ISSN 0377-4910

ICMR BULLETIN

Vo1.25, No.3 March, 1995

BCG : DO WE HAVE AN ALTERNATIVE

Vaccination is generally used as a form of immunoprophylaxis, so that administration of the vaccine even a long. time before exposure to the wild-type infectious organism should afford protection. Since effector T and B cells are short-lived, a prime requisite for a vaccine is to generate immunological memory.[1] In the case organisms such as mycobacteria which are obligate intracellular pathogens and which elicit granulomatous tissue reactions, artificial immunisation with live bacteria is required to induce protection.[2,3] The only existing vaccine against tuberculosis is the BCG (Bacille Calmette - Guerin), an attenuated strain of *M.bovis* and it is mandatory or officially recommended in 182 countries or territories. Under the Expanded Programme on Immuisation (EPT) started by the Government of India in 1978, BCG is recommended to be given to all infants 3-9 months after birth.[4]

History of BCG Vaccine

The history of BCG vaccination and the trials conducted to assess its effectiveness in humans have been reviewed by many workers.[5-10] BCG, the bile-tolerant, attenuated strain of *M.bovis*, was isolated by Calmette and Guerin.[11] Ox-bile was originally added to these cultures to prevent clumping of bacilli. This led to the fortuitous observation that growth in the presence of bile also resulted in attenuation or gradual loss of virulence. Such attenuated organisms will multiply only to a limited extent in the animal or human body and can bring about an increase in the resistance of the host to a subsequent fully virulent infection by the same or other antigenically closely related organisms. Calmette further attenuated this strain by cultivation of the organism on a potato-glycerol-bile medium for 230 serial transfer! between the years 1908 and 1918.

The bacilli resulting from this attentuation have never been cloned. The original strain of BCG has been lost and has been replaced by a variant while it was being transferred serially on artificial culture media at the Pasteur Institute[12] and have since been maintained by many different laboratories, using many different methods. As a result, the BCG strains used today are not bacterioiogically identical.[13,14] In 1966, a WHO Expert Committee on Biological Standardisation adopted a series of recommendations for the production of BCG vaccine.[15] These recommendations stated that the vaccine should be freeze-dried, and that the vaccine strain should be maintained by the seed-lot-system whereby no vaccine is produced from a seed more than 12 passages removed from a primary freeze-dried lot. Such a method of maintenance was soon adopted by most laboratories and this eliminated the possibility of more attenuated variants in later BCG vaccine lots.[16]

BCG Vaccine Production in India

In India, the BCG Vaccine Laboratory was started in Madras in 1948 for the production of BCG vaccine for use in India and also for supply to some of the neighbouring countries. Since 1966, Danish strain 1331 is being used here for the preparation of both the liquid and the freezedried BCG vaccines, based on the seed-lot-system[17].

For preparing the liquid and freeze-dried vaccine. the BCG Laboratory, Madras, uses the method followed at the State Serum Institute, Copenhagen, but using Sauton potato medium for maintaining the BCG strain. The prepared vaccine is tested for purity by Ziehl-Neelsen smear for acid fast bacilli, and by culture on nutrient broth, thioglycollate medium and Sabouraud's agar medium. Total bacterial count and the number of culturable particles in the preparation are estimated. Biological tests are carried out in guineapigs to estimate the degree of virulence of the BCG vaccine, allergenicity and safety. In addition to the above tests, in the case of the freeze-dried vaccine, tests are carried out to estimate residual. moisture and heat stability. Both types of vaccines are to be stored at refrigeration temperature, protected from light, Under these conditions of storage, the liquid vaccine can be used for 4 weeks from the date of manufacture while the freeze-dried vaccine can be used for 3 months.

BCG can be administered intracutaneously, orally, by scarification or by multiple puncture. The most widely used method of administration is by intracutaneous injection. The dose is usually 0.1 ml and the site of injection is the upper arm. In the newborn, the dose used is 0.05 ml. The liquid BCG vaccine prepared by the BCG Laboratory, Madras, is to be administered by an intracutaneous injection of 0.1 ml of the vaccine containing 0.075 mg (moist weight) of BCG. The freeze-dried vaccine prepared here is reconstituted by the addition of sterile distilled water or sterile saline to contain 0.1 mg (moist weight) in 0.1 ml of vaccine which is given intracutaneously.

Efficacy of BCG Vaccine

BCG was used successfully in humans for the first time in 1921 by Weil-Halle, a colleague of Calmette and Guerin.[18] Scepticism concerning the safety an; efficacy of BCG vaccine, and the Lubeck disaster in which 72 of 240 children vaccinated with BCG did as a result of being fed a batch of vaccine containing virulent tubercle bacilli, delayed the acceptance of BCG. A series of controlled trials were begun in the 1930s. Despite inconsistent results from the trials, WHO encouraged widespread dissemination of BCG vaccines, starring in the 195Os. By the 1970s, BCG became the most widely used vaccine in the world. About 3 billion doses have been given in the last four decades, and more than 70 per cent of the world's children now receive BCG.[5,19]

Between the years 1935 and 1955, at least eight controlled trials were conducted to assess the efficacy of BCG vaccine against tuberculosis. The protective efficacy obtained ranged from none to 80 per cent (Table).[8]

Table: Protective efficacy of BCG vaccine against tuberculosis

Population group	Period of intake	Protective efficacy (%)
North American Indians	1935-1938	80
Chicago infants	1937-1948	75
Georgia school children	1947	None
Illinois children	1917-1948	None
Puerto Rico general population	1949-1951	31
Georgia and Alabama general population	1950	14
British children	1950-1952	78
South Indian rural population	1950-1955	31

The South Indian Trial

A study was started in Chingleput, South India, in 1968 in an attempt to avoid the methodologic errors that might have affected previous trials.[10,20,21] The south Indian BCG trial was organised by the Indian Council of Medical Research (ICMR) in collaboration with the WHO and Centre for Disease Control (CDC), US Public Health Services. The intake for the study started in 1968 and was completed in 1971, including about 2.6 lakh participants out of a population of 3.6 lakhs. The entire population of all ages was eligible and tuberculin reactors were not excluded, in contrast with previous trials. Two BCG strains, Copenhagen and Paris, were tested at two doses, 0.1 mg and 0.01 mg. Neither of the vaccines, whether in full or reduced dosage, had given any protection against the bacillary form of pulmonary tuberculosis as assessed over a 7.5 year follow up period. No data are available from the study to evaluate protection in children. Very little disease was observed in the period immediately after infection.[22] Incidence peaks were absent in young children and in young adults but the incidence increased logarithmically with age.

The findings of the south Indian trial were disappointing. The ICMR convened an expert committee meeting to scrutinise the trial methodology, wherein it was agreed that no errors in the conduct of the field operations or in the data processing could have been so serious as to invalidate the results.[10] In the first meeting of the ICMR/WHO Scientific Group[23] it was stated that the data obtained in this trial are unique and of great importance for tropical countries. and should be considered as the starting, point for further intensive investigations into the epidemiological, bacteriological and immunological problems related to BCG vaccine and tuberculosis, as well as studies to test certain hypotheses, *eg,* that the immune response of the population was unusual, that the vaccine were inadequate to confer immunity, that the south Indian variant of *M. tuberculosis* acted as an attenuated immunising agent, and that mycobacteria other than *M. tuberculosis* may have partially immunised the study population.

Explanations for the Varying Efficacy of BCG

The explanations and hypotheses for the varying efficacy of BCG have been discussed in detail.[5,7] BCG varying efficacy due to interactions with the immune responses to other mycobacterial infections still remains one of the most popular explanations. Palmer and associates[24,25] showed in animal experiments, and in studies of US navy personnel, that infections with certain non-tubercuious mycobacteria could impart some protection against infection with the tubercle bacillus and such naturally acquired protection could mask any protection due to BCG vaccination, partially or totally. This explanation was criticised by Hart[26] as being inadequate to explain all the differences between the various BCG vaccine trials. Comstock *et al*[27] also could not find any evidence for lowered protection by BCG in those with intermediate levels of tuberculin reactivity, and this was thought to be due to non-tuberculosis mycobacterial infection. in the Puerto Rico trial.

in the 1980s, Rook, Stanford and associates[28-30] proposed that exposure to non-tuberculous mycobacteria (NTM) can result in two types of cell-mediated responses, the ‘Listeria type’ and the ‘Koch type’. Which of these two types of responses is evoked depended, among other factors, on the mycobacterial species inducing the response and the immunomodulating cells and the pathway brought into play. They further proposed that the ‘Listeria type’ of response enhances the protective effect of subsequent vaccination with BCG while the ‘Koch type’ response opposes the protective effect of BCG. Once Koch-like responsiveness is present, this blocks subsequent recognition of further species by Listeria-like responses. BCG vaccination of a person with a pre-existing Koch-like response will temporarily boost this response, but completely fail to reconvert to Listeria-like responsiveness or induce protection from pathogenic challenge. According to them. this is likely to have been the situation in the south Indian trial.[31,32]

Investigations carried out since then have been able to produce some evidence supporting the hypothesis that infection with NTM induces a protective response and does not interfere with the immunity produced by BCG. Attempts to demonstrate that prior infection with any of the mycobacteria induced a suppressive effect against BCG have failed.[33-36]

The study population in the south Indian BCG trial was characterised by a very high prevalence of nonspecific sensitivity[29]. Further, nearly 20 percent of the NTM obtained from sputum samples of subjects in this area belonged to the *Mycobacterium avium-intracellulare-scrofulaceum* (MAE) complex,[38] and a recent study on the isolation profiles of environmental mycobacteria present in soil, water and dust samples, and sputum samples of symptomatics in this area has shown that isolates belonging to the MAIS complex are predominant in water. dust and sputum samples while organisms of the *M.fortuitum* complex are predominant in soil samples.[39]

The hypothesis that oral immunisation with *M.avium intracellulare* complex might induce tolerance which might interfere with the immune response to subsequent BCG immunisation was studied at the Tuberculosis Research Centre (TRC)[40] in guineapigs challenged with *M.tuberculosis*, and it was found that there was no interference with the protective immunity induced by BCG. A later study using intradermal route showed that while there was no interference with the immunity due to BCG by prior exposure to NTM on the early course of challenge infection, modulation could be taking place during the later course.[41]

The variation in the efficacy of BCG has also been attributed to the differences between the BCG preparations.[42,43] Another view is that BCG is more effective in stopping haematogenous spread of the bacteria as occurring in primary progressive disease and endogenous reactivation versus exogenous reinfections.[44] Other explanations include the genetic or physiological differences between the trial populations.

More recently, another explanation for the varying efficacy of BCG has been proposed based on the observation that a subgroup of the population may be actually adversely affected by vaccination.[45] Several trials include in the assessment many subject with weak initial tuberculin sensitivity, due either to environmental mycobacterial infection or to infection with *M. tuberculosis.* While it is accepted that vaccine efficacy may be moderately reduced in the former subgroup, it has been postulated that the lattar subgroup may be at risk of reactivation of tuberculosis soon after vaccination perhaps from focal reactions due to enhancement of their weak sensitivity. The low levels of efficacy in several trials, and the early adverse effect in the south Indian trial are broadly consistent with this hypothesis.

In a search for identifying the correlates of vaccine-induced protective immunity, more than 70000 subjects in northern Malawi were skin tested with soluble antigens of the tubercle and leprosy bacilli, and then followed up for 5 years for tuberculosis and leprosy incidence. Incidence rate ratios were calculated to compare subjects with different levels of prior skin test sensitivity.[46] It was found that delayed type hyper-sensitivity to mycobacterial antigens has different implications for tuberculosis and leprosy: low level hypersensitivity, probably attributable to environmental mycobacteria, was associated with protection, but persistent vaccine associated hypersensitivity to mycobacterial antigens was not a correlate of vaccine derived protection against mycobacterial diseases.

BCG Vaccination and HIV Infection

With regard to BCG vaccination in HIV infected individuals, there are reports of BCG abscesses in HIV seropositives and of disseminated infection due to BCG in at least one case given BCG.[47] However, in all these cases, the infection could be successfully treated. Since the risks and known consequences of natural infection with tubercle bacilli are likely to be more serious than the risks associated with live attentuated vaccines, the WHO has recommended that all asymptomatic HIV infected children should receive all standard vaccines both live and inactivated; and those with symptoms of AIDS Related Complex (ARC)/AIDS should receive all vaccines other than BCG. However, developing countries like India, where extensive HIV testing is not possible, the WHO Expert Group has recommended that all infants should continue to receive immunisation against all the major preventable diseases.[48]

There is no evidence that BCG activates HIV infection.[49] Further, it has been observed that the incidence of disease due to *M.avium intracellulare* (MAI) in AIDS patients varies from region to region and it has been postulated that this difference is the result of a protective effect of neonatal BCG vaccination.[50] In the USA, 30 per cent of patients with AIDS develop MAI disease in contrast to only 10 per cent of AIDS patients in Sweden. This difference in incidence between the two countries could be due to BCG vaccination: most Swedish patients with AIDS would have received BCG in infancy while those in the USA would be unvaccinated. This is further supported by the fact that over 50 per cent of AIDS patients in Netherlands, where BCG vaccination is not given, developed disease due to MAI or *M.scrofulaceum*. Also, in a limited follow up of HIV infected individuals at the TRC, Madras, it has been found that while a few HIV infected individuals developed disease due to *M.tuberculosis* no case has been encountered so far with disease due to MAI (Tuberculosis Research Centre - Unpublished observations). It has been suggested that MAI disease in AIDS is not due to direct infection hut that it arises from long standing silent foci of MAI in the lymphatic tissue of the patient.[51] It is possible that neonatal BCG vaccination prevents overt infection by MAI and may therefore prevent inapparent persisting infection of lymphoid tissue thus removing the internal reservoir of these bacilli from which AIDS-related MAI disease may arise later in life.[52]

BCG as an Immunopotentiating Agent

The widespread use of BCG has demonstrated its safety and its potent immunogenicity. This has also led to its suggested use as a carrier to vaccinate against other diseases.[53,54] BCG and other mycobacteria are highly effective adjuvants. It is one of the few vaccines that can be given at birth, and with a single dose it induces long-lived immune responses. Till now, nearly 3 billion vaccinations have been carried out using BCG with a long record of safe use in man. There is also a worldwide distribution network with experience in BCG vaccination. The adjuvant properties of BCG and its cell wall components have previously been made use of in experimental vaccines. Mixtures of BCG and schistosomal antigens have been used successfully to protect mice in a model of schistosomiasis.[55] Mixture of muramyl dipeptide, which is one of the mycobacterial cell wall components that contributes to the adjuvant properties, and killed simian immunodeficiency virus (SIV) has been shown to provide partial protection against SIV infection in monkeys.[56] Mixtures of BCG and killed *M.leprae* have been used in large scale trials to assess the efficacy of this leprosy vaccine candidate."'

Recombinant BCG and BCG as a Multiple Vaccine Vehicle

Recently developed genetic engineering techniques for mycobacteria have provided the means for the introduction and expression of foreign genes in BCG.[53,58] Recombinant BCG vaccine vehicles can induce immune responses to foreign proteins produced by the bacillus, indicating that BCG can act simultaneously as an adjuvant and as a vehicle to produce and deliver specific antigens to the immune system. A BCG recombinant may provide a longer lasting immunity to a pathogen than a simple mixture of BCG and the antigen because the antigen continues to be produced by BCG multiplying in the host.

There is no ready answer for the question whether there is an alternative for BCG vaccine for protection against tuberculosis. It is possible to improve the protective efficacy of the existing BCG vaccine against tuberculosis by using the tools of genetic engineering even though very little has been achieved in this direction to date. Such an approach requires a full understanding of the factors important in the virulence of *M.tuberculoosis*, pathogenesis of tuberculosis, and protective response against tuberculosis. Genetic deletion or modification of mycobacterial virulence factors or the addition of appropriate mycobacterial antigens important for protection might improve the effectiveness of BCG as an antitubertulosis vaccine.

CONCLUSION

Fine and Rodrigues[7] state that several factors, especially the differences in BCG strains and regional differences in mycobacterial ecology, in addition to differences in trial methods, have all contributed to the observed variation in BCG's efficacy. They conclude that despite our inability to predict its precise effect. BCG is still judged worthwhile in many countries because there is a possibility that the vaccine might provide reasonable levels of protection against childhood forms of the disease in most populations.[7] Recent retrospective studies of BCG vaccine efficacy among newborns and children have reported a protective effect against all forms of tuberculosis ranging from 17 to 90 per cent, and protection against tuberculous meningitis and against cavitary, miliary and bone and joint tuberculosis has been estimated to be 75 per cent or greater.[59-61] BCG vaccine, when effective, apparently does not prevent infection hut interferes with the haematogenous spread of tubercle bacilli, thus reducing the risk of severe primary disease and its complications.[60] A meta-analysis of 14 trials and 12 case-control studies showed that the protective effect of BCG against tuberculosis was 51 and 50 per cent respectively.[62] Combining data from 7 trials reporting on deaths from tuberculosis, the relative risk for death among the vaccinated was 0.29 (7 1% protective effect). Five case-control studies reporting on tuberculous meningitis showed a 63 per cent protective effect, and 3 case-control studies reporting efficacy of BCG in preventing disseminated tuberculosis showed a 78 per cent protective effect. The conclusion was that BCG reduces the risk for active tuberculosis on an average by 50 per cent, and the risk for tuberculosis death. meningitis and disseminated tuberculosis. The fact that

BCG provides variable though significant protection against leprosy increases its value in those countries with high prevalence of leprosy.[63]

It has been concluded that vaccination alone, at least with the present vaccine, cannot substantially influence the epidemiological situation but should be continued for children when its use is justified for prevention.[64] BCG vaccination of the newborn usually protects against serious forms of tuberculosis is safe and cheap and should be used in developing countries, including India, where tuberculosis is more prevalent. In such highly endemic areas, due to the frequent occurrence of exogenous reinfection and also due to the waning of protective effect over the years after vaccination, BCG vaccination of the newborn may not offer protection in the later years of life when revaccination, perhaps at the school going age, may have to be considered. In developed countries with low prevalence of tuberculosis, BCG should be given to high risk groups such as immigrants, their newborn, contacts of patients with tuberculosis and hospital staff.[65]

References

1. **Ada, G.L. The immunological principles of vaccination. *Lancet, 335*: 523, 1990.**

2. **Lagrange, P.H., Hurtrel, B. and Stach, J.L. Vaccines against mycobacteria and other intracellular multiplying bacteria. *Ann Inst Pasteur/Immunol, 136D*: 151, 1985.**

3. Mackaness, G.B. Cellular resistance to infection. *J Exp Med,* 116: 381, 1962.

4. Sokhey, J., Bhargava. I. and Basu, R.N. In: *The Immunisation Programme in India: A Handbook for Medical Officers.* Government of India, Ministry of Health and Family Welfare, New Delhi, 1984.

5. Fine, P.E.M. BCG vaccination against tuberculosis and leprosy. *Br Med Bull, 44:* 691, 1988.

6. Fine, P.E.M. The BCG story: Lessons from the past and implications for the future. *Rev Infect Dis. 11 (Suppl 2):* 5353, 1989.

7. Fine, P.E.M and Rodrigues, L.C. Mycobacterial diseases. *Lancet, 335:* 1016, 1990.

8. Luelmo, F. BCG vacination. *Am Rev Respir Dis. 125:* 70, 1982.

9. Smith, D.W. BCG. In: *The Mycobacteria* (Part B). Eds. G.P. Kubica and L.C. Wayne, Marcel Dekker, Inc., New York and Basel, 1984, p. 1057.

10. Ten Dam, H.G. Research on BCG vaccination. *Adv Tuberc Res. 21,* 79, 1984.

11. Calmette, A. and Guerin. C. Sur quelques proprietes du bacille tubercluleux cultive sur la bile. *CR Acad Sci, 147:* 1456, 1908.

12. Guerin, C. In: *BCG Vaccination Against Tuberculosis* Ed. S.R. Rosenthal. Little. Brown, Boston, Massachuuets. 1957, p. 48.

13. Frappier, A., Portelance, V., St Pierre, J. and Parisset. M BCG strains: Characteristics and relative efficacy. In: *Status of Immunisation in Tuberculosis* Ed. E.C. Chamberlayne Fogarty Int Cent Proc 14, Washington, 1972, p.157.

14. Milstein, J.B. and Gibson, J.J. Quality control of BCG vaccines by the World Health Organisation: A review of factors that may influence vaccine effectiveness and safety. *WHO EPI/GEN/89:* 3, 1989.

15. WHO Expert Committee on Biological Standardisation. Requirements for dried BCG Vaccine. *WHO Tech Rep Ser, 392,* 23, 1966.

16. Collins. EM. Tuberculosis In: *Bacterial Vaccines* Ed R. Germanier. Academic Press. inc.. 1984. p. 373.

17. Suri, J.C. in: *Text-book on Tuberculosis* Ed. K.N. Rao. The Kothari Book Depot. Bombay. India, 1972, p. 495.

18. Calmette. A. *La Vaccination Preventive Contre la Tuberculose par le BCG.* Masson, Paris. 1927.

19. *WHO EPI Update.* August, 1989.

20. Tuberculosis Prevention Trial. Trial of BCG vaccines in south India for tuberculosis prevention: First Report. *Bull WHO, 57:* 819, 1979.

21. Tuberculosis Prevention Trial. Madras. Trial of BCG vaccines in south India for tuberculosis prevention. *Indian J Med Res, 72 (Suppl):* 1, 1980.

22. Baily, G.V.J. and Toman, K. Notes on the epidemiology of [illegible] in India. *WHO/TRV/ScG/79.9*, 1979.

23: ICMR/WHO Scientific Group: Vaccination against tuberculosis. *WHO Tech Rep Ser. 651:* 1, 1980.

24. Palmer, C.E. and Edwards, L.B. Identifying the tuberculous infection. *J Am Med Assoc, 205:* 167, 1968.

25. Palmer, C.E. and Long, M.W. Effects of infection with atypical mycobacteria on BCG vaccination and tuberculosis *Am Rev Respir Dis, 94,* 553, 1966.

26. Hart, P.D. Efficacy and applicability of mass BCG vaccination in tuberculosis control. *Br Med J 1:* 587, 1967.

27. Comstock, G.W., Livesay, V.T. and Woolpert, S.F. Evaluaation of BCG vaccination among Puerto Rican children. *Am J Pub Hlth, 64:* 283, 1974.

28. Rook, G.A.W. The importance to the International Union against Tuberculosis of some recent advances in our understanding of cell mediated immunity to microorganisms. *Bull Int Union Tuberc, 58*: 50, 1983.

29. Rook, G.A.W., Bahr, G.M. and Stanford, J.L. The effect of two distinct forms of cell-mediated response to mycobacteria on the protective efficacy of BCG. *Tubercle, 62*: 63, 1981.

30. Stanford, J.L., Shield, M.J. and Rook, G.A.W. How environmental mycobacteria may predetermine the protective efficacy of BCG. *Tubercle, 62* : 55, 1981.

31. Shield, M.J. The importance of immunologically effective contact with environmental mycobacteria. In: *The Biology of Mycobacteria, (Vol.2).* Eds. C. Ratledge and J.L. Stanford. Academic Press. London. 1983. p. 343.

32. Stanford, J.L. and Rook, G.A.W. Environmental mycobacteria and immunisation with BCG. In: *Medical Microbiology, (Vol.2). Immunisation Against Bacterial Disease.* EL. C.S.F. Easmon and J. Jeljaszewicz Academic Press. London and New York. 1983. p.43.

33. Collins, F.M. Kinetics of the delayed hypersensitivity response in tuberculous guinea pigs and mice tested with several mycobacterial antigen preparations. *Am Rev Respir Dis. 127:* 599, 1983.

34. Edwards, M.L., Goodrich, J.M., Muller, D., Pollock, A., Ziegler, J.E. and Smith, D.W. Infection with *Mycobacterium avium intracellulare* and the protective effects of Bacille Calmette-Guerin. *J Infect Dis, 145*: 733, 1982.

35. Orme, I.M. and Collins, F.M. Efficacy of *Mycobacterium bovis* BCG vaccination in mice undergoing prior pulmonary infection with a typical mycobacteria. *Infect Immun. 44:* 28, 1984.

36. Smith, D., Reeser, P. and Musa, S. Does infection with environmental mycobacteria suppress the protective response to subsequent vacc ination with BCG? *Tubercle, 55:* 17, 1988.

37. Narain, R., Vallishayee, R.S. and Venkatesha Reddy. A. Value of dual testing with PPD-S and PPD-B. *Indian J Med Res, 68:* 204, 1978.

38. Paramasivan, C.N., Prabhakar, R., Somasundaram, P.R., Subbammal, S. and Tripathy, S.P. Species level identification of non-tuberculous mycobactcria from south Indian BCG trial area during 1981. *Tubercle, 66:* 9, 1985.

39. Kamala, T., Paramasivan, C.N., Herbert. D., Venkatesan, P. and Prabhakar, R. Isolation and identification of environmental mycobacteria in the *Mycobacterium bovis* BCG trial area of south India. *Appl Environ Microbiol; 60:* 2180, 1994.

40. Narayanan, S., Paramasivan, C.N, Prabhakar, R. and Narayanan, P R. Effect of oral exposure of *Mycobacterium avium intracellulare* on the protective immunity induced by BCG. *J Biosci, 10:* 453, 1986.

41. Herbert, D., Paramasivan, C.N. and Prabhakar, R. Protective response in guineapigs exposed to *M.avium intracellulare/ M.scrofulaceum*, BCG and south Indian isolates of *M.tuberculosis. Indian J Med Res, 99*: 1, 1994.

42. Guld, J. BCG as an immunising agent. In: *Status of Immunisation in Tuberculosis.* Ed. E.C. Chamberlaynne. Fogarty Int Cent Proc 14, Washington, 1972, p. 149.

43. Willis, S. and Vandiviere, M. The heterogenicity of BCG. *Am Rev Respir Dis. 84:* 288, 1961.

44. Wiegeshaus, E.H. and Smith, D.W. Evaluation of the protective potency of new tuberculosis vaccines. *Rev Infect Dis. 11:* S484, 1989.

45. Springett, V.H. and Sutherland. I. A re-examination of the variation in the efficacy of BCG vaccination against tuberculosis in clinical trials. *Tuberc Lung Dis. 75:* 227, 1994.

46. Fine, P.E.M. Delayed-type hypersensitivity, mycobacterial vaccines and protective immunity. *Lancet, 344:* 1245, 1994.

47. Von Reyn, C.F, Clements, C.J. and Mann. J.M. Human immunodeficiency virus infection and routine immunisation in childhood. *Lancet, ii:* 669, 1987.

48. Indian Council of Medical Research. HIV infection - Current status and future research plans. *ICMR Bull, 21:* 125, 1991.

49. Nunn, P.P. and McAdam, K.P.WJ. Mycobacterial infections and AIDS. *Br Med J. 44:* 801, 1988.

50. Kallenius, G., Hoffner, S.E and Svenson, S.B. Does vaccination with Bacille Calmette-Guerin protect against AIDS? *Rev Infect Dis, 11:* 349, 1989.

51. Good, R.C. Opportunist pathogens in the genus *Mycobacterium. Ann Rev Microbiol, 39:* 347, 1985.

52. Grange. J.M. Is the incidence of AIDS-associated *Mycobacterium avium intracellulare* disease affected by previous exposure to BCG. *M. tuberculosis* or environmental mycobacterial? *Tuberc Lung Dis 75:* 224, 1984.

53. Aldovini, A. and Young, R.A. Humoral and cell mediated immune responses to live recombinant BCG-HIV vaccines. *Nature, 351:* 479, 1991.

54. Young, D.B. and Cole, S.T. Leprosy, tuberculosis and the new genetics. *J Bacteriol. 175:* 1, 1993.

55. Pearle, E.J., James. SL., Hieny, S., Lanar, D.E. and Sher, A. Induction of protective immunity against *Schistosoma mansoni* by vaccination with *Schistosoma paramysin* (Sm97), a nonsurface parasite antigen. *Proc Natl Acad Sci USA, 85:* 5678, 1988.

56. Desrosiers, R. C., Wynad, MS., Kodama, T., Ringler, D.J , Arthur, L.O., Sehgal, P.K., Letvin, N. L., King, N.W. and Daniel MD. Vaccine protection against simian immunodeficiency virus infection. *Proc Natl Acad Sci USA, 86:* 6353, 1989.

57. Bloom, B.R. Learning from leprosy: A perspective on immunology and the third world. *J Immunol, 137:* 1, 1986.

58. Jacob, W.R., Tuckman, R. and Bloom, B.W. Intro duction of foreign DNA into mycobacteria using a shuttle plasmid. *Nature, 327:* 532, 1987.

59. Padungchan, S., Konjanarat, S., Kasiratta, S., Daramas, S. and ten Dam, H. G. The effectiveness of BCG vaccination. of the newborn against childhood tuberculosis in Bangkok. *Bull WHO, 64:* 247, 1986.

60. Snider, D. E. Jr., Rieder, H.L., Combs. D., Bloch, A.B., Hayden, C.H. and Smith M.H.D. Tuberculosis in children *Pediatr Infect Dis J, 7:* 271, 1988.

61. Tidjani, O., Amedome, A. and ten Dam, H.G. The protective effect of BCG vaccination of the newborn against childhood tuberculosis in an African community. *Tubercle, 67:* 269, 1986.

62. Colditz, G.A., Brewer, T.F., Berkey, C.S., Wilson, M.E., Burdick, E., Fineberg, H.V. and Mosteller, F. Efficacy of BCG vaccine in the prevention of tuberculosis: Meta-analysis of the published literature. *J Am Med Assoc, 271:* 698, 1994.

63. Ponnighaus, J.M., Fine, P.E.M., Sterne, J.A.C., Wilson, R.J., Msosa, E., Gruer, P.J.K., Jenkins, P.A., Lucas, S.B., Liomba, N.G. and Bliss, L. Efficacy of BCG vaccine against leprosy and tuberculosis in northern Malawi. *Lancet, 339:* 636, 1992.

64. Styblo, K. Overview and epidemiologic assessment of the current global tuberculosis situation with an emphasis on control in developing countries. *Rev Infect Dis, 11 (suppl 2):* S339, 1989.

65. Citron, K.M. BCG vaccination against tuberculosis. International perspectives. *Br Med J, 306:* 222, 1993

This write-up has been contributed by Dr. C.N. Paramasivan, Dy. Director (Sr. Grade), Dr. Daniel Herbert, Research Officer and Dr. R Prabhakar, Director, Tuberculosis Research Centre, Madras.

ABSTRACTS

Some Research Projects Completed Recently

Study of delayed type sensitivity in enteric fever using outer membrane proteins of *Salmonella typhi* as the eliciting antigens:

Immune response specific to outer membrane proteins (OMPs) of *Salmonella typhi* was studied *in vitro* in 30 bacteriologically proven patients of typhoid in the acute phase and at follow-up after 3 months. Fifteen normal healthy controls matched for age and sex and 15 bacteriologically proven patients of septicaemia caused by Gram negative bacteria other than *S. typhi* were also studied. Cell mediated immunity (CMI) against *S. typhi* was studied by leucocyte migration inhibition test (LMIT). Lymphocyte transformation test (LTT), and production of interleukin-1 (1L- 1 α) and IL-2, and leukotriene B_4 and C_4 (LTB_4 and LTC_4). Enumeration of per cent and absolute peripheral lymphocyte subpopulations was performed using fluorescein-isothiocyanate conjugated mouse monoclonal antibody.

It was observed that *S. typhi* induced specific CMI response in typhoid, both in the acute phase and on follow up. Of the various *in vitro* parameters of CMI. LMIT showed significant inhibition response to OMPs of *S.typhi* compared to OMPs of *S. typhimurium*. The inhibition was more significant to OMP of *S. typhi* in the typhoid group than in the septicaemic group. Lymphoproliferative response was also higher to OMP of *S. typhi* in typhoid patients in the acute phase as well as follow up compared to normal controls. OMPs of *S. typhi* induced lower amounts of IL-1α and IL-2 during the acute phase of typhoid than on follow up, though proliferative response to these antigens was significant. Lipopolysaccharide (LPS) which formed an integral part of OMPs induced only transient immune response in the acute phase of typhoid. *In vitro* IL-1α and IL-2 production in response to OMPs and LPS was impaired.

Transient immuno-suppression in the acute phase of typhoid was observed by LMIT and LTT using PHA as mitogen. On follow up these parameters of CMI reverted to normal, possibly due to rise in T helper cells. Antibodies specific to OMPs of *S. typhi* were present in both acute phase and follow up in all typhoid patients. There were few cross-reactions with OMP of *S. typhimurium*.

The study thus provided evidence of elicitation of specific cellular immune response by OMPs of *S.typhi* in typhoid. It also indicated that though there may be