (Marburg), Hungary (Budapest), Iran (Teheran), Poland (Warsaw) and Vietnam (Dalat). Other substrains are no longer produced for licensed use and include the Berlin substrain formerly produced by the former German Democratic Republic, the Phipps substrain formerly produced at the Trudeau Institute, Lake Placid, New York and the Glaxo substrain prepared in the UK.

The Process for BCG Culture

Like TB, the BCG organism is slow to grow in culture although some other mycobacterial species are considered to be rapid growers. As originally described by Guérin (1980), the organism grew on potato slices in beef bile containing 5% glycerin, but later synthetic media, including those due to Sauton or Middlebrook, were substituted. The process has been described by Rosenthal (1980). Grown as a surface pellicle or veil in broad-based glass flasks at 37°C for up to three weeks, the medium is poured away. The collected veil is pressed or drained to remove growth medium in some processes or, in the Tice process, drained under vacuum. The flask is then rotated vertically for 12–15 min in the presence of $\frac{1}{4}$-inch stainless steel balls to produce a more or less uniform cellular dispersion which is then diluted with 15% lactose in $\frac{1}{4}$-strength Sauton medium before filling into ampoules and freeze-drying.

The initial culture was from a freeze-dried seed lot which should be a direct descendent of the original BCG strain from the Institut Pasteur in France. However, we now doubt this contention, and even Rosenthal (1980) reported that his Tice substrain was a combination of several substrains maintained in the Tice laboratory since March 1934 and one of the standard substrains received from Paris in October 1951. Nevertheless, freeze-dried seed ampoules obtained from cultures of some initial master cultures are used in order to minimize mutation and it is recommended that not more than 12 passages should be made of any one seed lot.

An alternative process is to grow the organism by deep fermentation in a liquid medium. Left to its own devices the organism will grow to form granules of up to 3–4 mm diameter which attach themselves to the glass walls and other components of the fermentor tank. This makes harvesting very difficult and a wetting agent, such as Tween 20 or in the case of the so-called Glaxo substrain (no longer manufactured), Triton WR1339, is usually necessary. The Glaxo suspension was grown to a moist weight of 0·3 mg mL^{-1} containing 8–20 $\times 10^6$ viable organisms per mL before placing (without milling) in ampoules and freeze-dried. A broadly similar process was used for the Swiss and Dutch BCG laboratories according to Rosenthal (1980).

Morphology

Individual mature organisms are a rounded cylinder shape, approximately 2·36 μm long and 0·47 μm wide (Groves et al 1991). This information was utilized to calculate the number of organisms present in the cellular aggregates that are usually found in reconstituted ampoules of BCG. When measured by either light blockage or Coulter Counter methods, Zhang & Groves (1988) found that aggregates were approximately 4–7 μm in diameter. This corresponds to up to 500 cells (alive or dead) being contained in one aggregate, although when tested for viability, each aggregate would count as only 1 colony forming unit (CFU). Measurement of the palmitic acid content as the methyl ester (PAME), using gas chromato-

graphy, confirmed this data (Olson et al 1990) and later work by Klegerman et al (1991a) on ATP measurements demonstrated that the CFU determination provides a significant under-representation of the number of viable organisms present in the reconstituted BCG suspension.

The organism is strongly pleomorphic and the complexity of the life cycle of BCG and other mycobacteria has been suspected for at least sixty years. Fontes (1910), for example, demonstrated the viability of filtered forms of *M. tuberculosis* as early as 1910. Rosenthal (1980) described the presence of granules inside the cylindrical cells and the budding of small granules 50–100 nm in diameter. This process was confirmed by scanning electron microscopy (Devadoss et al 1990, 1991a) and these filtrable forms are apparently capable of an independent existence. Devadoss et al (1990) suggested that the internal space available for the necessary DNA and RNA was likely to be limited but may be capable of growth if a number of the microscopic coccoidal forms were capable of fusing together after filtration.

What has proved to be more interesting, however, is the integument surrounding mature cells. Calmette and Guérin (1909) suggested that the hydrophobic aggregates of cells were dispersed to individual cells by the addition of bile salts. This was shown to be unlikely by Zhang & Groves (1988) who measured particle size as a function of surfactant concentration. Hardham & James (1980) had observed an amorphous layer around aggregates of the Glaxo substrain of BCG and the presence of this integument was confirmed in other substrains by Devadoss et al (1991b, c). Failure to disperse the microorganisms completely is due to the fact that the cellular aggregates are held together by this integument.

The nature of the integument has been explored and it appears to be substantially polysaccharidic in nature, although it can be removed with protease enzymes without killing the cells themselves (Klegerman & Groves 1992). The suggestion has been made that the integument is produced in response to environmental stress, especially high levels of oxygen or lack of nutrients. Moreover, our recent work (unpublished), has indicated that the integument is the source of the antineoplastic glycans previously isolated from BCG cultures (Wang et al 1995). Attention is therefore turning to methods of continuously harvesting the integument from deep fermentation cultures of BCG (Garrido et al 1996; Klegerman et al 1996).

The structure of the BCG cell wall is like that of other gram-positive mycobacteria, composed for the most part of complex peptidoglycan structures with an inner and outer cell wall (Fig. 2).

The Safety of BCG

The use of BCG as a TB prophylactic increased significantly during and immediately after the Second World War as con-

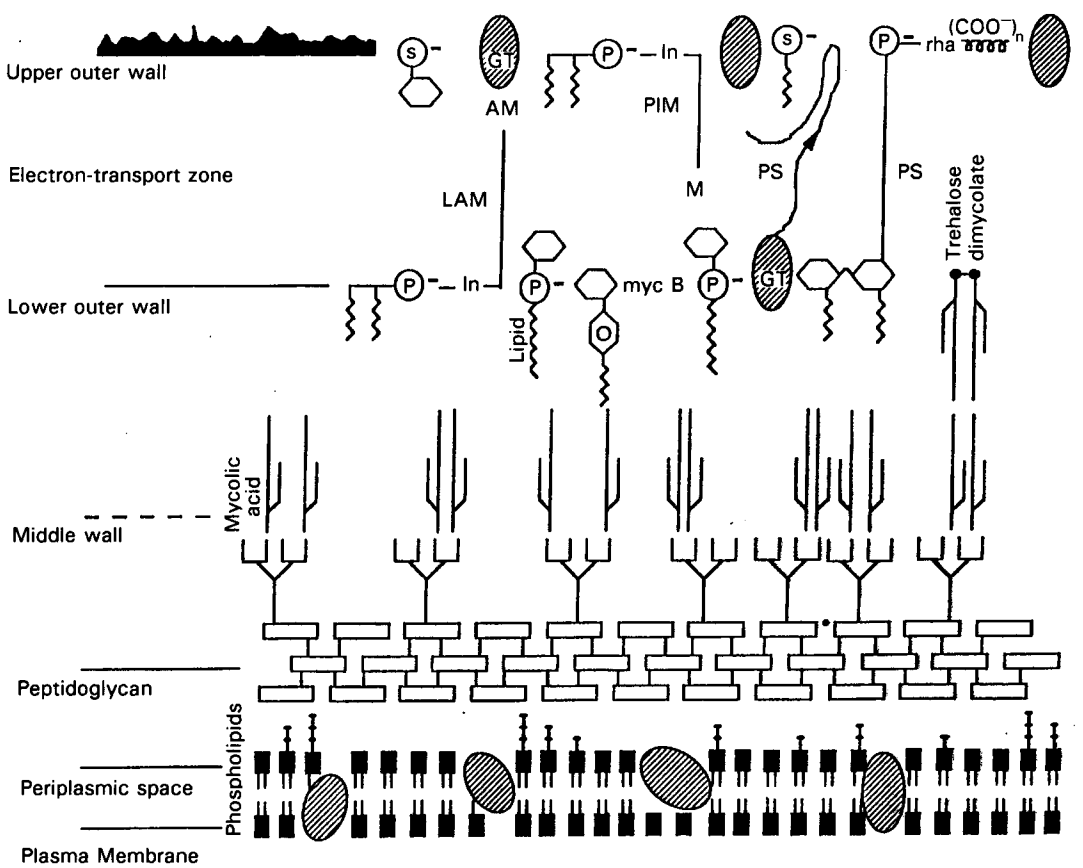

FIG. 2. A schematic view of the cross-section of cell wall of *Mycobacterium bovis* (BCG). S sulphate, P phosphate, hexagons oligosaccharides, AM arabinomannan, LAM lipoarabinomannan, PIM phosphatidylinositol mycolate, IN inositol, M mannan, rha rhamnose, GT glycosyltransferase, PS polysaccharide chain, myc B mycolate B (Klegerman, unpublished)

fidence returned following the Lübeck incident. With approximately 100 million doses administered in 1992 alone, it is believed that approaching three billion doses have been used in total, with very rare neurological damage and only 26 deaths attributable to the vaccine (World Health Organization (WHO) statistics). This is a truly impressive safety record for any vaccine, with an estimated serious side-effect frequency far below those reported for smallpox, for example (Bloom & Fine 1994). This makes BCG the world's most widely used vaccine, and probably the safest. However, the vaccine does produce some non-life threatening side effects. A common minor adverse effect is the production of induration and ulceration of the vaccination site. This has been claimed by some authorities to be an advantage since it leaves a scar which is visible evidence that the recipient has been vaccinated. Other minor effects include a low-grade fever, with a frequency of about 0·1%.

An area where BCG administration is likely to be dangerous is where the patient already has an impaired immune system such as a pre-existing HIV infection. A small number of disseminated BCG-osis cases have been reported in patients who were subsequently found to be HIV seropositive. Deaths have also been reported in patients with fulminating AIDS who were given BCG in a last desperate attempt to stimulate their diminished immune systems. The major current use of BCG is in Africa where it seems probable that BCG administration to patients already infected with HIV has occurred and it is equally probable that the side-effects and deaths have been under-reported. It was recommended by the WHO that BCG immunization of newborns continue, only excluding those children who are already showing signs of the immunodeficiency brought on by HIV. Bloom & Fine (1994) commented that there may be a "window of time" between birth, where the risk is minimal, and the point where the immune system is so damaged that BCG-osis becomes a substantial risk.

The Efficacy of BCG

BCG, until very recently, was hardly used in the USA, and even from a world-wide perspective varying results from controlled trials have left this area controversial, to say the least. Different patterns of protection have been observed around the world and it is now evident that the reasons for this are multifactorial. Factors are likely to be divided into two sections, the BCG itself and the environment in which the vaccine is used.

As noted previously, BCG is now obtained from a number of sources, Bloom & Fine (1994) reporting that over 90% of vaccines are derived from only three parent substrains (Glaxo-1077, Tokyo-172 and Pasteur-1172P). Of these, the Glaxo strain is no longer available, but different cultural conditions, variable viability due to storage conditions and other characteristics, all combine to produce different growth rates, viabilities, morphologies and, most important of all, antigen expression. As discussed earlier, the viability itself, determined as colony forming units (CFU), is critically affected by the state of dispersion. These interrelated factors have been found to vary according to the age of the stored ampoules from the same substrain (Groves et al 1991) and these factors are most likely to vary even more between different sources. A vitally needed assessment of the types and quantities of surface secreted antigens remains to be carried out for the various BCG strains currently used.

The environment in which the vaccinations themselves are carried out is likely to be even more critical. Methodological and statistical reappraisals of trials carried out up to 1983 suggested that BCG could confer a high degree of protection against TB (Clemens et al 1983) but the subject has remained controversial. Protective efficacy against TB (%) is defined as:

> (Incidence rate in unvaccinated population – incidence rate in vaccinated population) × 100/Incidence rate in unvaccinated population

Using these criteria the protective efficacy of BCG against TB in various trials has varied from below 20 to above 80%. In the meta-analysis by Colditz et al (1994), it was suggested that, under optimal conditions, the protection rarely fell below 50% and was usually as high as 80%. However, the major trial against TB carried out by the WHO in Chingleput, southern India, was a complete failure, with a zero protection rate being observed in a trial involving over a quarter of a million patients. It is now believed that this trial was doomed to fail in the first place because of the exogenous re-infection of the population with TB which occurs continuously. Survivors, in other words, were already protected before the BCG was given and no additional protection was evident. Bloom & Fine (1994) stated that interference by environmental mycobacteria provides the best explanation for the observed results in BCG immunization in both experimental animals and for the pattern of protection observed in different parts of the world. This argument was extended by Griffin & Buchan (1993) who suggested that a combination of immuno-diagnosis, chemotherapy and immuno-prophylaxis would be required to eradicate TB throughout the world.

There is now some agreement that BCG should be given at birth and certainly within the first three months of life. The way in which BCG protects is not completely rationalized although it does seem that the vaccine does not prevent the primary infection but it minimizes the spread of the disease from the lungs. What is even more impressive is the fact that miliary TB and tubercular meningitis have been almost eliminated in vaccinated children. This alone is sufficient justification for use of BCG when TB is being reported in epidemic proportions, as it currently is in Africa. Another reason is also becoming evident as BCG is now being reported to be effective against leprosy. This would be a major advantage if confirmed.

Some of the controversy surrounding BCG vaccination may be due to the short-range viewpoints of some medical authorities. In the USA, for example, the Centers for Disease Control (CDC) have consistently stated that BCG should not be used because it produces the same response to Tuberculin as a TB infection, thereby confusing and obscuring the disease diagnosis. Certainly our experience is that the medical profession here is unable to distinguish between the two situations. The real problem is one of attitude but it must be appreciated that the reaction to tuberculin is not just a simple or unequivocal correlation with a tubercular infection. The other problem is that a primary exposure to tuberculosis is often suppressed naturally, only becoming manifest if the immune system is affected. There is a considerable difference between infection and the disease state, and most infected

patients could remain healthy for the rest of their lives. BCG vaccination at birth would likely be beneficial, but the legal and medical systems in the USA do not encourage its use at present.

In Western Europe, where BCG has been used, the medical climate is generally more favourable. In the UK, the TB notification rate has fallen from 250 per 100 000 population to 10·5 over a twenty-year period. This may be due to an extensive use of BCG combined with other factors such as an improved economic climate and a generally better level of health following the disturbance of Second World War. However, with the incidence of HIV-infections increasing, combined with an expanding drug-abuse environment, it might be premature to suggest that BCG vaccination be stopped in order to save money in the National Health Service.

Undoubtably, there would be benefits from the introduction of a new or improved vaccine. However, realistically the regulatory situation is such that even if such a vaccine were available today, it would take a decade of testing before approval. BCG is available now, has an impressive safety record and an efficacy of 70–80%, something few other vaccines can claim.

Some Indications of How BCG Works

Although the exact mechanism by which BCG prevents TB is not fully understood, some immunological clues are already available. Macrophage stimulation does appear to play a major part in the mode of action of intact BCG against tumour cells as well as "foreign" cells such as tuberculosis, and this has also been observed for heat stable glycans extracted from BCG cultures. Certainly it is unlikely that BCG has any direct cytostatic activity on its own (Rajala et al 1992; Devadoss et al 1993). BCG appears to make macrophages bactericidal and tumouricidal through the release of oxygen metabolites such as hydrogen peroxide and superoxide anions (Heisse et al 1965; Nathan et al 1976; Hibbs et al 1977). Recently, an attempt to formulate a BCG glycan in collagen gel demonstrated clear evidence of macrophage stimulation, enough in fact, to severely corrode the drug delivery system (Friess et al 1996). We have also observed the activity of a BCG glycan against a human breast cell cancer line growing in nude mice—animals that have virtually no T-cells but which do retain macrophages (Donmez & Groves 1997). These observations all suggest that BCG and at least some of its components are capable of macrophage stimulation although the exact mechanism remains unclear at this stage.

BCG vaccination induces hypersensitivity to soluble mycobacterial antigens which is reflected in the production of CD4 T-cells and various lymphokines such as interferon (IFN) γ, interleukin (IL) 2 and tumour necrosis factor (TNF) β. Macrophages are activated to kill mycobacterial cells by IFN-γ and TNF-β. Moreover, IFN-γ is produced by Type 1 (Th1) subsets of human CD4 and CD8 T-cells. On the other hand, some cells also produce a Th2 response in which IL-4 and IL-10 are produced and these suppress CD4 T-cell production and the expression and transcription of IFN-γ and IL-2 as well as blocking macrophage activation. These processes inhibit clearance of intracellular pathogens. The contrary mechanisms may in part explain why low doses of BCG are occasionally capable of enhancing tumour growth (Klegerman et al 1991b).

It is also generally agreed that viable BCG vaccines are more effective than killed vaccines. This may be due to a continuous release of bacterial surface antigens into the body, the bacterial cells effectively behaving as a controlled release vaccine and thereby being better able to produce a cell-mediated response.

An interesting exception to this generalization is the new killed *M. vaccae* vaccine currently being tested in South Africa (Rook, private communication). This vaccine appears to behave as a general immunostimulant, in the same way as BCG. Since the culture is autoclaved, a treatment generally unfavourable to retention of protein structure or function, it would be interesting to know what the active component of this product is, from a chemical point of view. Groves et al (1995) reported the presence of a heat-stable complex glycan in boiling water extracts of *M. vaccae* cultures and it is quite possible this material (identified as PS4) could be a major active component of this killed vaccine.

BCG and Cancer

BCG has been known to be a general non-specific immunostimulant for some time. For example, Rosenthal (1980), in his in-depth study of the effect of BCG on the citizens of the South Side of Chicago, then, as now, identified with the poor of the city, administered BCG to 85 356 newborns from 1957 to 1969 and compared their data with that of an unvaccinated population of 534 870. What was remarkable about this trial was the length of follow-up—in some cases over twenty years. As anticipated, the TB death rate dropped (from 4·39 per 100 000 per year to 1·17, a protection rate of 74%). What was unexpected from this trial was the significant drop in deaths from malignancies, particularly leukaemia, lymphoma and soft tumours of the CNS and bone and connective tissue (Crispen & Rosenthal 1976). This paper was one of the first to suggest that BCG had a more pronounced general immunostimulant activity. Perhaps one of the most remarkable papers along the same lines was that of Pearle (1929) who had reviewed the literature over the previous century and concluded that TB patients tended not to have cancers, suggesting that mycobacteria in general stimulated the immune system. Holmgren (1935) was probably the first to try BCG against cancer, but by 1976, Morales et al were showing in controlled clinical trials in Canada that BCG was an effective treatment for bladder cancer. Indeed in the USA, BCG was approved for this purpose by the FDA in 1990 and is now proving to be the preferred clinical treatment for this disease. Although Rosenthal demonstrated that BCG vaccine produced a non-specific stimulation of the lymphoreticuloendothelial system (Rosenthal 1980) the exact details of the mode of action in bladder cancer remain obscure. Like its action against TB, the effect of BCG on various experimental cancer models remains multifaceted and to some degree variable. The review by Rosenthal covers this subject up to 1980 but since then others have confirmed this variability. Harboe & Nagai (1984), Harboe et al (1986) and Abou Zeid et al (1988), have all confirmed differences in antigen expression by BCG. In our laboratory, Klegerman et al (1991b) demonstrated both high dose tumour inhibition and low dose tumour stimulating activity for both viable and heat-killed BCG. This suggested that there may be as yet unidentified heat-stable inhibiting factors present in the

vaccine. This observation led to the identification of a series of heat-stable antineoplastic glycans in the BCG (Lou et al 1994a; Wang et al 1995; Garrido et al 1996). This work, which is irrelevant to the TB situation under consideration here, is ongoing and is providing an interesting insight into the possible mode of action of BCG, perhaps as an immunostimulant but certainly as a direct anticancer treatment.

One other aspect of the use of BCG in bladder cancer that needs attention is the way in which the bacterial cells appear to be able to target the tumour cells. Ratliff et al (1987, 1988), Ratliff (1989) and Kavoussi et al (1990) demonstrated that some tumours either express fibronectin or are associated with increased concentrations of fibronectin at the junction of the invading tumour and the healthy tissue. This observation has also been confirmed in our laboratory and we suggested that a major component in this reaction was the BCG cell wall protein identified as Antigen 85 (Ag 85)(Klegerman et al 1993; Öner et al 1994). This is a homologous group of three proteins with molecular weights of 29–30 kDa which act as fibronectin antibodies. However, this antigen is only loosely attached to the cell wall and Ratliff's later suggestion that other fibronectin antibodies of higher molecular weight which remained more firmly attached to the bacterial cell wall (Ratliff et al 1993) makes more sense from a mechanical point of view. Nevertheless, with pharmaceutical targeted drug delivery systems in mind, we have recently explored the use of Ag85A, trypsin-digest fragments of Ag 85, gelatin and now trypsinized gelatin (Lou et al 1994b, 1995; Öner et al 1994; Klegerman et al 1994; Gao 1996).

The Future of BCG

The USA can be seen to be looking in two directions on the issue of tuberculosis. On one hand, there is concern about the rising incidence of TB, especially of the multiple drug resistant types, and on the other hand there is an apparent unwillingness to accept that BCG is available. Currently there are demands for new tuberculosis vaccines from individuals who are apparently forgetting that it takes federal authorities up to ten years to approve new therapeutic entities, despite political assurances to the contrary. A vaccine is needed now, as indeed are better diagnostic tools. However, we already have a TB vaccine that has been in use for 75 years, is demonstrably safe and, as noted in the meta-analyses of previous clinical trials by Clemens et al (1983) or Colditz et al (1994), significantly reduces the risk of TB by at least 50%, with up to 80% or more being observed under optimal conditions. This has been seen in many populations, study designs and forms of TB but, as Colditz et al (1994) observed, the protection against death, tubercular meningitis and disseminated (miliary) disease is significantly higher still. Moreover, a vaccine that coincidentally inhibits leprosy is surely advantageous. Nettleman (1993) pointed out that even at 50% efficacy, a decision analysis of the benefits of vaccination (cost $9) against the cost of isoniazid treatment of a case of TB at $7500, suggested that 26 TB cases per 1000 would be prevented and 8 life-years gained. Thus, BCG vaccination would be economically favourable and preferable to disease treatment. In a review provocatively titled "Vaccination against tuberculosis: is BCG more sinned against than Sinner?", Griffen & Buchan (1993) concluded that it was naive to expect that what was, after all, a prototype attenuated BCG vaccine, could produce complete protection. Retrospective analysis prompted the question not so much why it has not been completely protective but, rather how has it worked so well?

The main point is that BCG is available now, its safety is well established and its efficacy is better understood than it was even a few years ago. New, more effective vaccines are needed, but where are they to be developed today? TB cases are currently estimated to be around 24 000 in the USA, and for the most part the cost of treatment and curing of the disease chemotherapeutically has fallen to around $11 (parenthetically, it is estimated to cost $250 000 to cure one multiple drug resistant case). Despite press claims to the contrary, TB is less of an issue in the USA but in some other parts of the world it has become a major problem. The question has to be raised about where any new vaccine can be tested since TB is endemic in every underdeveloped country. The problem encountered by the WHO when testing BCG in India is still with us. Moreover, funding for TB-related research is not considered to be a high priority. New vaccines are being developed but may not be totally effective in a disseminated wild-type TB infected population and it is this question that must be addressed by suitably designed clinical trials.

BCG is known to trigger antitumour activity and IL-1 secretion by macrophages (Seledtsova et al 1995) but is it possible to develop an improved BCG vaccine? Surprisingly the answer may be "yes". Work at the Whitehead Institute for Biomedical Research, Cambridge, Massachusetts, by Dr Richard Young and his colleagues have demonstrated how constructed recombinant strains of BCG can be produced that secrete enhanced levels of the cytokines IL-2, IL-4, IL-6, granulocyte–macrophage colony stimulating factor and IFN-γ (Murray et al 1996). Although only carried out for murine cytokines so far, the potential for producing an enhanced antitubercular or antitumour response in man is obviously considerable. Although some years away from a commercial product, the concept is interesting.

In conclusion, we can say that BCG vaccine has had a chequered past, may have a significant role to play in the present situation and may even form part of the strategy needed to eliminate TB in the future. If we add to this the benefits of actual and potential application to cancer treatments, BCG may continue to play a role in therapy as we make progress in understanding how it and its components work and how they may be applied clinically.

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