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The Immunological Basis for Immunization Series

Module 5:

Tuberculosis

GLOBAL PROGRAMME FOR VACCINES AND IMMUNIZATION
EXPANDED PROGRAMME ON IMMUNIZATION

World Health Organization
Geneva

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Module 5:

Tuberculosis

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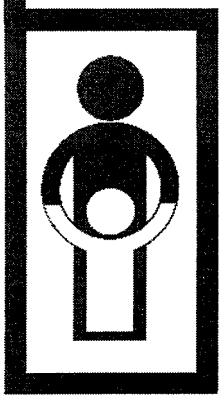

GLOBAL PROGRAMME FOR VACCINES AND IMMUNIZATION
EXPANDED PROGRAMME ON IMMUNIZATION

World Health Organization
Geneva

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Module 1: General Immunology
Module 2: Diphtheria
Module 3: Tetanus
Module 4: Pertussis
Module 5: Tuberculosis
Module 6: Poliomyelitis
Module 7: Measles
Module 8: Yellow fever

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Contents

Preface

This series of modules on the immunological basis for immunization has grown out of the experience of persons working with the WHO Expanded Programme on Immunization (EPI). The EPI was established in 1974 with the objective of expanding immunization services beyond smallpox, with emphasis on providing these services for children in developing countries.

Six vaccine-preventable diseases have been included within the EPI since its beginning: diphtheria, measles, pertussis, polio, tetanus, and tuberculosis. To protect newborns against neonatal tetanus, tetanus toxoid is administered to the mother either during her pregnancy or prior to pregnancy during the childbearing years.

Two more vaccine preventable-diseases will be addressed by the EPI during the 1990s. The World Health Assembly has set the target of including yellow fever vaccine in the EPI by 1993 in countries where this disease poses a risk. Hepatitis B vaccine is being added gradually, with the target date of 1997 for incorporation of this vaccine in the immunization programme in all countries.

Titles of the nine modules in this series are listed inside the front cover of this module. They are intended to provide information on the immunological basis for WHO-recommended immunization schedules and policies. They have been prepared for the following main audiences:

- immunization programme managers, whose questions and concerns caused this series to be written,
- consultants and advisers on immunization activities,
- teachers of courses on immunization at the university level and facilitators of workshops,
- medical and nursing students as part of the basic curriculum,
- laboratory scientists providing diagnostic or research services for vaccine-preventable diseases, and
- scientists involved in basic research aimed at improving the delivery of vaccines or providing improved vaccines.

Other modules in this series and additional materials on the EPI are available from the Expanded Programme on Immunization, World Health Organization, 1211 Geneva 27, Switzerland.

Tuberculosis

1. The Organism and the Disease

Tuberculosis is one of the most important health problems in developing countries and, as infection with human immunodeficiency virus (HIV) becomes more prevalent, tuberculosis is becoming a serious problem in developed countries as well *(Styblo 1989).*

Tuberculosis is caused by the bacillus *Mycobacterium tuberculosis. M. tuberculosis* is responsible for some eight million new illnesses and three million deaths per year; mostly in developing countries, although there are over 400 000 new cases annually in industrialized countries. There are several other forms of mycobacterium-caused diseases. The best-known of these is leprosy, caused by *Mycobacterium leprae.* Table 1 shows schematically the antigens shared by various mycobacteria. *Mycobacterium bovis,* the variant of M. tuberculosis which is used in preparation of BCG vaccine, shares group I and species-specific antigens with *M. tuberculosis,* but does not share species-specific antigens with *M. leprae,* against which it has been shown to provide protection.

By far, the most important source of human infection is an already infected person who spreads the highly infectious bacilli via respiratory droplets. Primary infections can occur at any age, but children are most often affected in areas of high incidence and high population density. Even after resolution, the disease can be reactivated and again spread. Agents that depress the immune system, such as corticosteroid therapy or HIV infection, facilitate reactivation.

Primary infection may be asymptomatic and often resolves spontaneously. However, it may progress by local spread in the lungs to cause pleurisy or bronchopneumonia. If the infection spreads through the bloodstream, it can affect many organs, including the meninges, the bones, or the internal organs. Disease can be accompanied by tuberculous lymphadenopathy, or this manifestation can occur in the absence of other features.

The bacillus contains a number of proteins or polypeptides which play important roles in the response to infection. Recent work with *M. tuberculosis* and *M. leprae (Melancon-Kaplan et al. 1988)*

Table 1. Relationship between antigens of various mycobacteria species, based on double-diffusion analysis against high titer rabbit antisera (adapted from *Stanford 1991).*

	Species	Group I	Group II	Group III
Slow growers	M. tuberculosis	xxxx	xxxx	
	M. ulcerans	xxxx	xxxx	
	M. intracellulare	xxxx	xxxx	
Fast growers	M. fortuitum	xxxx		xxxx
	M. flavescens	xxxx		xxxx
	M. phlei	xxxx		xxxx
Non-cultivable	M. leprae	xxxx		

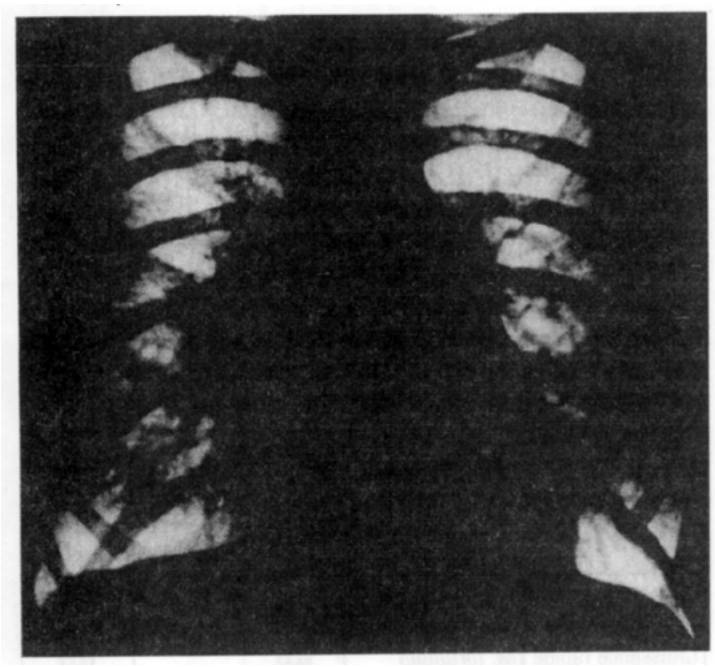
indicates that cell wall protein is a major contributor to cell-mediated immune reactivity to these organisms. In contrast, the delayed hypersensitivity response elicited by Bacille Calmette-Guerin, derived from *M. bovis,* is related to a 15 100 molecular weight polypeptide called MPB70, a breakdown product of the secreted protein, methoxymycolate *(Harboe & Nagai 1984).* Much work needs to be done to clarify the role of different proteins of the bacillus in the host response.

2. The Response to Natural Infection

Primary infection by tubercle bacilli induces both immune and nonimmune inflammatory reactions. Production of humoral antibodies is not the major kind of immunological defense in tuberculosis; rather, there are several different types of immune responses, including circulating antibody, delayed hypersensitivity, increased macrophage activity, and granulomatous inflammation.

The granulomatous reaction is an immune response that limits the dissemination of the organisms. The first exposure of the host to the tubercle bacillus may be completely asymptomatic, with a small "Ghon focus" of inflammation seen in the lung. A Ghon complex, as shown in Figure 1, is a healed granuloma

Figure 1. Primary tuberculosis, right lung in a 10 year old girl *(Grzybowski 1983).*



in the lung and the draining lymph nodes. It does not always protect the host, and may even serve to "hide" the bacillus from other defensive reactions of the host. The granulomatous reaction of the host is augmented by a T-cell-mediated response (see Module 1 for a more detailed discussion on cell-mediated immunity).

This T-cell-mediated response to the tubercle bacillus is a delayed hypersensitivity reaction. In 1891, Koch noted that the injection of tubercle bacilli subcutaneously into a normal guinea pig was followed by the slow development of a nodule at the site of injection in 10 to 15 days. Later; the nodule developed into a chronic ulcer, with lymphadenitis of the regional lymph nodes. When the same dose of tubercle bacilli was injected subcutaneously into the same guinea pig after a period of at least three weeks, a local reaction developed at the injection site, but the ulcer healed quickly and the regional lymph nodes remained unaffected. The tissue reaction seen in the tuberculin test is the result of the activity of the lymphocyte mediators and the macrophages. An inflammatory reaction, with redness and swelling, is seen 24 to 48 hours after antigen injection (skin test reaction).

Whether the primary exposure results in fulminant disease or not, or whether reactivation occurs after subsequent exposure may depend on the state of the host. Fulminant disease or reactivation is more likely to occur when the immune system is depressed, for example by corticosteroid therapy, other diseases, malnutrition, pregnancy, or HIV infection.

3. Characteristics of BCG Vaccines

For all mycobacterial diseases, only one vaccine, based on *M. bovis,* exists. In 1908, Calmette and Guérin at the Pasteur Institute of Lille, began a series of 230 passages of the virulent *M. bovis (Calmette et al. 1921).* The original virulent strain[1] was grown for 13 years on potato slices cooked in beef bile supplemented by glycerol. The resulting culture was stable to reversion to virulence but retained limited invasiveness. The first human vaccination with this attenuated strain, named Bacille Calmette-Guérin (BCG), was applied in 1921 in Paris *(Weill-Hallé & Turpin 1925).* After acceptance by the League of Nations in 1928, BCG vaccine was widely used.

For prevention of tuberculosis, BCG vaccination is accepted as one of the most important measures. It is compulsory in 64 countries and is officially recommended in an additional 118 countries and territories *(Ildirim et al. 1992).* BCG is the most effective known adjuvant in animals and humans. It is also cheap, stable, and safe.

As BCG came into general use, a number of different substrains were generated in a number of production laboratories. Some of these substrains, derived from the original strain by additional culture passages, lost residual invasiveness and were devoid of efficacy. Therefore, any strain used for vaccine production should be documented and approved by WHO. At present, the four most widely used strains are derivatives of the Pasteur-1173P_2, Tokyo-172, Copenhagen-1331, and Glaxo-1077 strains. Table 2 summarizes the characteristics of some BCG strains.

There is considerable evidence that heterogeneity exists among the different isolates of *M. tuberculosis (Rado & Bates 1980),* though the impact of these differences on the antigenic properties is not clear. One possible reason for the failure of BCG vaccine in the South Indian trial *(Tuberculosis Prevention Trial 1979, Tripathy 1987)* is that there was a high proportion of disease caused by antigenic variants. Despite the fact that BCG is a strain of *M. bovis* and tuberculosis is caused by *M. tuberculosis,* studies indicate that BCG protects against tuberculosis and leprosy (caused by *M. leprae).* Recent data confirm the assignment of BCG as *M. bovis (Collins & De Lisle 1987),* but also show great antigenic variation among BCG strains.

Although there is considerable heterogeneity among strains of BCG vaccine in use, several studies have failed to demonstrate significant differences in protective efficacy between these strains *(Milstien & Gibson 1989).*

[1]Strictly speaking, these are not strains as they have not been cloned; however, this module will refer to BCG strains throughout.

Table 2. Some characteristics of strains of BCG vaccine *(Milstien & Gibson 1989).*

BCG strain	Characteristics of strain				
	MPB70	Dimer	Methoxymycolate	Mycocide B	Colony Morphology
Tokyo-172	++	+	+	+	Spreading
Moreau (Brazil)	++	+	+	ND*	ND*
Russian	++	+	+	ND*	ND*
Swedish	++	ND*	+	ND*	ND*
Glaxo-1077	+	+/-	-	-	Nonspreading
Copenhagen-1331	-	-	-	+	Spreading
Pasteur-1173P_2	-	-	-	+	Spreading

** ND= not done.*

In 1966, the WHO Expert Committee on Biological Standardization established the first requirements for BCG vaccine *(WHO Expert Committee on Biological Standardization 1966).* These requirements have subsequently been revised *(WHO Expert Committee on Biological Standardization 1987, 1988).* They outline procedures for production of BCG vaccine to ensure potency, safety, and efficacy, and describe certain tests which should be done on the vaccine seeds, and on the final vaccine itself. The WHO requirements were designed to reduce the variability among BCG strains, seen in clinical and animal trials, by requiring each manufacturer to correlate laboratory test results with clinical efficacy data.

At present, there is no laboratory test that correlates with protective efficacy of any BCG vaccine preparation. For this reason, the strategy used has been to evaluate the protective efficacy of several different preparations of BCG through careful clinical trials, using vaccines whose safety and *in vitro* characteristics have already been verified. Once vaccine efficacy in humans is demonstrated, repeated measurement of tuberculin sensitivity and lesion size and various *in vitro* tests on cultured BCG bacteria are used to verify that later lots of vaccine grown from these preparations are being reproduced satisfactorily.

4. Response to Immunization

Clinical trials have confirmed that infection with mycobacteria other than *M. tuberculosis,* including the BCG vaccine, may induce some protection against tuberculosis. Artificial infection with BCG spreads from the inoculation site via the lymphatic system to local lymph nodes and produces an immunity equivalent to that produced by natural primary infection with virulent bacilli. As in the case of natural tuberculosis infection, the resistance is cell-mediated and is largely attributable to activated macrophages. BCG-induced immunity develops about six weeks after vaccination.

Experimental studies indicate that the mechanism of protection by BCG vaccination consists in reduction of the hematogenous spread of bacilli from the site of primary infection *(Smith & Harding 1979)* mediated by memory T lymphocytes induced by the first exposure to BCG. There is no evidence that BCG reduces the risk of becoming infected with tuberculosis bacilli, but it prevents forms of tuberculosis depending on hematogenous spread of the bacillus *(Heimbeck 1929).* This inhibition of the hematogenous spread of bacilli thus reduces the risk of immediate disease and of disease due to reactivation. Because there is reduction in risk of immediate disease, but not of infection, there is a difference in the protective effect of BCG, depending on the type of tuberculosis infection. Myint et al. *(1987),* in studies in newborns, showed a wide range of protective efficacy (that is, the measure of protection against tuberculosis afforded by BCG vaccination), depending on the form of tuberculosis (Table 3). The highest efficacy seen in this study was 80%, and efficacy was significantly higher for the more severe forms of disease.

Table 3. Protective efficacy of BCG against various clinical forms of tuberculosis *(Myint et al. 1987).*

Clinical form of tuberculosis	Protective efficacy (%) of BCG
Primary complex formed in the lung	20
Primary complex with local extension	32
Lymphadenitis	32
Tuberculosis of the bone	39
Tubercular meningitis	52
Disseminated tuberculosis	80

4.1 Route of administration of BCG vaccine

Early attempts were made to give BCG by mouth *(Weill-Hallé 1925)* using the fluid, not the lyophilized, form of the vaccine, but that route gave little success because of the low dosages used. Better response was seen with massive (hundreds of milligrams) doses (for a review of oral administration, see *Rosenthal 1980,* Chapter 11).

A number of different injection methods were tried. BCG administration by jet injector was found to deliver less than the full dose and give a variable vaccination lesion. Attempts to increase the dose gave rise to large ulcers and the method was discarded *(Ten Dam et al. 1970).* For this reason, BCG administration by jet injector is not advised.

Intradermal injection is the method of choice. This method was introduced in 1927 (for a review, see *Pontecorvo 1985).* For intradermal injection, the injection site is the lower deltoid area so as to involve the axillary instead of the upper clavicular lymph nodes. This is to minimize complications from post-vaccination lymphadenopathy.

Multiple puncture is an alternative technique for BCG administration, which is not recommended by WHO. Several drops of BCG are rubbed on the same site as for intradermal injection, and an appropriate device with multiple points (see Heaf test, section 4.3) is used to introduce the vaccine under the skin. To obtain a result similar to intradermal injection, 40 punctures are needed. This requires a large volume of vaccine, and it is operationally difficult. Use of a bifurcated needle has been studied *(Darmanger et al. 1977),* but this was also found to be inferior to intradermal injection.

Mee and Thwaites *(1977)* have studied the response to the tuberculin test in neonates given BCG by intradermal injection or by multiple puncture with a Heaf gun or a bifurcated needle. They found minimal difference in the conversion rates of the three groups (Table 4). However, other authors *(Darmanger et al. 1977)* have found that uniformity of dose is more readily assured by intradermal injection, along with a superior level of tuberculin sensitivity and lower cost. For this reason, intradermal injection is the WHO-recommended method for administration of BCG.

4.2 BCG vaccination scars

Following intradermal injection of live BCG vaccine into humans, a papule with induration appears within two to three weeks. The papule ulcerates at six to eight weeks, followed by a scar at the end of three months. The presence of such a scar in the appropriate place (generally the right arm just below the insertion of the deltoid) has been used as evidence for prior BCG vaccination. With multiple puncture inoculation, there are many small papules which disappear more quickly and often without scarring.

Although the size of the scar follows a simple dose-response, various other factors have been shown to influence the size and shape of the scar, including the technique of administration of vaccine (intradermal administration is more likely to leave a uniform scar, while improper, i.e. subcutaneous, administration may not); the characteristics of the recipient (keloid formation may be associated with race); and the strain of BCG used.

Fine et al. *(1989)* found that of children vaccinated in infancy fewer than 60% retained a recognizable scar after two years (Table 5). Thus, scars are poor indicators of BCG vaccination in infancy. This may be due to the lower dose used in infants, the difficulty of administering vaccine truly intradermally in infants, or an immature immune response in infants, although cell-mediated immunity is normal at birth.

A number of studies have reported on the agreement between a documented history of receipt of BCG vaccine and the presence of a BCG scar at one to two years after immunization: in Ivory Coast *(Expanded Programme on Immunization 1979b),* Lesotho *(Expanded Programme on Immunization 1986),* Malawi *(Expanded Programme on Immunization 1989),* Sri Lanka *(Expanded Programme on Immunization 1982),* and Zimbabwe *(Expanded Programme on Immunization 1983a).* Other studies have shown more than a 10% loss of scars: in Algeria *(Expanded Programme on Immunization 1979a),*

Table 4. Results of the tuberculin test at 72 hours after three different methods of administration of BCG vaccine *(Mee & Thwaites 1977).*

Method of administration	No. infants tested	Percentage with tuberculin reactions by induration (mm)			
		<1	1-4	5-9	10-14
Intradermal injection	39	36	15	33	15
Multiple puncture with 20-point Heaf gun applied two times	42	33	12	36	19
Multiple puncture with bifurcated needle applied 20 times	45	38	11	33	18

Table 5. Percentage of children recorded as BCG scar positive, by age at vaccination and interval since vaccination, Malawi *(Fine et al. 1989).*

Age at vaccination	Percentage with BCG scar positive by interval since vaccination (months)					
	0-2	3-6	7-12	13-18	19-24	25+
0 to 2 months	82	88	100	96	78	43
3 to 5 months	83	90	95	88	75	67
0 to 14 months	83	89	95	91	70	54

Botswana *(Expanded Programme on Immunization 1983c),* Tunisia *(Expanded Programme on Immunization 1983b),* and Zambia *(Expanded Programme on Immunization 1985).* Poor immunization technique or loss of vaccine integrity might explain the failure to retain a scar. It has been suggested that immunization programme managers might use a systematic assessment of the variability of scar size in BCG recipients to evaluate the proficiency of vaccinators, since a uniform scar of a certain minimum size would indicate a consistency in dose of vaccine administered. However, this suggestion may not be feasible when BCG is given over a wide range of ages or if different strains of BCG are administered, since there is known variability in scar size with vaccine strain used and with age at which the vaccine is administered.

It is possible that there is an association between the tendency for vaccination to leave a scar and its protective efficacy. This may be because improperly administered vaccine may not be efficacious. Another explanation could be that if the vaccine recipient did not mount an adequate immune response to the vaccine, no scar would be seen, and the vaccination would not be protective. It is worth noting, however, that misclassification of vaccination status because of lack of scar formation would tend to reduce the apparent vaccine efficacy. For this reason, studies on BCG vaccine efficacy should rely on documentation of immunization by immunization card.

It must be borne in mind that the immune system has not evolved around artificial immunization with syringes and needles; thus, injection of BCG vaccine may not be the optimal method of presentation of the vaccine. Nevertheless, data comparing tuberculin conversion as a function of method of administration of the vaccine (Table 4) have found no significant differences. These data were obtained with intradermal rather than subcutaneous injection. Subcutaneous injection may reduce protective efficacy, particularly if the replication of the BCG bacilli is decreased in the deep subcutaneous tissue. Since the immune response to BCG vaccine is dose-dependent, the extent of replication must correlate positively with response, at least up to a certain level.

It is known also that administration of the vaccine by other than the intradermal route (that is, subcutaneously or intramuscularly) is likely to result in a significant increase in local reactions. One factor which may be important is that vaccinators may calibrate the size of the dose by the size of the "blister" raised on intradermal administration. Improper administration of the vaccine or leaking syringes may influence this rough calibration and cause larger doses of BCG to be inadvertently administered. Thus, use of syringes which hold only the indicated dose would be an improvement.

4.3 Delayed hypersensitivity reaction

Generally, delayed hypersensitivity is measured not by injection of BCG into the immunized (or possibly tuberculin-exposed) patient, but by use of a purified protein derivative (PPD) of the tubercule bacillus. 'Tuberculin' refers to a preparation of *M. tuberculosis.* 'PPD', purified protein derivative, may be a purified product of tuberculin or may be derived from other Mycobacteria, such as *M. bovis.* Thus, detection of tuberculin reaction to PPD might not necessarily correlate with existence of an immune response to BCG or to *M. tuberculosis.*

The general test for quantitation of the delayed hypersensitivity reaction is the Mantoux test. Other puncture tests, such as the Heaf test, are useful for screening patients for evidence of prior exposure to tuberculosis. The tests differ in the concentration of tuberculin used, the method of introducing it into the patient, and the method of reading results.

In the *Mantoux test,* 0.1 ml containing 5 TU (tuberculin units) of PPD solution is injected intradermally on the volar surface of the upper third of the forearm. The results are read 48 to 72 hours later as the area of induration, with at least 5 mm in diameter the threshold for indication of a positive reaction.

The *Heaf test* uses a drop of undiluted (100 000 IU/ml) PPD spread with a glass stick on the forearm, after which an apparatus with six needles is used to inject about 50 IU into the epidermis. The results are read five to seven days later and recorded as grade 1 to 4, depending on the appearance of the papules at the puncture sites. Grades 2 to 4 are interpreted as positive. The Heaf test uses quite a lot of tuberculin and does not give quantitative results.

Figure 2. Responses for the tuberculin test by Mantoux (left) and Heaf (right) methods *(Grist et al. 1993).*

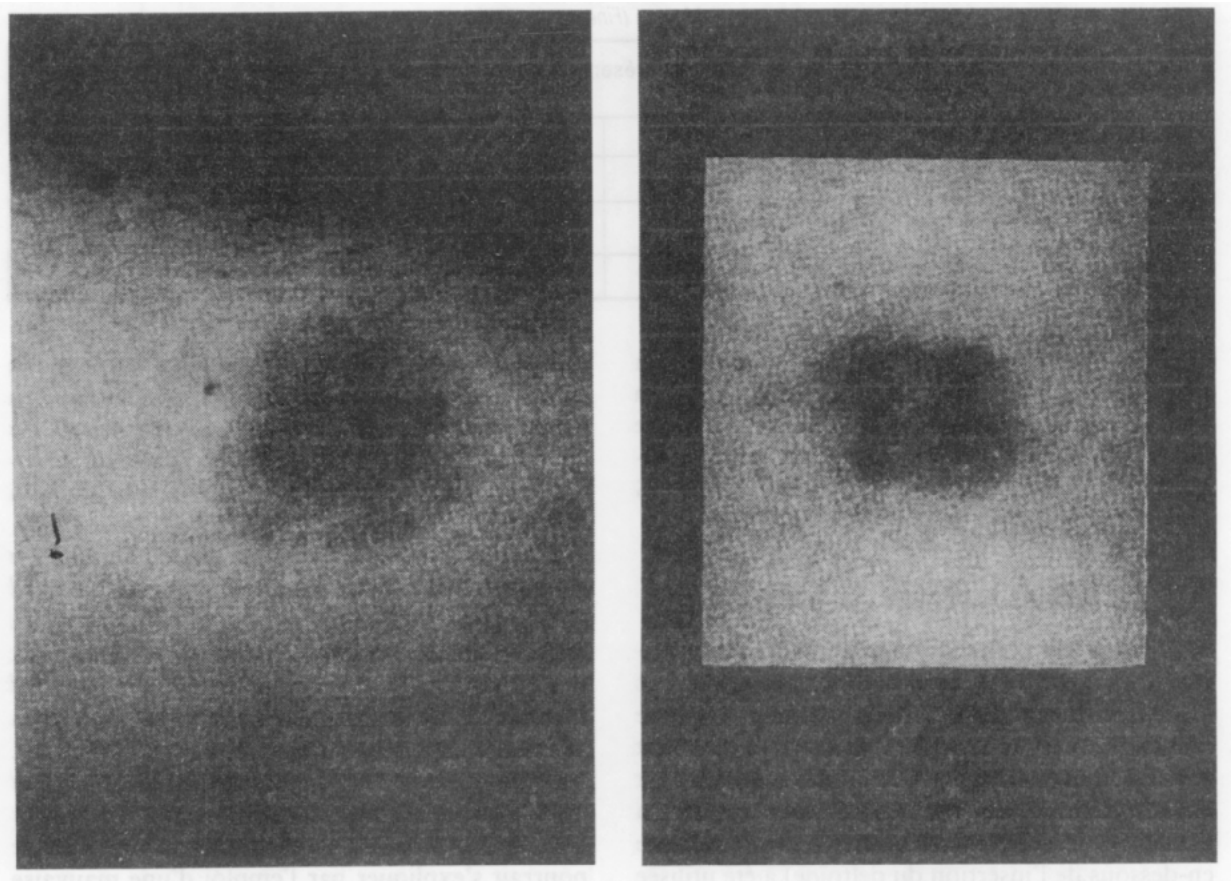


Figure 2 shows responses for the tuberculin test as measured by the Mantoux and the Heaf methods. Because the hypersensitivity induced by the denatured protein (which is not identical to the intact living tubercle bacillus) may not completely overlap with that induced by the live mycobacterium, the results of the Mantoux test and the Heaf test do not completely correlate with those which would be seen if the infecting organism itself were used. Nonetheless, the potency of BCG vaccine is traditionally determined by measuring the tuberculin sensitivity induced by that vaccine in children who were tuberculin-negative before vaccination. The test is good for quality control of different batches of the same vaccine and for assessing techniques of vaccinators. It is not generally useful for comparing vaccines from different producers.

The tuberculin reaction follows a simple dose-response, as does scar size. Table 6 shows an illustration of this in a study performed on newborns in India *(Narain et al. 1978).* It can be seen that the dose-dependence of the tuberculin reaction does not correlate with that for scar formation. This is probably because the scar (assuming standard administration technique) reflects the total bacillus mass (living or dead), while the tuberculin reaction measures viable bacilli. Vallishayee et al. *(1974)* found variation in mean size of the tuberculin reaction which did not necessarily correlate with skin lesion size when a number of different vaccines were tested (Figure 3).

Edwards et al. *(1953)* found that halving the dose of vaccine decreases both the tuberculin reaction and the scar size by about 1 mm, but use of BCG vaccine containing a large proportion of dead organisms decreases the scar size less than the tuberculin sensitivity. Thus, the immunogenic potency in the delayed hypersensitivity reaction is lower for killed organisms or derivatives than for live BCG *(Tuberculosis Program, Public Health Service, USA, 1955).*

Despite the fact that delayed hypersensitivity is postulated to be mediated by the same mechanisms as protective effect, these two properties do not cor-

Table 6. Dose-dependence of skin test response and scar size in infants *(Narain et al. 1978).*

Dose of BCG vaccine	Number of infants	Mean response (in mm)	
		Skin test	Scar
placebo	73	3.7	—
0.01 mg BCG	70	13.5	3.7
0.10 mg BCG	74	15.1	4.8

relate exactly. Ladefoged et al. *(1970)* found that the Tokyo strain of BCG, which induces a high degree of delayed hypersensitivity, is inferior in terms of minimum protective dose in a model system using the bank vole. However, available data suggest that the Tokyo strain shows satisfactory protective efficacy in humans *(Milstien & Gibson 1989).*

The association between delayed-type hypersensitivity and protective effect has been examined in human populations. Comstock *(1971)* reviewed data from trials and concluded that "the lack of correlation is obvious and underscores the futility of predicting potency [e.g. protective effect] from conversion rates [e.g. delayed type hypersensitivity]." Hart et al. *(1967)* studied the relationship using the population from the British Medical Research Council trial *(Fourth Report to the Medical Research Council 1972).* After grouping all vaccines according to the degree of tuberculin sensitivity after vaccination, they found no difference in vaccine efficacy. They concluded that "with highly effective tuberculosis vaccines, the degree of protection conferred on the individual is independent of the degree of tuberculin skin sensitivity induced in that individual by the vaccination."

Fine and Rodrigues *(1990),* after reviewing the available data, concluded that there is no evidence for a correlation between post-vaccination tuberculin conversion and BCG-induced protective efficacy in human populations.

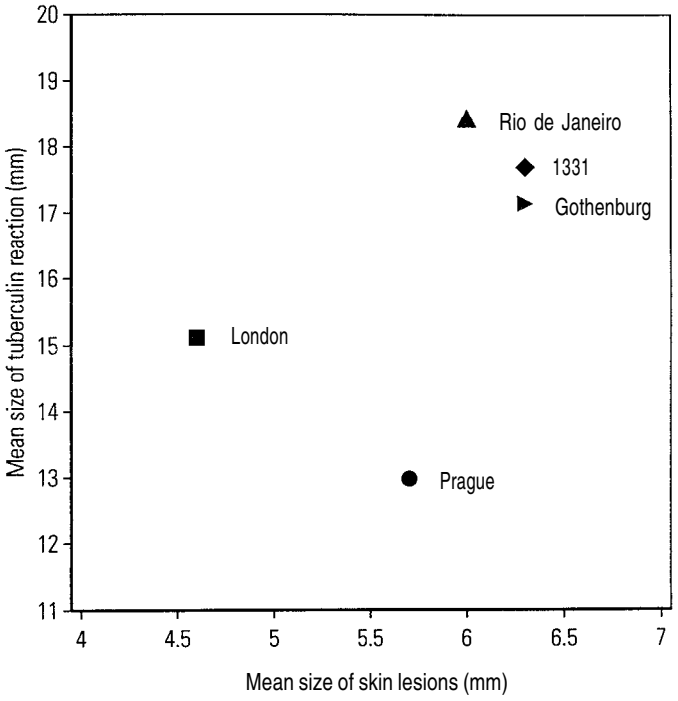
Moreover, the loss of tuberculin sensitivity and protective immunity with time have not been observed to follow parallel kinetics *(Fine et al. 1986),* based on data from the British Medical Research Council Trial *(Fourth Report to the Medical Research Council 1972)* and the South Indian trial *(Tuberculosis Prevention Trial 1979).* Fine et al. *(1986)* make the point that the monitoring of vaccine-attributable tuberculin conversion, while it may not correlate with protection, may be justified in the trial setting, as a means of ensuring that viable BCG was administered.

4.4 Adverse events

Since BCG is a live attenuated vaccine, it can be expected that occasionally its use will result in complications. Systemic effects have been observed following administration of BCG vaccine, including regional lymphadenitis, systemic BCG infection, and bone tuberculosis. Some authors feel that unless BCG induces lymphadenitis to some extent *(Gheorghiu et al. 1978),* no protective immune response has been induced.

Although vaccine efficacy (as measured by the delayed hypersensitivity reaction) and vaccine safety (as measured by incidence of adverse reactions such as BCG-lymphadenitis) both show a dose-response relationship, the slopes of these dose-response curves differ for BCG vaccines of different strains. The acceptability of a strain of BCG vaccine will depend on the relative slopes of these two curves. For strains with a higher tendency to elicit lymphadenitis (or, more commonly, lymph node swelling), the dose at which good efficacy and low reactogenicity is found may be difficult to determine. Figure 4 shows this curve for Copenhagen strain vaccine.

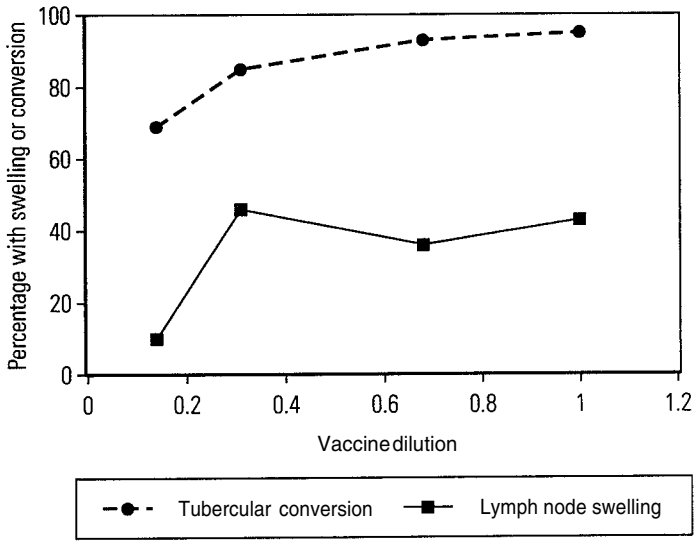
Figure 3. Mean size of tuberculin reactions and skin lesions 8 to 10 weeks after vaccination for 4 different BCG vaccine strains *(Valishayee et al 1974).*



Host characteristics will affect the incidence of adverse events as well. The major host characteristics which may affect adverse reactions to BCG in immunization programmes are age (there is a much higher incidence of adenitis in neonates as compared to older infants and children) and the increased risk of disseminated reactions (and possibly local reactions) in recipients with serious immune deficiency involving the T-cell-mediated system.

In practical terms, the major risk of concern is abnormal T-cell function secondary to HIV infection, which is rarely present until several months after birth in perinatally infected infants. A recent study *(Lallemont Le Coeur et al. 1991)* has supported the absence of increased risk of BCG vaccination for perinatally HIV-I-infected babies after 36 months of follow-up. Because of the demonstrated risk of disseminated BCG infections in patients with immunodeficiency syndromes *(Gonzalez et al. 1989),* particularly those due to HIV infection, WHO has

Figure 4. Dose-dependence of tuberculin conversion and lymph node swelling with Copenhagen strain *(Oehme & Siegle-Joos 1976).*



issued a recommendation not to give BCG to infants with symptomatic AIDS *(Special Programme on AIDS and Expanded Programme on Immunization 1987):*

> In countries where human immunodeficiency virus (HIV) infection is considered a problem, individuals should be immunized with the EPI antigens according to standard schedules. This also applies to individuals with asymptomatic HIV infection. Unimmunized individuals with clinical (symptomatic) AIDS in countries where the EPI target diseases remain serious risks should not receive BCG, but should receive the other vaccines.

Thus, EPI managers should aim to deliver BCG vaccine as early in life as possible, before symptomatic AIDS develops.

4.5 Protective efficacy of BCG and duration of immunity

The best method for determining the protective efficacy of a vaccine is a prospective, randomized, double-blind, placebo-controlled trial. These studies are difficult and expensive, and have rarely been performed. WHO has recently sponsored studies to evaluate the protective efficacy of BCG immunization in infants and/or children by two low-cost methods: case-control studies *(Smith 1982, 1987)* and contact studies *(Ten Dam 1987).*

These studies have recently been reviewed by Milstien and Gibson *(1989),* who concluded that there is good evidence that the efficacy of modern BCG vaccines is in the range of 60% to 90% for disseminated tuberculosis or meningitis in young children, but somewhat lower for other forms of primary tuberculosis disease. They also found no evidence that one BCG preparation tested was more efficacious than any other under the conditions of the trials, and thus no evidence to support the choice of one preparation or manufacturer of BCG over another on the basis of protective efficacy. A recent matched case-control study in Bangkok *(Sirinavin et al. 1991)* found an adjusted protective efficacy for neonatal BCG vaccination of 83%, and provided evidence to support the thesis that the accuracy of tuberculosis diagnosis, the types of tuberculosis, the length of time after vaccination, and the household tuberculosis exposure contribute to variation in the reported protective efficacy of neonatal BCG vaccination. There is a need for the development of a single *in vitro* test capable of predicting the induction of immune resistance of humans to infection or dissemination of *M. tuberculosis.*

Despite the difficulty in interpretation of data, several trials have shown that efficacy of BCG is highest among the youngest recipients, and that it falls with increasing age at vaccination *(Fine et al. 1986, Tuberculosis Prevention Trial 1979).* Tuberculosis has been mainly seen as a disease of older individuals, although primary infections affect younger children, while BCG vaccination is targeted at children under one year of age, and mostly at newborns. It is therefore difficult to assess the impact of BCG vaccination programmes. However, when BCG immunization of newborns was stopped in Sweden, the incidence of the disease in infants rose sixfold *(Romanus 1987).*

There are several publications which suggest that the protection BCG provides against tuberculosis is a function of the relative importance of disease due to endogenous reactivation as compared to reinfection from the outside. Thus, BCG protects against hematogenous spread of infection *(Fine 1988, Ten Dam 1984).* This suggestion predicts the greater protective efficacy seen against miliary tuberculosis and tuberulous meningitis, compared with pulmonary tuberculosis *(Smith 1987)* (see Table 3).

Some studies suggest that protection due to a cell-mediated immune response to BCG is of long duration. The Medical Research Council Trial in the UK *(Fourth Report to the Medical Research Council 1972)* showed that BCG offered 70% to 80% of its original level of protection for at least ten years after vaccination in adolescents (Table 7).

A study in Chicago *(Rosenthal et al. 1961)* followed recipients of BCG for up to 23 years and showed 75% protective efficacy when vaccine was given to infants under three months of age. These data suggest that a booster dose may not be necessary for maintenance of BCG-induced immunity. Certainly, if BCG shows protective efficacy reproducibly only

when administered to young infants, a booster dose would not be indicated whether or not immunity wanes. One recent case-control study in Australia *(Patel et al. 1991)* failed to show more than a modest protective efficacy from immunizing children aged 12 to 14 years. More studies on the utility of booster immunization would be useful. One gap in knowledge about BCG vaccine is the efficacy of repeated booster immunizations.

5. Current Practice and Schedules

Most countries (except for the United States of America and the Netherlands) recommend the use of BCG. The schedule differs from country to country. WHO recommends one dose at birth or at the first contact of the infant with the health system. Other countries use other schedules, the differences mostly attributable to differing interpretations of the available data.

The protective response to BCG vaccine against infection by *M. tuberculosis* depends on a number of factors. These include the substrain of the vaccine, the dose and the method of presentation of the organism, and the characteristics of the recipient, including age, concomitant illnesses and vaccinations, and nutritional status.

The effect of the age of the recipient on the immune response to BCG has been discussed in section 4. Although the immune system may be slightly immature at birth, studies in neonates show that BCG vaccine is efficacious when given at birth *(Cartwright 1978, Myint et al. 1987).*

There are likely to be ethnic effects on response to BCG vaccine. Certainly the frequency and severity of some adverse reactions to BCG vaccine varies between ethnic groups *(Lotte et al. 1984).* For example, persons of Swedish and Finnish national origin may have a substantially higher risk of developing BCG osteitis, even after differences in the use of BCG vaccine preparations are taken into account *(Böttiger et al. 1982).* There is convincing evidence for a substantial association of risk of local cutaneous reactions (i.e. keloids) with racial group *(Lotte et al. 1984).* It is known that components of the T-cell response are genetically controlled.

Table 7. Protective efficacy over 15 years of BCG vaccine given in the Medical Research Council Trial *(Fourth Report to the Medical Research Council 1972).*

Time since BCG vaccination (years)	Protective efficacy	
	% found	% of original
0	81	100
2.5	87	107
5	79	86
7.5	68	84
10 to 15	59	73

It has been reported *(Fourth Report to the Medical Research Council 1972)* that the efficacy of BCG vaccine varies with variation in the nutritional status of the population. However, Mehta et al. *(1976)* have reported that poor nutritional status is not responsible for poor development of immunity. It is known that very severe (third-degree) malnutrition gives thymus involution, decreased lymphocyte counts, and thus lowered level of cell-mediated immunity.

Some data exist regarding the adverse impact of malnutrition on BCG-mediated tuberculin conversion *(Chandra 1983, Epstein 1990).* Although it appears likely that nutritional status affects the cell-mediated immune response, the response to BCG vaccine is complex. Many factors are likely to be involved, of which nutritional status is one whose relative role may be difficult to assess.

6. Future Prospects (and Needs)

The discussions above indicate some needs in BCG vaccinology. They are summarized as follows, in decreasing order of likelihood:

A decreased number of BCG preparations. At present there are many, which may not be well characterized in terms of their tuberculin response and reactogenicity. Efforts are being made, primarily through the UNICEF tender, to decrease this number, by imposing limits on the tuberculin response and reactogenicity. Programme managers can help by assuring continuity of supply of their BCG vaccine and by discouraging proliferation of strains in local production.

Development of an *in vitro* assay which would relate to human tuberculosis immunity. As more is being learned about the structure of the *M. tuberculosis* bacillus and the components of the immune response, this possibility looks increasingly more probable.

Development of a vaccine which is well defined in terms of its molecular structure, so that it can be tested in a quantitative manner. Such a vaccine will become feasible as studies on the molecular structure of the organism and on the cellular immune response are completed.

Development of a vaccine which will work against exogenous reinfection, that is, will pre-

vent implantation of the tubercle bacillus from outside the host organism. This will probably necessitate a vaccine which works at the level of the respiratory tract. Only by designing a vaccine which will fulfill this characteristic will a tuberculosis vaccine be able to be truly protective.

7. Implications for Immunization Programmes

The best available data indicate that the maximal effect of BCG vaccine in terms of protective efficacy occurs when it is given to young infants. This is because the vaccine is given and an immune response is induced before the infant has been infected.

Since cell-mediated immunity is lifelong, there may be little advantage in giving a booster dose, even if there is an increase in tuberculin sensitivity on revaccination. Since early BCG immunization is effective in preventing the most dangerous forms of tuberculosis (miliary forms and meningitis), all efforts should be made to achieve high coverage with BCG vaccine in infants.

The EPI Global Advisory Group recommends the following *(Expanded Programme on Immunization 1991):*

> BCG should be given to newborns as protection against the most severe forms of childhood tuberculosis. BCG should continue to be given as early in life as possible in all populations at risk of tuberculosis infection...Research should be initiated or continued on the long-term effectiveness of BCG given in infancy...

In routine immunization programs, BCG is often given in conjunction with vaccination against diphtheria, tetanus, pertussis, and poliomyelitis. These antigens do not interfere with the immune response to BCG.

Unless the vaccines are administered simultaneously, however, it is best to allow an interval of one month between BCG vaccination and vaccinations against measles and other similar vaccines such as mumps vaccine *(Ajjan 1986).* This is because it is postulated that some vaccines, for example, measles vaccine and mumps vaccine, may temporarily depress the cellular immune response. To date, it is not known how important the role of the T-cell response is in developing immunity to measles and mumps following immunization with these two live virus vaccines. Thus the above recommendation is made to avoid any possibility of interference.

It is standard medical practice of perform tuberculin testing when diphtheria-pertussis-tetanus vaccine, oral polio vaccine, and measles vaccine are given. However, the Mantoux test may be negative during the incubation period of tuberculosis, measles, influenza, chickenpox, mumps, and during corticosteroid therapy. The impact on immune response to BCG vaccination during these periods is not known. As they are effectors of the T-cell response, the immune response to BCG may be depressed.

Questions have arisen as to when BCG immunization can be stopped with no consequences. This is under consideration at WHO. It is projected that guidelines will be developed for the use of BCG in countries where serious forms of tuberculosis are not of major public health importance.

Abbreviations

BCG	Bacille Calmette-Guérin, vaccine against tuberculosis
HIV	human immunodeficiency virus
IU	international units
PPD	purified protein derivative
TU	tuberculin units

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