Tuberculin Specific T Cell Responses in BCG Vaccinated Children

Vijaya Lakshmi V., Sunil Kumar, Surekha Rani H., Suman Latha G. and Murthy K.J.R.

From the Immunology Unit, Bhagawan Mahavir Medical Research Center, 10-1-1, Mahavir Marg, Hyderabad 500 004, India.

Correspondence to: Dr. Vijaya Lakshmi V., Immunology Unit, Bhagawan Mahavir Medical Research Center, 10-1-1, Mahavir Marg, Hyderabad 500 004, India, E-mail: vijayavalluri@hotmail.com

Manuscript received: February 18, 2003, Initial review completed: July 21, 2003; Revision accepted: July 5, 2004.

The effector mechanisms of BCG protection were examined 5-7 years after vaccination. The in vitro lymphoproliferation, following stimulation with tuberculin, in normal, (A) vaccinated and (B) unvaccinated children and children with tuberculosis (C), were assayed. The mean stimulation index (SI) of lymphocyte transformation in normal subjects were significantly ($P<0.05$) higher than those with tuberculosis. The ratio of tuberculin-specific CD4 to CD8 cells in short-term cultures were significantly ($P<0.05$) higher in the vaccinees. In group (A), 70% had positive ratios as against 20% and 0% in groups (B) and (C), respectively. Secretion of IL-2 by the cells was significantly ($P<0.05$) high in the vaccinated. None of the unvaccinated children had positive levels of IL-2. The vaccinees also had highly significant ($P<0.01$) levels of IFN-γ in the supernatants of cell-cultures, following tuberculin stimulation. In majority of the BCG vaccinated children, the stimulation of specific TH1 cells seem to be considerably high, in short-term in vitro cultures. While these responses were not so marked in the unvaccinated, they were almost totally absent in the patients.

The incidence of tuberculosis is on the rise. The rate of increase cannot decrease by chemotherapy alone. Immunoprophylaxis has a pivotal role in the control of the disease(1). BCG, a vaccine currently in use in several countries including India, was developed nearly 80 years ago, when many of the aspects of cellular immunity were still unknown. The efficacy of BCG is in doubt. There is a need therefore, to elucidate whether BCG specifically stimulates those subsets of T cells, which are beneficial to the host.

Subjects and Methods

This study was conducted in normal children in the age-group of 5-7 years and categorized into the following groups: (Group A) normal and vaccinated with BCG during the first year (vaccination was confirmed by presence of BCG scar and interrogation of the parents), n = 45; (Group B) normal and without a BCG scar and with no evident history of vaccination, n = 31; and (Group C) children with active tuberculosis (meningitis, miliary and lymphadenitis forms), n = 31. Peripheral venous blood was collected following an informed consent from the parents.

Briefly, the assays were carried out by culturing lymphocytes in complete RPMI 1640 medium and stimulated with either concanavalin-A or tuberculin. Stimulation index (SI) in lymphoproliferation and levels of interferon-IFN-(γ) levels and interleukin-2 (IL-2) were measured. Also, specific CD4 and CD8 Cells were measured by ELISA (1,2).

Results

The results indicated that the stimulation

index (SI) in lymphocytes stimulated by concanavalin-A was positive in all the normal children. With tuberculin, the mean value of the patients was significantly (P <0.05) lower than that of the normal children, irrespective of the vaccination status. When SI value of 2 and above were considered to be positive, 34/34 (100%) of the vaccinated, 18/21 (85.7%) of the unvaccinated and 15/30 (50%) of children with tuberculosis had positive values. The individual values are shown in (*Fig. 1*).

The levels of specific CD4 and CD8 cells in the vaccinated group (group A) were significantly (P <0.05) higher than the levels in the other groups, i.e. unvaccinated (group B) and those with tuberculosis (group C). When 1.5 was considered as a cut-off value, 70%, 20% and 0% of the children in the three groups respectively, had positive ratios (*Fig. 2*).

The difference in IL-2 levels between groups A and B was significant (P <0.05). This assay was not done in group C. While 20/30 (66.7%) of the children in group A had positive values, none in group B had a SI of 2 and above (*Fig. 3*). Mean values of IFN-(g) levels were statistically different in the three groups (P <0.01). When 100 pg/ml was considered as the cut-off value, 55.0 %, 47.6% and 3.1% of the children in groups A, B and C respectively, had positive values (*Fig. 4*).

Discussion

The results of a large-scale trial conducted

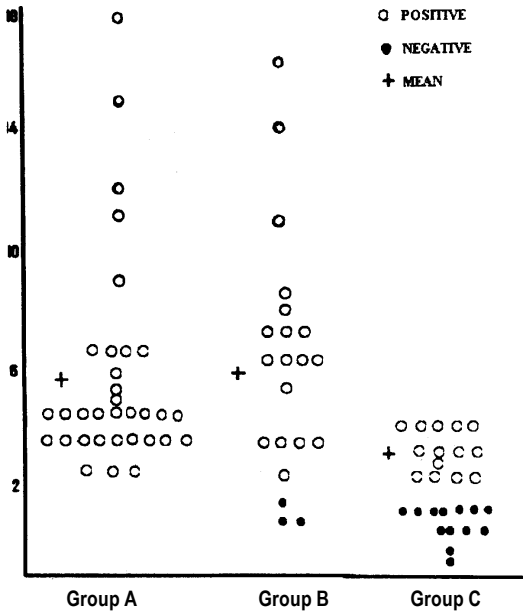

Fig. 1. Stimulation indices against tuberculin in lymphocyte transformation test of vaccinated (group A), unvaccinated (group B) and tuberculous children (group C).

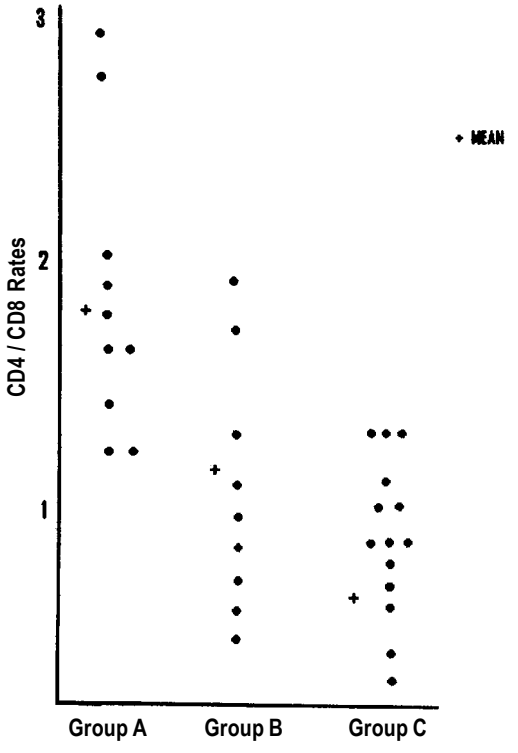

Fig. 2. Ratios of CD4 to CD8 cells after stimulation with tuberculin in short-term cultures of BCG vaccinated(group A), unvaccinated (group B)and tuberculous children (group C).

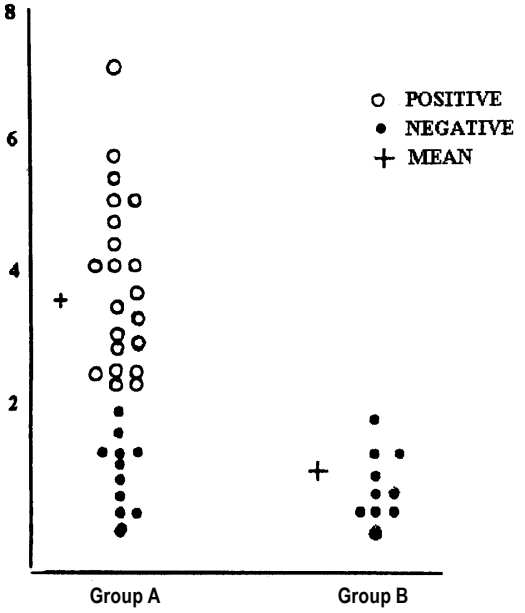

Fig. 3. Stimulation indices in cultures of IL-2-dependent cells supplemented with supernatants of short-term cultures of lymphocytes from BCG vaccinated (group A) and unvaccinated children (group B).

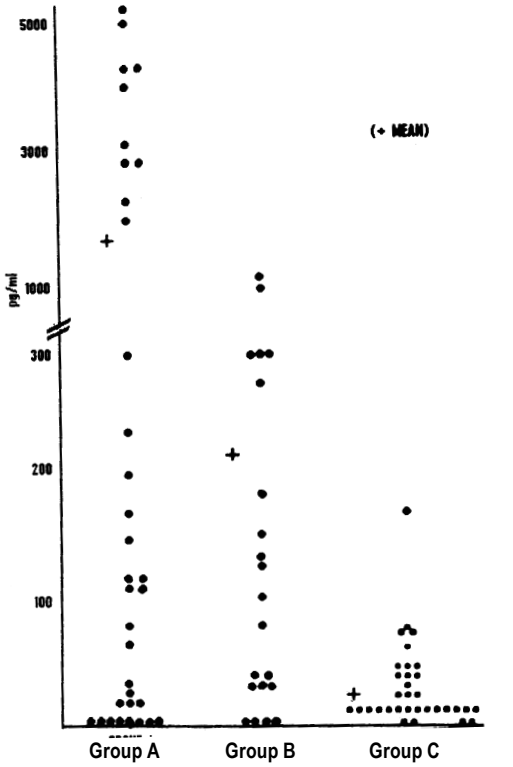

Fig. 4. IFN-g levels in supernatants of short-term cultures of lymphocytes from BCG vaccinated (group A), unvaccinated (group B) and tuberculous (group C) children.

in India on BCG, indicated that the efficacy of the vaccine was 0%(3). Ever since, neither the level of protection that the vaccine provides in children, nor the duration of its effect have been firmly established in children who receive the vaccination. In a previous study, it was shown that 33% of the unvaccinated and 70% of the vaccinated children had positive *in vitro* cell mediated immune responses to PPD; the vaccinated children had higher levels which started to decline after about four years (4). In another study from India, the overall vaccine effectiveness estimated was 60%(5).

In the present study, conducted in children aged 5-7 years, the results suggested that the in vitro lymphoproliferation of tuberculin-specific cells were more or less similar in both the vaccinees and those who did not receive the vaccination. The information gained from both the above tests was that tuberculin-sensitized cells were present in children irrespective of the vaccination status; the magnitude of the response, was albeit, higher in the vaccinated group. However, whether these cells are of the beneficial type or not was the issue addressed in this study.

Within the complex immunoregulatory response to mycobacterial infection, it is established that T cells provide protection(6). It is also known that CD4 subset of T cell is the primary cell responsible for regulating immune responses to M. tuberculosis(7). Bulk CD4 cell populations and CD4 clones from *M. tuberculosis*-infected individuals have been found to be directly cytotoxic for monocytes

pulsed with mycobacterial antigens(6). CD4 cells cross inflamed endothelial surfaces to reach the sites of mycobacterial infection. Infections by *M. tuberculosis* were substantially enhanced by CD4 depletion in mice(7). In this study majority (70%) of the BCG vaccinees had elevated levels of specific CD4/CD8 cell ratios, as against a minor (20%) proportion of the unvaccinated, suggesting that BCG vaccine specifically stimulates CD4 T cells in children. Whether the efficacy wanes in the remaining 30% of the vaccinated children or whether the vaccine provided any protection in the first place, needs to be clarified.

IFN-g an essential component of the host defence against mycobacteria, is responsible for the activation of macrophages, stimulation of anti-mycobacterial properties(8,9) and secretion of IL-1, tumor necrosis factor, granulocyte macrophage-colony stimulating factor and platelet derived growth factor(10) by the macrophages(11) . That BCG vaccine stimulates the secretion of IFN-g by lymphocytes from vaccinated children has been shown in this study, wherein the levels of the cytokines were elevated after *in vitro* stimulation. However, about 40% of them still had low levels of the cytokine. The levels of the cytokine present soon after vaccination may throw light on whether there has been a decline in the immunity. Majority (66.7%) of the vaccinated children in this study had significantly elevated (positive) levels of IL-2, while all of the unvaccinated children had low (negative) levels, suggesting that BCG selectively induces human *TH1* cells.

Tuberculin-specific *in vitro* lympho-proliferative responses in patients with tuberculosis (group C) were low, as also the secretion of IFN-g. It was reported earlier that patients with newly diagnosed, pulmonary tuberculosis had a tuberculin-specific defect in IL-2 production(12). Poor CD4 T cell responses, both in vivo (skin test anergy) and in vitro (with PBMC), were reported in patients with advanced disease(13). A reciprocal relationship between T cell responsiveness and the extent of disease in patients has been demonstrated several times. According to Orme(9), when mice or guinea pigs are immunized with BCG and later challenged in the lungs with virulent M. tuberculosis, the progression of the infection is slowed and there is an accelerated development of granulomatous response, probably a result of rapid recognition of the primary lesion by memory T-cells.

The results of this study indicate that BCG vaccination in children entails positive TH1 immune responses in the majority of them. These responses were not marked in the unvaccinated and absent in the tuberculous children. Future research needs to be directed towards augmentation of the magnitude and incidence of the beneficial effects of BCG.

Acknowledgements

The authors thank Prof. Indira Nath, All India Institute of Medical Sciences, New Delhi; Dr. K. Sridhar Rao, Center for Cellular & Molecular Biology, Hyderabad; Director, State TB Center; General Secretary, TB Association of Andhra Pradesh; and Superintendent, Nilofer Hospital for Women and Children, Hyderabad.

Contributors: VLV, SK, SRH, SLG were involved in the conception, design, acquisition and analysis of data. VLV and MKJR drafted and reviewed the manuscript. VLV will be the guarantor of the study.

Funding: Department of Science and Technology, Government of India.

Competing interests: None declared.

REFERENCES

1. Mosmann TR, Rapid colorimetric assay for cellular growth and survival: application to

proliferation and cytotoxicity assay. J Immunol Methods 1983; 65: 55-63

2. Voller A, Bidwell D. Enzyme linked immunosorbent assay. *In:* Editors Rose NR, Friedman H, John L.Fahey. Manual of Clinical Laboratory Immunology. Washington, American Society for Microbiolgy, 1986; pp. 99-109.
3. Collins FM. Tuberculosis: The return of an old enemy. Crit Rev Microbiol 1993; 19: 1-16.
4. Vijayalakshmi V, Devi PS, Murthy KJR, Rao DV, Jain SN Cell mediated immune responses in BCG vaccinated children. Indian Pediatr 1993; 30: 899-903.
5. Murhekar MV, Kulkarni HR, Zodpey SP, Dehankar AG. Effectiveness of mass neonatal BCG vaccination in the prevention of pulmonary tuberculosis: a case-control study in Nagpur, India. Tuberulosis and Lung Disease 1995; 76: 545-549.
6. Mutis T, Cornelisse YE, Ottenhoff TH. Mycobacteria induce CD4+ T cells that are cytotoxic and display Th1-like cytokine secretion profile: Heterogeneity in cytotoxic activity and cytokine secretion levels. Eur J Immunol 1993; 9: 2189-2195.
7. Flory CM, Hubbard RD, Collins FM. Effects of *in vivo* T lymphocyte subset depletion on mycobacterial infections in mice. J Leukoc Biol 1992; 51: 225-229.
8. Hirsch CS, Yoneda T, Averill L, Ellner JJ, Toossi Z. Enhancement of intracellular growth of Mycobacterium tuberculosis in human monocytes by transforming growth factor-beta 1. J Infect Dis 1994; 170: 1229-1237.
9. Orme I. Beyond BCG: The potential for a more effective TB vaccine. Mol Med Today 1999; 5: 487-492
10. Wangoo A, Taylor IK, Haynes AR, Shaw RJ. Up-regulation of alveolar macrophage platelet-derived growth factor-B (PDGF-B) mRNA by interferon-gamma from Mycobacterium tuberculosis antigen (PPD)-stimulated lymphocytes. Clin Exp Immunol 1993; 94: 43-50.
11. Fong TAT, Mosman TR. The role of IFN-gamma in delayed type hypersensitivity mediated by Th1 clones. J Immunol 1989; 143: 2887-2893.
12. Toossi Z, Kleinhenz ME, Ellner JJ. Defective interleukin 2 production and responsiveness in human pulmonary tuberculosis. J Exp Med 1986; 163: 1162-1172.
13. Ribera E, Espanol T, Martinez-Vazquez JM, Ocana I, Encabo G. Lymphocyte proliferation and gamma-interferon production after *in vitro* stimulation with PPD. Differences between tuberculous and nontuberculous pleurisy in patients with positive tuberculin skin test. Chest 1990; 97: 1381-1385.