# COMPARISON OF THE IMMUNE RESPONSES IN CHILDREN VACCINATED WITH THREE STRAINS OF BCG VACCINE

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## ABSTRACT

*The present study was conducted to evaluate and compare the specific cellular responses of children vaccinated with three different strains of BCG. The study comprised of normal children with normal weight and normal general responses (PHA) to in vitro leukocyte migration inhibition test (LMIT). The three strains of BCG under study were Japan-BCG, Glaxo-BCG and Madras-BCG. One hundred children were selected at random from each group. The mean ages of these infants were 9.9 ±9.5, 9.8 ±7.6 and 9.8 ±8.3 weeks, respectively. Six weeks after vaccination, the diameter (in mm) of induration at the vaccination site was measured. Three months after vaccination, in vitro LMIT was performed against PPD tuberculin antigen. This test was done again after 3 months in all the children who tested negative.*

*The mean value of the diameter of the Glaxo-BCG group (10.0 ± 13.5 mm) was significantly higher (p <0.05) than the mean values of Japan-BCG (9.10 ± 3.9 mm) and Madras-BCG (8.38 ± 4.1 mm). The mean LM1 values were similar in all the three groups. There was no correlation between the in vitro and in vivo parameters. The number of children positive to LMI (PPD) were 59, 58 and 63, for the Madras, Japan and Glaxo-BCG groups, respectively. A total number of 91, " 91 and 95 were positive to LMIT at the end of 6 months after BCG in the Madras, Japan and Glaxo-BCG groups, respectively. The observations suggested that there were no major differences between the three strains of BCG in their capacity to induce cellular responses.*

**Key words:** *BCG vaccine, Leucocyte migration inhibition test.*

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The Expanded Programme on Immunization (EPI), now recommends BCG vaccination as part of routine childhood immunization. However, trials on BCG provided controversial results, with protective efficacy ranging from 0% to 80%(l). One of the reasons offered to explain the discrepancy in results was that it reflects differences in the sub-strains of BCG used in different trials(2). The main objective, therefore, of the present study was to evaluate and compare the specific cellular responses of children vaccinated with different strains of BCG.

## Material and Methods

Normal healthy infants who were brought for immunization to the State Tuberculosis Centre and Chest Clinic, Tuberculosis Association of A.P., Hyderabad, during the period 1990 to 1992 were included in the study. After an 'informed' consent, a reference card was given to them and they were asked to visit the clinic 6 months after BCG vaccination. A detailed history of the child including date of birth, weight,

skin-fold thickness and general health status was recorded.

During the period of the study, various strains of BCG *(e.g.,* Madras, BCG, Glaxo-BCG, Pasteur BCG, Canada-BCG, Japan-BCG) were made available to this centre. Only the children vaccinated with Madras-BCG, Glaxo-BCG and Japan-BCG were included for analysis in the present report.

The total number of children examined for induration were 150, 254 and 179, and those investigated for LMI were 237, 360 and 238 for the Madras, Japan and Glaxo groups, respectively. The first 100 children from each group were selected for analysis. The mean ages of the children selected from each group was 9.9 ± 9.6; *7.8* ± 7.6; 9.8 ± 8.3 weeks, respectively. The dosage of BCG administered for infants below 3 months was 0.05 ml and for children above 3 months was 0.1 ml.

After 6 weeks of vaccination, the diameter of induration at the site of vaccination was measured. After 3 months of vaccination, blood was collected in a heparinized tube and leukocyte migration inhibition test (LMIT) was performed by the capillary method(3), using PHA and PPD as mitogen and antigen, respectively. Children who had negative responses to PPD were retested 3 months later (6 months after BCG).

Six children out of 300 had positive histories of contact and 40 children had negative LMI responses to PHA and were excluded from analysis. None of the vaccinated children had BCG lymphadenitis. For statistical analysis, the mean values (± 1 SD) were calculated in each group, and were compared by the t-test at $p<0.05$ and also by analysis of variance.

## Results

The mean diameter (in mm) of induration of the children vaccinated with the Glaxo strain was 10.0 (±13.5) and significantly higher ($p < 0.05$) than the mean values of the Japan-BCG group (9.10 ± 3.0) and the Madras BCG group (8.38 ± 4.1); the differences between the values in the latter groups were not statistically significant. *Table I* depicts the mean values (±SD) of % LMI (PPD) at three months after vaccination. Amongst the negative responders at 3 months, 32/41, 33/42 and 32/37 converted to LMI-positive in the Madras, Japan and Glaxo groups, respectively *(Table II)* at 6 months. The total number of children with positive LMI values at the end of six months were 91, 91 and 95, respectively. There were no significant differences amongst the strains, within the strains amongst" the children and within the children by analysis of variance.

## Discussion

Although the origin of the BCG vaccines presently being used is from a single strain, these bacilli may have undergone changes as a result of being prepared at several centres following different methodologies leading to various sub-strains of BCG(4,5). There are many reports on these differences. Biochemical differences were reported by Bonicke in 1957(6), and others reported differences in their immunogeniciry(7), skin test sensitivity(8), the ability to protect against challenge with tubercle bacilli and virulence in animals(9). The precise relationship between these properties and the vaccine's efficacy in humans was questioned by some authors(10,ll). The relevant *in vitro* cell-mediated immune re-

**TABLE I-***Per cent LMI (PPD) Values in Children Three Months post-vaccination*

| BCG Strain | | No. of Children | | |
|---|---|---|---|---|
| | | Total | With (+) ve values | With (-) ve values |
| Madras | No. | 100 | 59 | 41 |
| | Mean (SD) | 22.8 (10.3) | 29.5 (7.2) | 12.7 (4.4) |
| Japan | No. | 100 | 58 | 42 |
| | Mean (SD) | 22.5 (9.8) | 29.5 (6.5) | 13.2 (4.2) |
| Glaxo | No. | 100 | 63 | 37 |
| | Mean (SD) | 22.5 (10.4) | 29.6 (5.5) | 10.6 (4.8) |

**TABLE** 11- % *LMI (PPD) Values in Children Vaccinated with Either One of Three Strains of BCG (Madras, Japan, Glaxo) who had Negative «20%) Values at* 3 *Months, Rtested,* 6 *months After BCG Vaccination.*

| BCG Strain | | 3 months | 6 months | |
|---|---|---|---|---|
| | | Total | With (+) ve values | With (-) ve values |
| Madras | No. | 41 | 32 | 9 |
| | Mean (SD) | 12.7(4.7) | 28.2(5.3) | 13.6(4.8) |
| Japan | No. | 42 | 33 | 9 |
| | Mean (SD) | 13.2 (4.2) | 25.8(7.4) | 11.3(6.0) |
| Glaxo | No. | 37 | 32 | 5 |
| | Mean (SD) | 10.6(4.8) | 32.6(6.6) | 9.0(13.6) |

sponse evoked by different strains in normal children were, therefore, tested in the present study. *In vivo* tuberculin testing was not carried out since this test is not useful as a post-vaccination check(12,13). Variations were observed in the indurations which developed six weeks after vaccination in different groups of children, the largest induration being observed in the children vaccinated with the Glaxo strain of BCG. This observation was similar to that of an earlier report wherein variations in the size and character of local lesions due to BCG strain differences were observed(ll). However, it is still not clear, whether this parameter in any way correlates with protection. No such correlation was observed in the present study.

It has been reported that tuberculin conversion is similar in all children irrespective of the formation of abscess after vaccination(14). Apparently, more or less equal number of children had positive *in vitro* responses in the three groups when evaluated three months after vaccination. The mean values of the test results were also similar. A majority

of the LMIT negative children, when retested after three months were positive in all the three groups. Although the total number of children with positive responses, the average values of the LMIT tests and the size of the local lesions appeared to be consistently higher in the group vaccinated with the Glaxo strain, the differences were negligible. It remains to be established by applying more sensitive methods whether these differences have any clinical significance. Some studies have suggested that Glaxo-BCG protects Asians living in Britain, better than Paris or Copenhagen BCG does in South India(14).

On the basis of the present study, it is concluded that the three strains of BCG vaccine, Madras, Japan and Glaxo, elicit more or less similar *in vitro* responses in children.

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