Immune Response to BCG Vaccination in Children

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The world-wide programme of mass BCG vaccination started in 1921[1] and used extensively since the forties has provided evidence that BCG vaccine has a protective value. This was confirmed by studies done on experimental animals.[2] Despite this, no vaccination has become more controversial than BCG vaccination in recent times. The protection conferred by BCG vaccination in children is important because of the serious consequences of tuberculosis in them.

The aim of this study was to assess the immunological response of children to BCG vaccination.

Materials and Methods

The study group consisted of children of employees of a private company attending a medical centre established for the benefit of the workers. The average income of the families was Rs. 800/– to Rs. 1000/– per month. During a screening programme for tuberculosis among all the employees and their families, 90 tuberculin negative normal children were vaccinated by the BCG team from the Government Chest Institute, Chetput, Madras. 0.1 ml containing 0.1 mg of BCG was administered.

Normal BCG reaction occurred after 4–6 weeks and healed after 1–2 months leaving an easily identifiable scar. Post vaccination allergy was tested in 15 children 6 months later and in 50 children 1 year later using PPD-S 1 tuberculin unit with Tween 80 (BCG Laboratories, Guindy) by the intracutaneous method (Mantoux). All the recommended precautions were taken to avoid false negative tests.[3,4]

With the consent of the parents, all the 50 were tested for reaction to 2,4 dinitrochlorobenzene (DNCB) using the method of Catalona *et al.*[5] but with the lower sensitizing dose of 500 µg as this was found to be as effective as 1000 µg.[6] The results were expressed as negative for no response to 4+ for very strong reaction.

Acknowledgement

Sarala Rajajee was a recipient of an Indian Council of Medical Research Senior Research Fellowship during the period of this research. The authors are grateful to the Director, TB Research Centre for permission to do this work.

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Fourteen children, all of whom were Mantoux negative, were tested for the lymphocyte response to phytohemagglutinin (PHA) and the antigens PPD and BCG. They were tested in parallel with controls. Ten of the controls had received BCG and were Mantoux positive; 8 controls had not been given BCG and were Mantoux negative.[7,8]

The immunological studies were done at the Immunology Department, Tuberculosis Research Centre (ICMR), Ghetput, Madras.

Lymphocyte transformation test (LTT)

Peripheral blood lymphocytes were isolated by Ficoll–Hypaque density gradient centrifugation. Cells were cultured in triplicate in 0.2 ml of RPMI 1640 supplemented with penicillin (100 µ/ml) streptomycin (100 µg/ml) glutamine (300 µg/ml) and 10 per cent autologous plasma in 96 well (u bottom) tissue culture plates (Laxbro) at a concentration of 0.5×10^6 cells/ml PHA was added to a final concentration of 1 µg/ml, PPD 50 µg/ml and BCG 50 µg/ml. Cultures were incubated at 37°C in 5 per cent CO_2 for 96 hours for PHA and 144 hours for PPD and BCG. The cultures received 1 uCi of 3 H Thymidine (Sp. act 13000 mCi/mol Babha Atomic Research Centre, Bombay) 16 hours before harvesting.

Cells were harvested with MASH II (Microbiological Associates USA) and deposited on fibre glass filter paper. Paper discs were then transferred to biovials containing 1 ml of scintillation fluid and counted in a β scintillation counter. (Packard Tricarb 300) Stimulation Index was calculated as follows:–

$$\frac{\text{CPM in stimulated cultures}}{\text{CPM in control cultures.}}$$

Indirect leucocyte migration inhibition test (ILMI)

Lymphokines; Lymphocytes were cultured in sterile Bijou bottles at a concentration of 1×10^6 cells/ml. In RPMI 1640 with Penicillin 100 µ/ml Streptomycin 100 µg/ml Glutamine 300 µg/ml along with 10 per cent sterile horse serum. The cells were stimulated using PPD 50 µg/ml and BCG 50 µg/ml with controls to which no antigen was added. The cultures were incubated at 37°C in 5 per cent CO_2 for 5 days. After centrifugation, the supernatant containing lymphokines, was separated and frozen at −20°C until use.

Guinea pig macrophages

Liquid paraffin 10 ml was injected intraperitoneally into guinea pigs. After 3 days, the peritoneal cells were collected and washed thrice with Hank's BSS. After the final washing, the cells were suspended in 0.1 ml of RPMI 1640, micro capillaries were filled with the cell suspension and one end sealed. The capillary tubes were centrifuged to pack the exudate cells. The capillaries were then cut at the cell fluid interface.

The exudate cells were shown to migrate in 12 well LM1 plates (Laxbro) in the presence of lymphokines obtained from the children. The plates were incubated at 37°C for 18 hours. Migration patterns were projected at fixed magnification and traced. The projected areas were measured by cutting out and weighing. The assay was set up in triplicates. The results were expressed as migration index.

Migration index (MI)

$$= \frac{\text{area of migration in the presence of lymphokines of stimulated lymphocytes}}{\text{area of migration in the presence of lymphokines of control lymphocytes.}}$$

Migration index of less than 0.8 was taken as indicative of positive reactors.

Results

The average age of the children was 6.5 years.

There were 28 boys and 22 girls. Forty of the 50 children were of normal weight for age and 10 had grade 1 under nutrition (Table 1).

TABLE 1
Nutritional status of 50 children graded using weight for age Gomez method of classification and ICMR reference standards

Number	Normal	Grade I	Grade II	Grade III
50	40	10	—	—

TABLE 2
Mantoux conversion after BCG

No. of children	Mantoux before BCG	Mantoux 1 year after BCG		
	< 5 mm	< 5 mm	5–10 mm	> 10 mm
50	50	45	2	3
		Mantoux 6 months after BCG		
		< 5 mm	5–10 mm	> 10 mm
15	15	15	—	—

* PPD-S.

Table 2 indicates that 45 out of 50 children (90 per cent) were non-converters with post-vaccination allergy of less than 5 mm. Five children showed reactions more than 5 mm in size of whom 3 had reactions more than 10 mm in size after BCG. All the 15 children tested at 6 months were tuberculin negative.

All the 50 children displayed normal cutaneous hyper-sensitivity to DNCB with majority having 3 plus and 4 plus reactions. Nine of them also had good response to PPD. This was significant when compared to non-vaccinated mantoux negative controls (Tables 3 and 4, Fig. 2).

Indirect leucocyte migration inhibition test also showed good response to antigens PPD and BCG in 13 out of 14 children (Table 5, Figs 3 and 4). This was highly significant when compared to the response in non-vaccinated tuberculin negative children.

TABLE 3
ITU with TWEEN 80

Mantoux	Number of children	DNCB		
		2 plus	3 plus	4 plus
< 5 mm	(45)	2	28	15
5–10 mm	(2)	—	1	1
> 10 mm	(3)	—	2	1

TABLE 4
Lymphocyte transformation response to PPD

Group	No.	Stimulation index Mean and S.D.
BCG vaccinated Mantoux negative	9	2.8 ± 2.24*
Non-vaccinated Mantoux negative	8	0.9 ± 0.6

* Highly significant $P < 0.05$.

TABLE 5
ILM1 in vaccinated and non-vaccinated group

Group	No.	Migration Index mean, S.D. PPD	BCG
BCG vaccinated children tuberculin negative	14	0.4 ± 0.3	0.6 ± 0.06*
Non-vaccinated children tuberculin negative	3	1.5 ± 0.2	1.2 ± 0.4

* Highly significant $P < 0.001$.

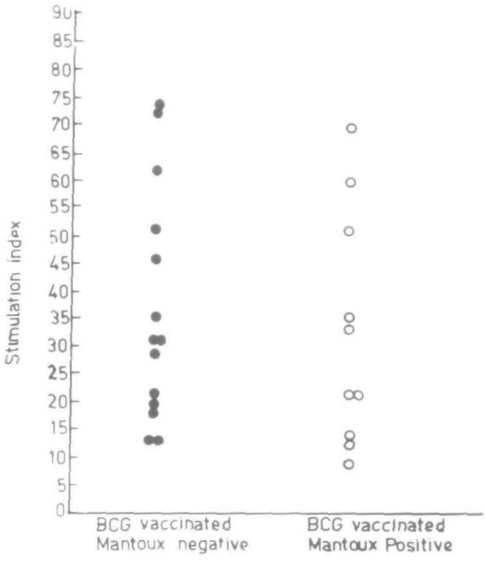

FIG. 1. Lymphocyte transformation response to PHA-P.

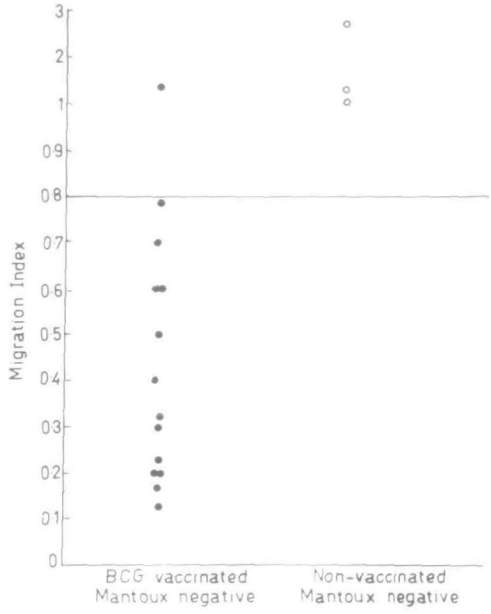

FIG. 3. Indirect leucocyte migration inhibition response to PPD.

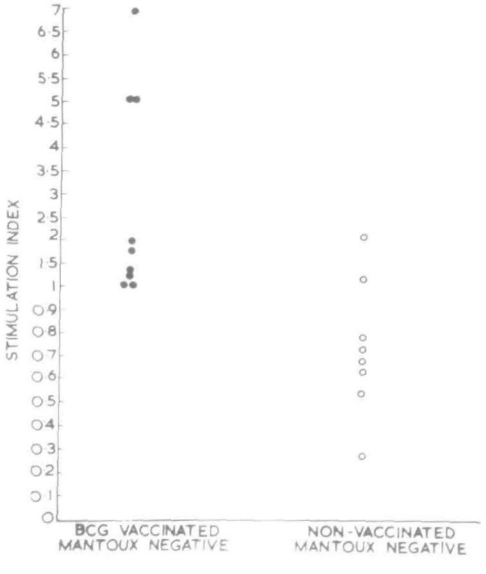

FIG. 2. Lymphocyte transformation response to PPD.

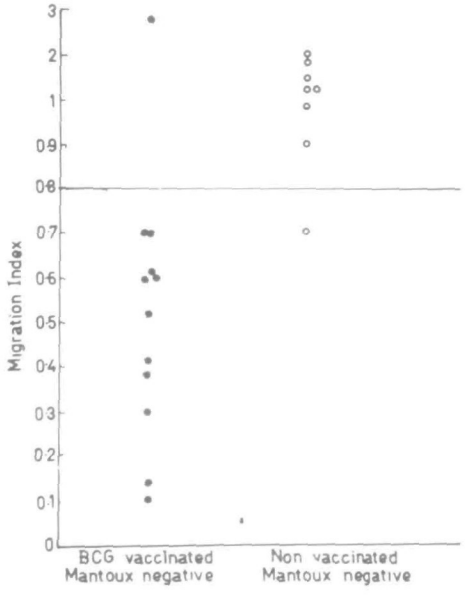

FIG. 4. Indirect leucocyte migration inhibition response to BCG.

Discussion

The protective value of BCG vaccine is controversial, controlled field trials have shown protection to range from none to as much as 80 per cent.

For measuring the degree of resistance against tuberculosis conferred by vaccination, Moller suggested that PVA can be accepted as indirect evidence of immunity.[10] Miller considered BCG vaccination to be successful if it produced a positive tuberculin reaction.[11]

The tuberculin test shows a high degree of negative reactions even in the presence of tuberculosis or after BCG vaccination and total reliance on PVA as a means of evaluating response to BCG will give misleading results regarding the value of BCG vaccination.[12–15]

Ninety per cent of the children in the present group were tuberculin negative at 1 year and 15 were negative even earlier at 6 months. Clinically, majority of the children were nutritionally normal for their age and came from middle income group families.

Cell mediated immunity (CMI) was normal as shown by positive DNCB skin reactions in all and good response to PHA in LTT.

In vitro tests showed significant positive response of the lymphocytes to PPD and BCG both in LTT and ILMI tests. This points to the presence of immunocompetent T cells in the absence of positive skin test.

This was also found in a study to assess the efficacy of BCG given at birth compared to at age 3 months by Mantoux skin test and lymphocyte migration inhibition (LMI) test.[16] In vitro positive cell mediated response was found even after the positive skin tests became negative.[17] Positive LMI response was recorded in 87.6 per cent of pre-school children after BCG vaccination in the newborn period irrespective of their nutritional status.[18] In a study on undernourished children it was found that though Mantoux was negative in children with kwashiorkor, LMI response to PPD was positive in these children.[19]

These results indicate that a negative skin test does not imply absence of in vitro CMI response.[20–22] Oppenheim[23] observed that in vitro response can occur before the development of reactive skin tests in persons vaccinated with BCG. He speculated that fewer immunologically committed lymphocytes might be needed to produce an in vitro response. The available circulating pool of tuberculin committed lymphocytes might not have been of sufficient magnitude to produce a local reaction in the skin but were adequate to produce an in vitro response. In our study, the non-vaccinated children who were Mantoux negative did not show positive Lymphocyte responsiveness to tubercular antigens in the in vitro tests. But, the BCG vaccinated group who remained Mantoux negative did show lymphocyte sensitivation to these antigens. We conclude that BCG vaccination induces positive immune response in children.

Summary

Ninety children attending a medical centre were administered the BCG vaccine after initial negative tuberculin reaction with 1 tuberculin unit (TU) of PPD-S. Fifty children were tested for post vaccination allergy (PVA) at one year, of whom 15 children were also tested at six months. Ninety per cent of the 50 children showed negative reaction to PPD-S (<5 mm) at 1 year, only 10 per cent had a positive reaction. All the 50 children had positive response to 2,4 dinitrochlorobenzene (DNCB) indicating normal delayed cutaneous hypersensitivity.

Fourteen children who were negative reactors after BCG showed normal response to phytohemagglutinin (PHA) in the lymphocyte transformation test (LTT) indicating normal cell mediated immune response (CMI). They all had significant response to PPD and BCG in indirect leucocyte migration inhibition test (ILMS). The stimulation index in the lymphocyte proliferative response to PPD was 2.8 ± 2.24 in these children.

Thus, in vitro tests indicate that the T lymphocytes were sensitised to PPD and BCG after BCG vaccination even though tests for post vaccination allergy were negative. This was significant as 8 non-vaccinated tuberculin negative children did not show evidence of lymphocyte sensitisation to PPD and BCG.

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Osmolality of Rice Water, Dextrose–Saline Solution and Formula Milk—Implication in the Management of Infantile Gastroenteritis

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In the management of infantile gastroenteritis, the use of oral rehydration therapy is the mainstay of the treatment for dehydration except in the more severe cases. The majority of the oral solutions are mixtures of sugar and electrolytes and the use of glucose and sucrose as the two common sugars has been extensively studied.[1–3] While the use of sugar-electrolyte solutions has been well established, the dangers of hyperosmolality cannot be overlooked.[4] Rice water, although not entirely new,[5] has been evaluated and found to be an effective, cheap and simple means of treating infantile gastroenteritis.[6,7]

We aim to study the differences in osmolality between rice water, dextrose–saline solution and formula milk, all of which are used as oral therapy in some form or another in the management of infantile gastroenteritis.

Materials and Methods

Twenty samples of rice water were collected from the Paediatric ward of the University Department of Paediatrics, Singapore General Hospital over a

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Acknowledgement

We thank Miss Heng Ye Yong for technical assistance and Miss Fannie Chan for preparing the manuscript.

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