CELL MEDIATED IMMUNE RESPONSES IN BCG VACCINATED CHILDREN

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ABSTRACT

The immunological status of BCG vaccinated and unvaccinated healthy children was evaluated to assess the efficacy of BCG. The duration of immunity conferred by the vaccine was also investigated. Of the 326 children studied, 170 (52%) had the BCG scar and only 24 (14%) showed a positive Mantoux response. Among the unvaccinated group, 14 of 156 (9%) showed a positive response. All cases had normal proportions of T and B cells in the peripheral blood. The mean values of the leukocyte migration inhibition (LMI) test with PHA were also normal. The per cent LMI values against PPD were compared in the children classified into groups based on their vaccination status and response to Mantoux test. A higher number of the vaccinated children had positive LMI values compared to those unvaccinated ($p<0.01$). The LMI values of children classified into three age groups decreased significantly ($p<0.01$) with increase in age. Hence, BCG seems to afford some protection in children and has to be administered at birth. Revaccination at the age of eight years, may boost the waning immunity and, may be considered in this age-group.

Key words: *BCG vaccine, Tuberculin test, BCG revaccination.*

Mass BCG vaccination programmes have been reported to lower the incidence of tuberculosis in many countries(1-3); however in a large-scale controlled trial conducted by WHO in South India, the results showed that BCG vaccination provided no protection against the disease in adults(4). The main objectives, therefore, of the present study were to evaluate the immunological status of BCG vaccinated and unvaccinated healthy children and to assess the duration of immunity conferred by the vaccine.

Material and Methods

The study was conducted in 326 randomly selected healthy children attending the Outpatient Department of the Gandhi Hospital and the Well Baby Clinic of Mahavir Hospital, Hyderabad during 1987 to 1989. Children who were malnourished (Grades III and IV)(5), or those having had measles or any other serious infection in the previous six months requiring treatment with antibiotics or children with a history of exposure to a case of tuberculosis, were excluded. The age group of children studied ranged from 3 months to 14 years. The presence or absence of a BCG scar was noted. Children were vaccinated either at birth or below three months of age.

Mantoux test was done with 10 tuberculin units(6) of purified protein derivative (PPD, Span Diagnostics, India) injected intradermally on the volar surface of the left

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forearm. PPD is calibrated against a batch of RT 23 (Statens Serum Institute, Denmark) and is diluted with a buffer containing Tween 80 as a stabilizer. Ten TU were used to rule out negative reactions with lower concentrations. An induration of 10 mm or more, 48 hours after injection was considered as a positive reaction. The person (VV) who recorded the skin test response was not aware of the BCG status of the child.

The percentage of B and T lymphocytes in the peripheral blood were evaluated by the EAC rosette and E rosette techniques, respectively(7). The leukocyte migration inhibition (LMI) test to assess the level of the lymphokine and LMI factor was done using the capillary method(8). The cells were allowed to migrate in the presence of phytohemagglutinin (PHA-P, Difco, USA) and purified protein derivative (PPD) of tuberculin. The percentage inhibition of migration was calculated as:

100 (Area of migration with antigen/ Area of migration without antigen × 100).

A value of 20% or more was considered to be a positive test.

The *in vitro* tests were performed only in those children for whom consent was obtained from the parents. The children included for different parameters were not necessarily the same. Statistical analysis was done using the 't'-test and the chi-square test of significance.

Results

Of the 326 children, 170(52.1%) had the BCG scar and gave a history of vaccination with BCG vaccine. Only 24 of 170 (14.1%) and 14 of 156 (9%) of the children with and without the BCG scar respectively, showed a positive Mantoux response.

The children were categorized according to the presence or absence of the BCG scar and Mantoux reactivity as Group I [BCG vaccinated: (*a*) Mantoux positive; (*b*) Mantoux negative] and Group II [BCG unvaccinated: (*a*) Mantoux positive; (*b*) Mantoux negative]. All cases had normal

TABLE I–*Percentages of T Cells, B Cells and LMI (PHA) in Normal Children Classified as per their BCG Vaccination and Mantoux Status*

Parameter	BCG vaccinated		BCG vaccinated	
	Mx+ve	Mx –ve	Mx+ve	Mx –ve
n	20	40	12	47
% T cells	54.0	51.7	50.0	51.2
	(3.3)	(2.4)	(2.7)	(2.4)
% B cells	20.5	22.5	19.0	20.3
	(1.8)	(3.4)	(3.0)	(2.2)
% LMI	72.0	69.4	74.9	76.9
(PHA)	(13.9)	(14.5)	(11.3)	(11.9)
n	13	17	10	19
%LMI	33.5	34.2	14.0	20.9
(PPD)	(14.9)	(17.4)	(15.3)	(18.4)

Mean values are mentioned with SD (in parentheses).

proportions of T and B cells and normal levels of per cent LMI using PHA as a mitogen, in the peripheral blood (*Table I*).

The mean (SD) values for LMI (using PPD as the antigen for Group I were (*a*) 33.5 (24.9)%, (*b*) 34.2 (17.4)%; Group II (*a*) 14.1 (15.4)% and Group II (*b*) 20.9 (18.4)%. The differences between Groups I(*b*) and II(*a*) were significant statistically ($p<0.05$). However, the differences within the other groups were not significant (*Table I*).

The per cent migration inhibition levels against PPD were compared in the four groups. Individual values are plotted in *Fig. 1*. A significantly higher number of Group I children, (*a*) 9/13 (69.2%) and (*b*)12/17 (70.6%) had positive LMI values compared to Group II children, (*a*) 2/10 (20%) and (*b*) 8/19 (42.1%) ($p<0.01$).

To find out the duration of protective immunity conferred by BCG, 31 vaccinated children were classified according to their age (*Fig. 2*). The mean (SD) values for LMI obtained were 38.1 (15)%, 21.3 (13)% and 15.0 (11.2)% for children less than 4 years (n=15), 4-8 years (n=9) and 8-12 (n=7) years, respectively. The LMI values decreased significantly ($p<0.01$) with increase in age for the vaccinated children.

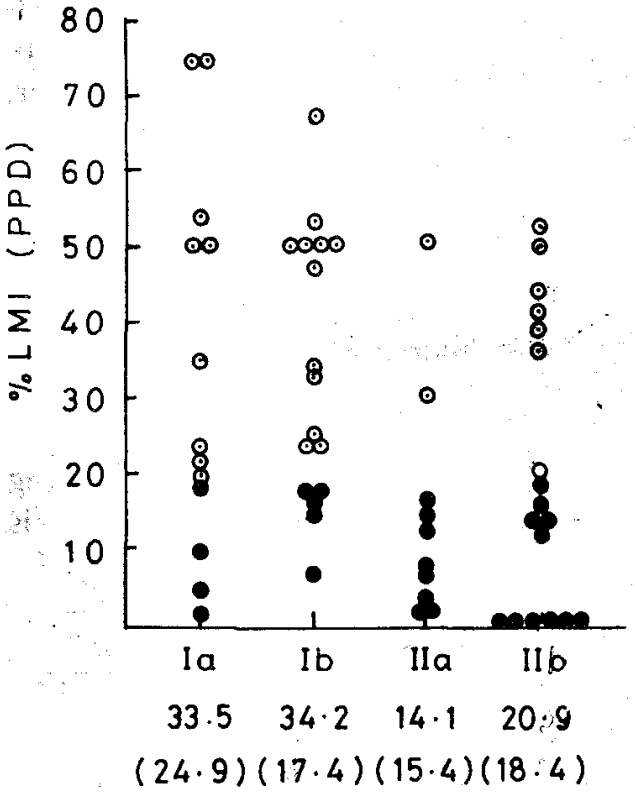

Fig. 1. Leukocyte migration inhibition (PPD) values in 4 groups of children: I BCG vaccinated: (a) Mantoux positive, (b) Mantoux negative, II BCG unvaccinated: (a) Mantoux positive, (b) Mantoux negative. Open and solid circles indicated LMI negative and positive values, respectively.

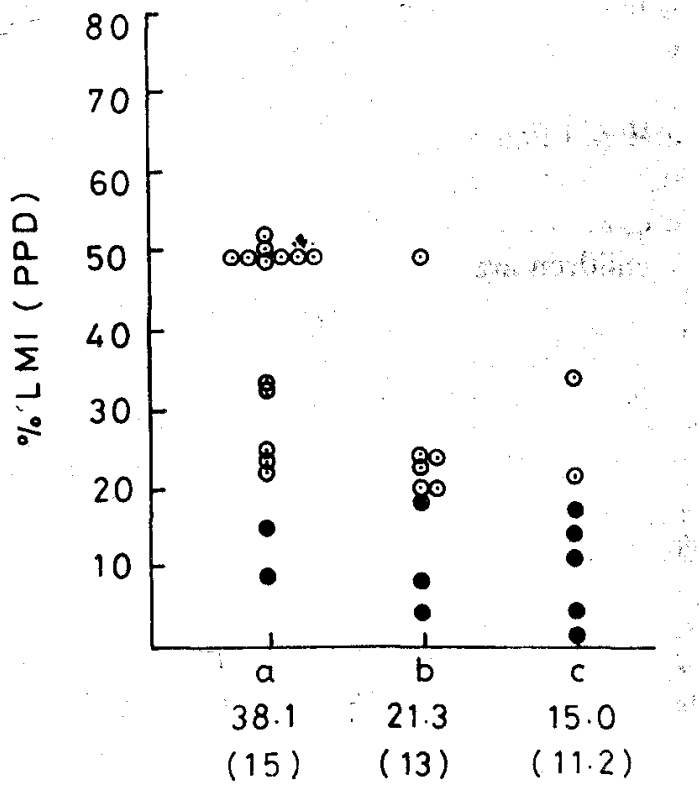

Fig. 2. Leukocyte migration inhibition (PPD) values in BCG vaccinated children of different age groups [(a) less than 4 years; (b) 4-8 years; (c) 8-12 years]. Open and solid circles indicated LMI negative and positive values, respectively.

Discussion

Prophylactic immunization with BCG is thought to confer resistance against infection with *M. tuberculosis*. BCG is a potent stimulator of T, B and reticuloendothelial system(9,10). The immunity afforded by BCG is expected to be indicated by a positive intradermal tuberculin test(6). However, in the present study only a small proportion (14.1%) of the vaccinated children showed a positive skin test response. Although the incidence was higher compared to the number of tuberculin reactors (9%) in the unvaccinated group, the difference was not statistically significant. Nevertheless, the absence of demonstrable post-vaccination sensitivity in the remaining 86% of the vaccinated children raised doubts regarding the efficacy of BCG vaccine and the reliability of the tuberculin test.

Similar reports, indicating a high incidence of tuberculin negativity in vaccinated children are reported in Nepalese(11) and Sri Lankan(12) children. It was felt that the presence of atypical or environmental mycobacteria contributed to the negative responses(13) due to cross reactions. An earlier study suggested that the Mantoux test is not reliable as a post-vaccination check although the *in vitro* cell mediated immune responses were positive(14). This has concordance with another report(15). Results of the present study confirm the earlier observations and it was observed that a higher proportion (70%) of the vaccinated children were positive to the *in vitro* test, as against 35% of the unvaccinated group. The differences could probably be attributed to the protective effect of the vaccine itself.

Similar observations, by other workers suggested that the BCG vaccine seems to be effective in majority of the children(16,17). Chandra(16) reported beneficial effects of the vaccine. Sensitization of T-lymphocytes to PPD and BCG after the vaccination in tuberculin non-reactors were also demonstrated in a previous study(17).

The *in vitro* responses of the vaccinated children declined significantly with age and at about eight years after the vaccine was administered, the mean values of the *in vitro* responses were negative. Waning of the efficacy of BCG vaccine with increasing age has been reported previously(12,18,19). When the protective effect of the vaccination was estimated in Norway, in the first ten years after it was administered, it was observed that the effect was higher during the first five years than during the next five(19). Revaccination has been advised by some authors to overcome the problem(12,18). However, decision about a revaccination could be arrived at only after a thorough understanding of the mechanism of action of the vaccine.

In conclusion, BCG vaccination, at or soon after birth seems to afford some protection in children. However, the protective efficacy of BCG cannot be assessed by the tuberculin skin test alone. Revaccination at the age of eight years may boost the waning immunity and may be contemplated in this age-group.

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