Effect of treatment on immune responsiveness in lepromatous leprosy patients

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Summary This study was performed in order to analyse whether the immune unresponsiveness to Mycobacterium leprae, largely seen in lepromatous patients, persisted after discharge from treatment. Lymphoproliferation and skin tests were performed using two mycobacterial antigens (M. leprae and BCG) in three groups of lepromatous patients grouped by treatment status. Forty-seven per cent of the lepromatous patients tested acquired reactivity to M. leprae after long-term treatment.

Introduction

Lepromatous leprosy patients (LL or BL forms) display a selective immunological unresponsiveness to Mycobacterium leprae antigen with the absence of delayed-type-hypersensitivity, T-cell proliferation, and deficiency in the production of growth factors such as IL-2. These patients also fail to produce interferon-gamma (IFN-γ) in response to M. leprae. Active suppression by macrophages and/or T cells may explain their inability to respond to leprosy bacilli. Lepromatous patients carry a high load of bacilli which may play a role in vivo in the induction of immune tolerance. Cellular anergy observed in lepromatous patients appears to be M. leprae specific since the immune response against other antigens is largely normal.

The effect of treatment on the recovery from the immunological anergy in lepromatous patients is a controversial subject. Findings from a number of studies suggest that an unresponsiveness to M. leprae seen in lepromatous patients is long-lasting and unrelated to the bacterial load. However, some studies have revealed different immunological reactivity to mitogens and mycobacterial antigens when cellular immune responses of short-term treated patients were compared with untreated patients.

To determine the effect of long-term treatment on the immune status of patients, we

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have assessed the cellular immune responses of 64 lepromatous patients to *M. leprae* and to BCG.

**Materials and methods**

**Patients**

Sixty-four multibacillary leprosy patients who attended the Outpatient Unit of the Oswaldo Cruz Foundation, Rio de Janeiro, Brazil, were included in this study. Forty-one patients were diagnosed as borderline lepromatous (BL) and twenty-three were classified as polar lepromatous (LL) according to Ridley–Jopling classification. The patients were grouped by length of treatment. Twenty-four patients were recently diagnosed and had received no treatment (NT) at the time of the study. Twenty-five patients were on multidrug therapy, ranging from 2 to 15 months (on treatment, OT). Fifteen patients had received monotherapy with dapsone from between 10 to 30 years (mean 16·6 years) and had terminated therapy from 1 to 6 years prior to participation in this study. Patients in this group, designated AT (after treatment) were without lesions and had negative lymph smears for acid-fast bacilli (AFB). None of the patients included in this study presented episodes of reaction during the study.

**Lepromin skin test**

Armadillo-derived lepromin (NHDC, Carville, USA, $3-4 \times 10^7$ bacilli/ml) was injected intradermally in the forearm and the reaction was measured 3 to 4 weeks after the injection (late lepromin reaction). Induration $\geq 3$ mm was considered positive.

**Lymphoproliferation assay**

Heparinized blood was collected under sterile conditions from the patients and mononuclear leukocytes (PBL) were isolated by Ficoll–Hypaque gradient centrifugation. The cells were resuspended in RPMI 1640 (Gibco Lab.) supplemented with 10% human AB serum, 100 U/ml penicillin and 100 $\mu$g/ml streptomycin and 2 mM L-glutamine (complete medium). All proliferation assays were performed in microtitre wells in a final volume of 0·2 ml complete medium. Stimulation with antigen was carried out for 6 days at 37°C in a 5% CO₂ atmosphere. For these experiments, $2 \times 10^5$ PBL were incubated with 20 $\mu$g/ml *M. leprae*, or 25 $\mu$g/ml BCG in triplicate. One $\mu$Ci per well of [³H]thymidine (Amersham Co., specific activity 6·7 Ci/mM) was added 18 h before harvesting cells for measurement of radiolabelled thymidine incorporated into newly synthesized DNA. The results are expressed as stimulation index (SI) derived as the ratio of mean cpm cultures with antigen to the cpm of cultures without antigen. Proliferation to the antigen was considered positive for $SI \geq 3·0$.

**Antigens**

*M. leprae* was kindly provided by Dr R. J. W. Rees (IMMLEP Bank, Mill Hill, England) and BCG was obtained from the Ataulfo de Paiva Foundation, Rio de Janeiro, Brazil.
STATISTICAL ANALYSIS

For comparison of the cellular immune response to *M. leprae* and BCG among the groups, Student’s *t*-test and the Mann–Whitney test were used.

**Results**

**LEPROMIN TEST**

As expected, all the patients in NT and OT groups showed a negative lepromin skin test. In AT group, four patients developed a skin-test reaction; however, no correlation with the duration of treatment was noted (Table 1). Before the onset of treatment all patients in the AT group had negative skin tests (data not shown).

**LYMPHOPROLIFERATION ASSAY**

The number of *M. leprae* nonresponders was significantly lower in the AT group in comparison to that of the NT and OT patients (*p* < 0.05). Of the AT patients 53.4% were unresponsive to *M. leprae* (SI < 3.0) (Figure 1). In contrast, 95.8% and 92% of the NT and OT patients, respectively, failed to respond to leprosy bacilli. There was no difference between the response of LL and BL patients in any of the groups studied. However, a significant difference was found between the mean SI of AT group as compared to the NT

<table>
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<tr>
<th>Name</th>
<th>Histopathology classification</th>
<th>Lepromin test†</th>
<th>In vitro test to <em>M. leprae</em> antigen</th>
<th>Time of treatment until became negative to bacilloscopy (years)</th>
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* Presence of frequent episodes of erythema nodosum leprosum (ENL) during the time of treatment.
† All patients had a negative lepromin skin test at the beginning of treatment.
‡ Relapse of the disease with leprosy lesion compatible to the indeterminate form with AFB+ in the skin biopsy.
Figure 1. Relationship between treatment and response to *Mycobacterium leprae*. PBL from patients after treatment (AT) (n = 15), on treatment (OT) (n = 25), and untreated (NT) (n = 25) were stimulated with *M. leprae* or BCG. Proliferation to antigen was considered negative for SI < 3·0.

Figure 2. Proliferative responses of PBL from patients with lepromatous leprosy. Each point represents the SI of PBL from each patient and the bars represent the mean SI in each group, as described in the legend of Figure 1. A SI ≥ 3 was considered a positive response. There was a significant difference between the response to *Mycobacterium leprae* (P < 0·05) and BCG (P < 0·02), when the AT group was compared to NT and OT groups (Student’s t-test).

and OT patients (p < 0·05). The AT group mean SI was 4·2 (ranging from 0·9 to 18·8) in comparison to mean SI of 1·5 (ranging from 0·5 to 3·0) in the NT group and 1·04 (ranging from 0·2 to 4·0) among the OT group (Figure 2). To determine whether the duration of treatment was correlated to the *M. leprae* response in the AT patients, we divided this group into responders and nonresponders. Among the 8 (53·3%) responder patients, the mean treatment time was 16·2 ± 4·5 years, while in the group that remained unresponsive, the mean duration of treatment was 17·0 ± 5·0 years. No statistical difference was observed between the nonresponder and responder patients with regard to the duration of treatment before their lymph smear became AFB negative (8·7 ± 5·3 years for the responder patients vs. 8·3 ± 5·2 years for the nonresponders).
During the course of these studies, two *M. leprae* responsive patients from the AT group developed lesions clinically and histologically compatible with indeterminate leprosy, and rare AFB was seen in the skin biopsies (Table 1). With regard to reactional states, it is important to note that 7 patients (46.6%) of the AT group had erythema nodosum leprosum during their course of treatment. All but one showed a positive response to *M. leprae* in this study.

Stimulation with BCG, similarly, evoked a higher response in PBL from patients in the AT group as compared to the OT and NT groups ($p < 0.02$). As shown in Figure 2, the mean SI for AT patients was 7.52 compared to 3.44 and 4.20 in the OT and NT patients. All patients showing a positive response to *M. leprae* (1 patient in the NT group, 2 patients in the OT group, and 7 patients in the AT group) were also responsive to BCG.

**Discussion**

The present study supports previous findings concerning the lack of cellular immune response to *M. leprae* in LL and BL patients. While the majority (80.7%) of the patients included in this study did not respond to *M. leprae* (SI < 3.0%), only 30% were unresponsive to BCG. However, when patients from the whole spectrum of leprosy were compared to household contacts, a good correlation was found between the response to *M. leprae* and BCG.

When lepromatous patients were grouped by their treatment status, the percentage of *M. leprae* nonresponders was significantly lower among the long-term treated patients (AT) compared to untreated, newly diagnosed patients (NT) and the short-term treated (OT) patients. Likewise, the number of BCG responsive patients also increased after treatment. The number of responder patients was higher, and an intensified response was observed to both *M. leprae* and BCG as evaluated by the mean SI. This is another indication that the continuous presence of mycobacteria could contribute to the depression of the host's cellular immunity.

The improved immune response to BCG demonstrated that the unresponsiveness in lepromatous patients is not restricted to *M. leprae*. Reitan et al. have, similarly, observed that PPD evokes a stronger reaction in PBL from treated patients as compared to the untreated leprosy patients. An improved response to mitogens in treated patients has also been reported. The long-lasting unresponsiveness seen in almost half of the long-term treated patients might support the hypothesis which attributes the absence of responsiveness in LL patients to genetic factors, absence of *M. leprae*-reactive T cells from the circulation or the presence of suppressor mechanisms. However, reversion of the unresponsiveness of lepromatous patients has been documented in many reports under different clinical and experimental conditions.

The improved reactivity observed after chemotherapy suggests that the unresponsiveness in lepromatous patients might not be long lasting and unchangeable in all cases. The inability to kill and clear bacteria during the early phase of infection could result in a high antigenic load which may in turn induce a tolerant state. Recent studies have demonstrated that immune tolerance may develop in the presence of a high concentration of antigens and this state may be reversed after decreasing the antigenic load.

The fact that many of the *M. leprae* patients in the AT group had previously presented episodes of ENL during the course of treatment raises the hypothesis that *M. leprae-*
reactive T cells had emerged during the reactional stages.\textsuperscript{24} Waldorf \textit{et al.}\textsuperscript{25} have reported similar findings using skin tests to assess immune responses in leprosy patients. Lepromatous patients with ENL showed higher positivity to DNBCB sensitization as compared to patients without ENL.

Taken together, findings from the present investigation support the hypothesis that reduction in \textit{M. leprae} post-therapy may contribute to the reversal of unresponsiveness in some lepromatous patients.

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