

CORRESPONDENCE

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Development of TT Leprosy in a BCG-Vaccinated Individual: Immunological Response During Disease and After Spontaneous Healing

TO THE EDITOR:

In the last decade, several studies have clearly demonstrated a protective effect of BCG (Bacille Calmette-Guérin) vaccination against the development of leprosy (⁴). Results of a large vaccine trial indicated that several doses of BCG offer additional protection, and that the use of killed *Mycobacterium leprae* did not improve the protective effect of BCG alone (³). In leprosy, a Th1-type response observed in the tuberculoid forms (TT/BT) has been associated with a high gamma interferon (IFN- γ) production and development of effective cell-mediated immunity resulting in elimination of the bacilli. As described recently, BCG vaccination in humans was able to induce a Th1 response and enhanced IFN- γ production to specific antigens (¹²) and to *M. leprae* antigens as well (¹⁰). Thus, the protection afforded by BCG seems to be related, at least in part, to the induction of IFN- γ secretion following vaccination. In the present study, clinical and immunological follow-up evaluation of a household contact who developed TT leprosy after BCG vaccination was assessed during active disease and after self-healing. A major participation of CD4+ T cells in the *in vitro* response is further demonstrated.

Clinical and immunological response. In her first clinical examination at the Lep-

rosy Outpatient Unit (Oswaldo Cruz Foundation, Rio de Janeiro, Brazil), a healthy household contact, aged 55, was vaccinated with 0.1 ml BCG (Moreau strain, Ataulpho de Paiva Foundation, Rio de Janeiro, Brazil) administered intradermally in accordance with the National Leprosy Program recommendation. Approximately 4 months after BCG vaccination, the contact returned to the clinic due to the appearance of skin lesions on her face and right upper limb that had become visible 2 months after vaccination. The lesions resembled the polar tuberculoid form similar to those seen in children and commonly referred to as infantum nodular leprosy (INL). This individual presented with a positive lepromin skin test (22 mm with central necrosis) and a negative bacterial index. A clinical and histological diagnosis of TT leprosy was established (¹³) and immunostaining of tissue biopsy using anti-CD3 antibody showed a large lymphocytic infiltrate in which more than 50% of the CD3+ T cells were also CD4+ cells, and less than 10% were CD8+. Although the patient had regular clinical and immunological follow-up examinations every 3 months no treatment was given (as recommended for INL) and progressive involution of the lesions was noted. Sixteen months later, all lesions had disappeared, showing atrophic scars similar to those that occur in INL. After 1 year, even these scars were not detected.

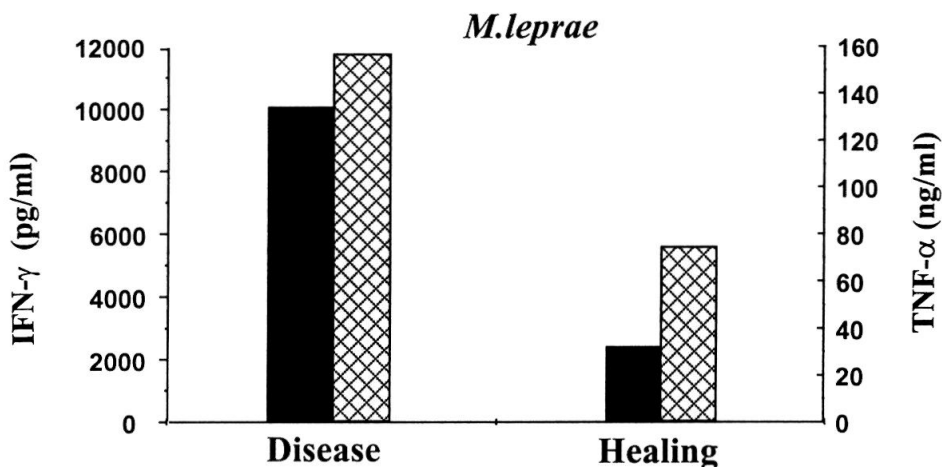


FIG. 1. *In vitro* immunological response of one TT leprosy patient during active disease (7 months of follow up) and after healing of leprosy (16 months of follow up). PBMC (1×10^6 cells) were stimulated with *M. leprae* for 5 days in 24-well culture plates. Cytokine levels in cell-free culture supernatants (24 hr culture for TNF- α ; 5 day culture for IFN- γ) were determined using specific ELISA (Pharmingen). ■ = IFN- γ ; ▨ = TNF- α .

The immunological response *in vitro* to whole *M. leprae* (20 μ g/ml) at the time of leprosy diagnosis showed a strong lymphoproliferative response (LTT, Δ cpm \pm S.D. = $10,414 \pm 1202$) and detectable IFN- γ levels (860 pg/ml) as measured by ELISA. Once the patient developed an INL-like form following BCG vaccine, the cytokine profile secreted by peripheral blood mononuclear cells (PBMC) obtained at different periods during the follow up was investigated. Upon stimulation with *M. leprae* or BCG (20 μ g/ml), very high levels of IFN- γ (10,090 and 801 pg/ml, respectively) and tumor necrosis factor-alpha (TNF- α) (157 and 6.2 ng/ml) were detected during active disease (a period corresponding to 7 months after BCG vaccination and 3 months after leprosy diagnosis). In contrast, IL-5 (*M. leprae* = 317 pg/ml; BCG = 125 pg/ml) and IL-10 levels (*M. leprae* = 478 pg/ml; BCG = 2016 pg/ml) were in the range observed for healthy leprosy household contacts (¹⁰). Moreover, IL-2 production following *M. leprae* stimulation was 3950 cpm as measured in the CTLL bioassay (⁶). Additional experiments were then carried out to determine whether mycobacteria-reactive Th1-like T cells were able to recognize a panel of proteins purified from the mycobacteria. The purification of mycobacterial antigens was as described by Hunter, *et al.* (⁸) and Pessolani, *et al.* (¹¹). A prominent response

(IFN- γ production >100 pg/ml) was detected to antigens shared by BCG and *M. leprae* (85 complex, GroEL/10 kDa and BFR/22 kDa) as well as to the *M. leprae* specific antigen, the MMP-I/35 kDa protein (not shown).

Assuming that the intense Th1-like response evoked by BCG vaccination could at least be partially responsible for the appearance of leprosy lesions, we monitored cytokine levels after complete regression of the patient's lesions (month 16 of follow up) as compared to the levels detected during active disease (month 7 of follow up). As expected (Fig. 1), a 53% and 84% decrease in the capacity of PBMC to secrete TNF- α and IFN- γ , respectively, in response to *M. leprae* was observed after healing.

To identify the subpopulation of T cells recognizing *M. leprae*, PBMC were depleted of CD4+ (PBMC-CD4) or CD8+ (PBMC-CD8) T cells by negative selection using magnetic beads (Bio Mag, Cambridge, Massachusetts, U.S.A.), according to the manufacturer's recommendation. Flow cytometry analysis and functional assays (LTT and cytokine measurement) were performed before and after T-cell depletion. As expected, the positive LTT seen in response to *M. leprae* (Δ cpm = 7727 ± 1777) in the PBMC population was reduced when CD4+ T cells (3385 ± 300), but not CD8 ($12,053 \pm 3223$), were depleted. In addition,

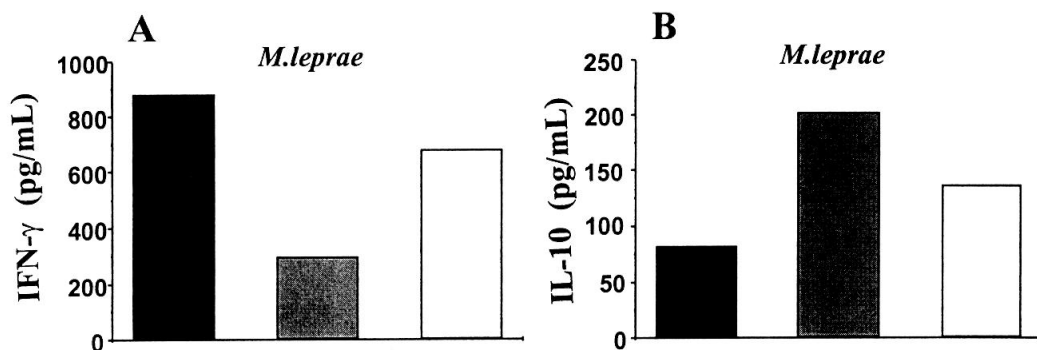


FIG. 2. PBMC depleted of the CD4+ T lymphocytes showed decreased IFN- γ secretion in response to *M. leprae* when compared with whole PBMC (19 months of the follow-up period). PBMC were depleted of the CD4+ or CD8+ T lymphocytes and stimulated with *M. leprae* (20 μ g/ml). Supernatants were harvested and levels of IFN- γ (A) and IL-10 (B) were determined by ELISA. ■ = PBMC; ▒ = -CD4; □ = -CD8.

reductions in IFN- γ production of 66% and 86% was reached in PBMC-CD4 cultures stimulated with *M. leprae* and BCG, respectively. Accordingly, no significant effect on IFN- γ was noted in response to *M. leprae* when CD8+ cells were depleted from the cultures (880 pg/ml in the PBMC versus 681 pg/ml in the PBMC-CD8 population). Analysis of IL-10 secretion in these cultures was also assessed. Interestingly, slight increases in IL-10 production were observed in response to *M. leprae* (Fig. 2) when both CD4 and CD8 T-cell populations were depleted.

It was interesting that, in our clinic, another three household contacts (one of them was the grandchild of the TT patient) have also developed tuberculoid leprosy after BCG vaccination (they also presented positive LTT and IFN- γ release in response to *M. leprae in vitro*), supporting the hypothesis, in accordance with others (1), that BCG vaccine could shift the immune response to the tuberculoid side of the leprosy spectrum. In order to determine the human histocompatibility leukocyte antigen (HLA) haplotype inherited by these two family members who developed leprosy following BCG vaccination, the family segregation of class I and class II molecules was analyzed using serological HLA typing by the microlymphocytotoxicity method (14). Our findings defined the TT patient as A2, A31, B35, B51, Cw4, -, DR16, -, DR51, -, DQ7 (for the grandchild it was A2, A23, B35, B62, Cw2, Cw4, DR7, DR13, DR52, DR53, DQ2, DQ6). Therefore, there can be two

different HLA associations contributing to the disease in this family, since they exhibited a split of HLA-DQ1 (DR16 and DQ6) and the HLA-DR13.

The ability of viable (vaccine) but not killed BCG to induce IFN- γ early in infection has been reported to play a critical role in the generation of protective antigen-specific T-cell response against BCG (5). Immunological follow up of household contacts who were BCG vaccinated demonstrated enhanced IFN- γ production and activation of an anti-inflammatory loop with increased IL-10 release and concomitant decrease in TNF- α production following stimulation of PBMC with whole *M. leprae in vitro* (10). Possibly, in this patient who presented with INL, the high levels of IFN- γ and TNF- α were related to the development of her leprosy lesions. The concomitant increase in IL-10 could have led to further downregulation of cytokine production *in vitro* which paralleled deactivation of the immune inflammatory response during self-healing of her leprosy.

In conclusion, the immunological follow up of a household contact who developed TT leprosy following BCG vaccination demonstrated a preferential Th1-type response during active disease. The enhanced levels of cytokines detected during the disease were reduced after self-healing, suggesting that at this moment an equilibrium between pro- and anti-inflammatory cytokines has been established with emergence of a Th0 response, as observed previously by Lima, *et al.* (10). Indeed, the major

T-cell subset involved in *M. leprae* responsiveness was shown to be the CD4+ T cells. It is likely that, in this patient, vaccination with BCG led to amplification of the inflammatory response resulting in an imbalance between pro- and anti-inflammatory cytokines, culminating with disease manifestation with hyper-responsiveness. Our findings and those of others (2) suggest that early and late responses to mycobacterial infection are influenced by previous exposure to vaccination and/or environmental mycobacteria in addition to genetic factors (7).

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Dapsone Drug Resistance in the MDT Era

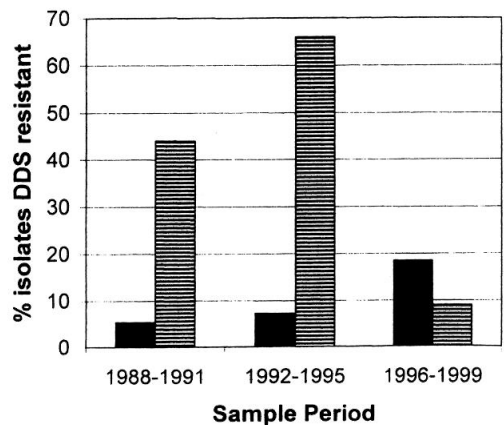
TO THE EDITOR:

The specter of drug-resistant leprosy has receded with the advent of multidrug therapy (MDT). However, there are few laboratories remaining which have the facility to measure drug resistance by the mouse foot pad method. Hence, it has been difficult to ascertain what the current state of drug resistance is and what the trends in resistance patterns after MDT implementation have been.

In Nepal, dapsone (DDS) monotherapy was introduced in 1956 and MDT in 1983, although the coverage of MDT only reached 95% more than a decade later. Since 1987, we have been testing all suitable patients for primary and secondary drug resistance using the mouse foot pad model⁽³⁾. We have analyzed the drug sensitivities of *Mycobacterium leprae* isolated from skin biopsies from 268 new and relapsed cases and from patients re-starting treatment (The Table). In the period 1988 to 1999, there was a decline in the prevalence of acquired dapsone resistance and a rise in the prevalence of primary dapsone resistance (The Figure). There was a complete absence of rifampin (RMP) resistance among our patients.

Primary dapsone resistance arises by the infection of new persons with resistant bacteria often shed by a person with acquired resistance to dapsone. During the late 1960s and 1970s, there were alarming reports of

increasing secondary and primary dapsone resistance at the end of the dapsone monotherapy era, which prompted the introduction of MDT^(2,4). From our data it appears that secondary resistance to dapsone does not develop under MDT. Hence, the decline in secondary resistance is due to the introduction of MDT in Nepal and to the cure of secondary dapsone-resistant cases with MDT. By contrast there was a significant increase in the prevalence of primary dapsone resistance in new, previously untreated cases (trend in proportion test $\chi = 4.98$, $p < 0.05$, The Table). All except one isolate was resistant to low-level dapsone



THE FIGURE. Prevalence of dapsone resistance in Nepal, 1988–1999. ■ = Primary resistance; ▨ = acquired resistance.