**In vitro Lymphocyte Stimulation in Patients with Lepromatous and Borderline Tuberculoid Leprosy. The Effect of Dapsone Treatment on the Response to *Mycobacterium leprae* Antigens, Tuberculin Purified Protein Derivative and Non-mycobacterial Stimulants**

Liv Jorun Reitan, Otto Closs, and Ayele Belehu

It is well established that patients with lepromatous leprosy (LL) are deficient in their cellular immune response to antigens from *Mycobacterium leprae* \(^{(1,20,30)}\). Many efforts have been made to clarify the mechanisms behind this defect \(^{(5,11,12,17,18)}\), one of the crucial questions being whether the defect is specific for *M. leprae* or generalized. Godal, *et al.* \(^{(12)}\) found that the lymphocytes of three LL patients who had been on antileprosy treatment with dapsone (DDS) for more than ten years still failed to respond to *M. leprae* antigen *in vitro* while responding strongly to BCG and tuberculin purified protein derivative (PPD). They concluded that the defect in LL is *M. leprae*-specific, long-lasting, and not merely the result of a large bacillary load. Nath, *et al.* \(^{(21)}\) found that the number of circulating T lymphocytes and their *in vitro* response to mitogens were depressed in untreated LL patients. After treatment with DDS the number of T cells and their response to mitogens became normal, whereas the failure to respond to *M. leprae* remained.

We have recently shown that lymphocytes from untreated LL patients failed to respond to an isolated cell wall fraction of *M. leprae* which induced strong lymphocyte responses in patients with tuberculoid (TT) and borderline tuberculoid leprosy (BT) and in healthy contacts of leprosy patients \(^{(7)}\). In the same study it was observed that the median response to PPD was also significantly depressed in untreated LL patients, although not to the same extent as the response to *M. leprae* antigens. This depression of PPD responses has been noted by many workers but has been either disregarded \(^{(1)}\) or taken as a generalized impairment of cell-mediated immunity (CMI) \(^{(13)}\). This could indicate either that the defect in CMI in LL is not restricted to *M. leprae* or that, although the defect is restricted to *M. leprae*, the response to other mycobacteria may become affected because they contain antigens that cross-react with *M. leprae*.

These findings called for further studies comparing the *in vitro* lymphocyte responses of leprosy patients to tuberculin PPD with their responses to both *M. leprae* and non-mycobacterial antigens. Furthermore, the effect of long-term DDS treatment on the immune response of leprosy patients to PPD needed further clarification.

In the present study the *in vitro* lymphocyte responses of groups of DDS treated and untreated leprosy patients and healthy contacts of leprosy patients are compared using *M. leprae* antigens, tuberculin PPD, four non-mycobacterial antigens and two non-specific mitogens as stimulants.
TABLE I. Basic information on the leprosy patients and the healthy contacts of leprosy patients.

<table>
<thead>
<tr>
<th>Group of individuals</th>
<th>Duration of infection or contact with leprosy (yr)*</th>
<th>Diagnosis</th>
<th>Sex</th>
<th>Age (yr)*</th>
<th>Duration of treatment (yr)</th>
<th>Bacteriology</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Clin.</td>
<td>F:M</td>
<td></td>
<td></td>
<td>BI</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Hist.</td>
<td></td>
<td></td>
<td></td>
<td>MI (%)</td>
</tr>
<tr>
<td>Untreated BT (BT₁₃)</td>
<td>10 ½</td>
<td>1 BT/TT</td>
<td>7</td>
<td>BT</td>
<td>25</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>(½₁₀⁻¹₀)</td>
<td>9 BT</td>
<td>3</td>
<td>ID</td>
<td>(13⁻⁻₄₅)</td>
<td></td>
</tr>
<tr>
<td>Treated BT (BT₇)</td>
<td>10 6½</td>
<td>2 TT</td>
<td>2</td>
<td>BT</td>
<td>28</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>(⁴₅½⁻¹₀)</td>
<td>1 TT/ΒΤ</td>
<td>8</td>
<td>ND</td>
<td>(19⁻⁻₄₅)</td>
<td>(4⁻⁻₉)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>7 BT</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Untreated LL (LL₁₇)</td>
<td>17 2</td>
<td>2 BL</td>
<td>6</td>
<td>BL</td>
<td>25</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>(½₁⁻⁻₀)</td>
<td>6 LL₁₇</td>
<td>5</td>
<td>LL₁₇</td>
<td>(14⁻⁻₅₁)</td>
<td>3.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>9 LL</td>
<td>6</td>
<td>LL</td>
<td></td>
<td>7.2</td>
</tr>
<tr>
<td>Treated LL (LL₇)</td>
<td>11 14</td>
<td>1 BL</td>
<td>11</td>
<td>ND</td>
<td>33</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>(⁹⁻⁻₂₅)</td>
<td>2 BL/LL</td>
<td></td>
<td></td>
<td>(22⁻⁻₆₅)</td>
<td>(2.⁵⁻⁻₁₄)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>8 LL</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Healthy contacts (HC)</td>
<td>15 2</td>
<td>6:9</td>
<td></td>
<td></td>
<td>26</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(⁴⁻⁻₁₀)</td>
<td></td>
<td></td>
<td></td>
<td>(1₈⁻⁻₃₆)</td>
<td></td>
</tr>
</tbody>
</table>

* Refer to median values; the range is given in brackets.

Abbreviations are used according to the classification of Ridley and Jopling (25) with the following additions: LL₁₇ = subpolar lepromatous leprosy, ID = indeterminate leprosy, ND = not done.

MATERIALS AND METHODS

Patients and controls. All the patients included in this study attended the All Africa Leprosy and Rehabilitation Training Centre (ALERT) in Addis Ababa. Using the Ridley-Jopling scale (25) the untreated patients and two patients in the treated BT group were classified both clinically and histologically, while the remaining patients were classified only clinically before the treatment started. Clinical classification, however, was done by experienced physicians. The treated patients had all been on dapsone (DDS) (100 mg/day) for a long period, had a negative skin smear and showed no sign of any reaction when tested. More detailed information about the number of patients in each group, diagnosis, duration of infection, Bacteriologic and Morphological Indices, duration of treatment, sex and age for all the groups of patients is given in Table I. Results from seven of the untreated lepromatous patients and all in the control group were included in a previous study (7). The control group consisted of 15 healthy contacts of leprosy patients. They were all staff members of ALERT or the Armauer Hansen Research Institute (AHRI) and were of a higher socioeconomic group than the patients. The duration of close contact with the patients and the sex and age of this group are given in Table I.

Antigens. The following seven antigens were used in the lymphocyte stimulation test (LST): a) A fractionated preparation of *M. leprae* of armadillo origin, called MLW 1, which has been shown to contain mainly *M. leprae* antigen 7 by crossed immunoelectrophoresis (7). b) Whole, washed bacilli of human origin (7). c) Tuberculin purified protein derivative (PPD), Batch RT23 obtained from Statens Seruminstitutt, Copenhagen, Denmark. d) *Leishmania aethiopica* parasites (7) were isolated from an Ethiopian patient with oriental sore and were grown on 2.3% Nutritional agar/10% sheep blood-medium overlayed with RPMI 1640 (Flow Laboratories, Ayshire, Scotland) containing 200 U/ml of penicillin and 200 µg/ml streptomycin. The parasites were boiled for 10 min and washed in PBS before they were used in the LST. e) Amoeba antigen, consisting of freeze-dried organisms of washed *Entamoeba histolytica*, was obtained from Wellcome Reagents Limited, Beckenham, England. f) Parotitis virus antigen was obtained from Behringwerke AG, Marburg, West Germany, and was a concentrated, purified, inactivated and preservative free suspension of mumps virus. g)
Candida antigen was an "allergenic extract" of *Monilia albicans*, lot L 54538015 from Hollister-Stier Laboratories, Spokane, Washington, U.S.A.

**Mitogens.** Phytohemagglutinin (PHA) was obtained from Wellcome Reagents Limited and concanavalin A (Con-A) from Sigma Chemical Company, St. Louis, Missouri, U.S.A.

**Cultures of lymphocytes.** Mononuclear cells were isolated from defibrinated blood as described for the patients’ cells by Closs, et al. (7). Briefly, the cells, 10% well, were cultured in triplicate in round-bottomed microtitre trays (ISMRC 90TC, Linbro Chemical Co., New Haven, Connecticut, U.S.A.) in RPMI 1640 medium (Flow) containing 20% pooled human serum and supplemented with glutamine (2 mM) and antibiotics (100 U/ml penicillin and 100 µg/ml streptomycin). The antigen stimulated cells were cultured for six days and the mitogen stimulated cells for seven days in 5% CO₂ in humidified air before being harvested. Each culture received 1 µCi of (methyl-¹³H)-thymidine (spec. act. 2.0 Ci/mmole, Radiochemical Centre, Amersham, England) 18 hr before harvest. The cells were harvested with a multiple harvester (Skatron A/S, Norway), placed onto glass fiber filters and washed in distilled water. Thymidine incorporation was measured in a liquid scintillation counter (SL30, Intertechnique, France). The median counts per minute (cpm) of each triplicate were used and the degree of stimulation expressed as Acpm = cpm of stimulated triplicate–cpm of unstimulated control triplicate.

**Statistical methods.** Wilcoxon’s rank sum test (*) was used to compare groups of patients and p < 0.05 was set as the limit of statistical significance.

**RESULTS**

The effect of dapsone treatment on the *in vitro* lymphocyte response to *M. leprae* antigen, PPD, a few non-mycobacterial antigens and the non-specific mitogens PHA and Con-A was investigated by comparing groups of treated and untreated patients with the same clinical diagnosis. A group of healthy occupational contacts (HC) of leprosy patients was included as a reference group. For all patients and contacts a dose/response curve was made with two or more concentrations for each of the seven antigens used. The concentration giving the highest stimulation was used in the comparisons. An individual with a response of Δcpm ≥5000 was defined as a responder. The mean background level in unstimulated cultures was 5000 cpm, and 68% of the controls were below this value. Consequently, an individual was defined as a responder to a certain antigen when he showed a response which was at least two times the mean background level.

The lymphocyte responses to the *M. leprae* cell wall fraction MLW I and to whole, washed *M. leprae* bacilli of human origin are shown in Figure 1, A and B, respectively. The responses in the HC group did not differ significantly from those in the BT groups. In the group of untreated patients with borderline tuberculoid leprosy (BT<sub>uT</sub>) there were two who were non-responders to both *M. leprae* antigens. In the group of treated borderline tuberculoid patients (BT<sub>T</sub>) two were found to be non-responders to both antigens, and two additional were non-responders to whole *M. leprae* antigen. In the HC group one non-responder to whole *M. leprae* was found. In conclusion there were fewer non-responders to MLW I than to whole *M. leprae*. Looking at individual responses, they were all higher for MLW I than for whole *M. leprae* in the HC and BT groups, except for five patients in the BT<sub>uT</sub> group where the responses were the same to both antigens (data not shown). As expected, the responses to both antigens were markedly and significantly lower (p < 0.005) in patients with lepromatous leprosy both untreated (LL<sub>uT</sub>) and treated (LL<sub>T</sub>) than in BT<sub>uT</sub> and BT<sub>T</sub> patients. In the LL<sub>uT</sub> group there was one patient who was a responder to both antigens, while in the LL<sub>T</sub> group there were two responders to MLW I and three to whole *M. leprae*. Only one of these patients was a responder to both antigens. Thus, in most patients the failure to respond to *M. leprae* antigens in LST remained after long-term treatment and the medians for the LL<sub>T</sub> group were even found to be below those of the LL<sub>uT</sub> group.

Since the groups compared in the present study were not matched for sex and there was a high proportion of lepromatous males, we wanted to know if sex could have influenced the results. Therefore groups of three
males and three females were selected randomly from each group of patients and controls and pooled so that the responses of 15 males were compared with those of 14 females (LL\textsubscript{T}, contained only 2 females). In the comparisons for the M. leprae antigens, the responses of the lepromatous groups are not included since they do not give any meaningful results. The ratios of the median responses of the females to those of the males are shown in Table 2 and varied from 0.5 to 1.2 for the antigens and were 1.4 and 1.0 for Con-A and PHA, respectively. The differences were significant only for the MLW 1 antigen. The responses to MLW 1, PPD and PHA in the two selected sex groups are shown in Figure 2. The ratios between the responses for females and males for both MLW 1 and PPD were 0.5.

Figures 3 and 4 show the responses to two parasites occurring among Ethiopians, Leishmania aethiopica and Entamoeba histolytica, respectively. Both antigen preparations were shown to be relatively strong stimulators with median responses in the various groups between 26,100 and 46,500 \( \Delta\text{cpm} \) for the leishmanial antigen and 21,800 and 41,500 \( \Delta\text{cpm} \) for the amoeba antigen. There were 4 non-responders to the leishmanial antigen with 2 each in both the HC and the BT\textsubscript{T} groups and 2 non-responders to the amoeba antigen with 1 each in both the BT\textsubscript{T} and LL\textsubscript{T} groups. The BT\textsubscript{UT} group showed very strong responses to the leishmanial antigen, significantly higher than both the HC and the LL\textsubscript{UT} group. For the parotitis virus antigen (Fig. 5) the median responses varied from 11,200 \( \Delta\text{cpm} \) in the LL\textsubscript{UT} group to 19,200 \( \Delta\text{cpm} \) in the BT\textsubscript{UT} group. Non-responders were seen in all patient groups, and the number varied from 1 in the BT\textsubscript{UT} and LL\textsubscript{T} groups to 3 in the LL\textsubscript{UT} group. For the candida antigen (Fig. 6) the median responses varied from 3700 \( \Delta\text{cpm} \)
TABLE 2. Female/male ratios of the in vitro lymphocyte responses to various stimulants.\textsuperscript{a}

<table>
<thead>
<tr>
<th>Stimulant</th>
<th>Ratio Female/male</th>
</tr>
</thead>
<tbody>
<tr>
<td>MLW 1</td>
<td>0.5\textsuperscript{b}</td>
</tr>
<tr>
<td><em>M. leprae</em>, whole</td>
<td>0.5\textsuperscript{c}</td>
</tr>
<tr>
<td>PPD</td>
<td>0.5\textsuperscript{c}</td>
</tr>
<tr>
<td><em>L. aethiopica</em></td>
<td>0.9\textsuperscript{c}</td>
</tr>
<tr>
<td><em>E. histolytica</em></td>
<td>0.9\textsuperscript{c}</td>
</tr>
<tr>
<td>Mumps</td>
<td>1.2\textsuperscript{c}</td>
</tr>
<tr>
<td>Candida</td>
<td>0.5\textsuperscript{c}</td>
</tr>
<tr>
<td>Con-A</td>
<td>1.4\textsuperscript{c}</td>
</tr>
<tr>
<td>PHA</td>
<td>1.0\textsuperscript{c}</td>
</tr>
</tbody>
</table>

\textsuperscript{a} The groups are composed of three females and three males randomly selected from each category of patients/controls, except for the *M. leprae* stimulations where the lepromatous responses are not included.

\textsuperscript{b} *p* = 0.005, Wilcoxon's rank sum test, comparing the responses of females to those of males.

\textsuperscript{c} Not statistically significant.

in the *LL\textsubscript{UT}* group to 20,300 \textDelta cpm in the *LL\textsubscript{T}* group. Non-responders were seen in all groups except the *LL\textsubscript{UT}* group, and the number varied from 3 in the *BT\textsubscript{UT}* to 10 in the *LL\textsubscript{UT}* group.

For all four non-mycobacterial antigens there was a tendency to lower responses in the *LL\textsubscript{UT}* group, but in no instances were the responses in this group significantly different from the HC group. For the candida antigen the responses in the *LL\textsubscript{T}* group were significantly higher than in the *LL\textsubscript{UT}* group (*p* < 0.005). In fact, the responses to candida antigen in the *LL\textsubscript{T}* group were significantly stronger than in all other groups except the *BT\textsubscript{UT}*. Because the pattern of response to tuberculin PPD in the various groups was different from that of both the *M. leprae* antigens and the non-mycobacterial antigens, the data concerning PPD are presented in greater detail. Figure 7 shows the responses to 0.1, 1.0 and 10 \textmu g/ml of PPD. The responses in all groups varied within a wide range although the range was somewhat narrower in the HC group than in the patient groups. There was a marked and significant depression (*p* < 0.005) in the responses to all three concentrations of PPD in the *LL\textsubscript{UT}* group compared with the HC group. Nevertheless, the three highest responses to 1.0 \textmu g/ml in the *LL\textsubscript{UT}* group were higher than the highest response in the HC group with the same concentration of PPD. To facilitate a comparison of the responses to PPD with the responses to the antigens, the results shown in Figures 1 and 3 to 7 have been summarized in Table 3, where the median response of each group is expressed in percent of the median response of the HC group. Table 3 clearly shows that the depression in the response to PPD in the *LL\textsubscript{UT}* was not as marked as for the *M. leprae* antigens but clearly was more marked than the slight depression which was seen in the responses to the non-mycobacterial antigens. For the leishmanial antigen and the mitogens Con-A and PHA, the responses in the *LL\textsubscript{UT}* group were even slightly higher than those in the HC group. At the concentration of 1.0 \textmu g/ml of PPD the
Fig. 3. In vitro lymphocyte stimulation to L. archa"opica (10^6 parasites/ml). For further explanation see legend to Figure 1.

responses in the LL_T group were markedly and significantly higher than in the LL_UT group. There were 5 non-responders in the LL_UT group versus 1 in the LL_T group. The difference between the responses in the LL_T and the LL_UT groups was even greater at the lower dose of 0.1 µg/ml. But at all doses the responses were higher in the LL_T group than in the LL_UT group. In fact, the responses in the LL_T group were not significantly different from the responses in the HC group. Notably, the median responses to PPD (1.0 µg/ml) were depressed in the LL_T group versus 1 in the HC group. Notably, the median responses to PPD (1.0 µg/ml) were depressed in the LL_T group versus 1 in the HC group. Thus the pattern of response to PPD was different from that to the M. lepra" antigens, the four non-mycobacterial antigens and the non-specific mitogens with respect to depression of responses in the LL_UT.
Fig. 7. *In vitro* lymphocyte stimulation to tuberculin PPD at three different concentrations: open bars (0.1 µg/ml), hatched bars (1.0 µg/ml) and double hatched bars (10 µg/ml). For further explanation see legend to Figure 1.

In view of the unexpectedly high number of low-responders to MLW 1 and PPD in the BT groups, we wanted to see whether this could be explained by assuming that these patients were general low-responders in the LST. According to Figures 3 to 6 both high- and low-responders were seen for all four non-mycobacterial antigens.

| Table 3. Median responses in the lymphocyte stimulation test in the various groups of patients* presented as percent of the median response in the group of healthy contacts (HC) for each antigen/mitogen separately. |
|---|---|---|---|---|---|
| Stimulant | HC | BT<sub>UT</sub> | BT<sub>T</sub> | LL<sub>UT</sub> | LL<sub>T</sub> |
| (Δcpm) = 100% | (%) | (%) | (%) | (%) |
| MLW 1 | 23,500 | 109 | 122 | 5<sup>b</sup> | 5<sup>b</sup> |
| *M. leprae*, whole | 10,200 | 180 | 90 | 0<sup>b</sup> | 0<sup>b</sup> |
| PPD | 80,700 | 30 | 25<sup>b</sup> | 9<sup>b</sup> | 85 |
| *L. aethiopica* | 26,100 | 175<sup>b</sup> | 113 | 106 | 117 |
| *E. histolytica* | 34,600 | 117 | 88 | 65 | 84 |
| Mumps | 13,700 | 146 | 127 | 85 | 123 |
| Candida | 9,100 | 100 | 60 | 40 | 205<sup>b</sup> |
| Con-A | 14,900 | 220 | 170 | 113 | 187 |
| PHA | 75,800 | 131 | 81 | 117 | 119 |

*For abbreviations of patient groups, see legend to Figure 1.

<sup>b</sup> Values that differ significantly from the controls (HC).
Non-mycobacterial antigens

FIG. 8. Correlation between responses to the four non-mycobacterial antigens (L. actinopica, E. histolytica, parotitis virus and candida antigens) and the MLW 1 antigen in: a) a combined BT group (N = 20) consisting of BT_{TT} and BT_{T}, and b) the HC group (N = 15). The responses are expressed as the rank of the response to MLW 1 and the average score of the ranks of the responses to the non-mycobacterial antigens. Open circles (○) indicate results of patients with a response to MLW 1 less than 10,000 Δcpm.

Since the data were partly not normally distributed, a non-parametric method was applied for analysis. To see whether the same individuals were high-responders or low-responders to all four antigens, they were ranked according to their LST response for each antigen and numbered starting with 1 for the lowest response. This was done for two groups, the HC group and a combined BT group consisting of both the BT_{TT} and the BT_{T} patients. The same individual tended to be either a high- or a low-responder to all four antigens (data not shown). An average score for all four non-mycobacterial antigens was therefore calculated for each individual.

The individuals were then ranked in a similar manner for their response to the MLW 1 antigen. Figure 8A shows the relationship between the average scores for the four non-mycobacterial antigens and the ranks for MLW 1 in the combined BT group. Nearly all high-responders to the non-mycobacterial antigens were high-responders to MLW 1. Figure 1A shows that in the BT groups there were altogether 6 patients with responses to MLW 1 lower than 10,000 Δcpm. In Figure 8A these patients have the six lowest ranks (open circles) for MLW 1, but 4 of them have high scores for the four non-mycobacterial antigens. In contrast, in the HC group (Fig. 8B) there seemed to be a very close correlation between the ranks for MLW 1 and the scores for the four non-mycobacterial antigens. These findings do not support the possibility that a weak response to MLW 1 in patients with BT leprosy is due to technical or other factors affecting the general responsiveness of patients in the LST.

Similar analysis of the PPD data did not allow any definite conclusions.

DISCUSSION

The lack of cell-mediated immune response to M. leprae antigens in LL may be mediated by two mechanisms: lack of M. leprae-reactive circulating T lymphocytes (11) or antigen-induced suppressor mechanisms (17). Godal, et al. (12) showed that three LL patients who had been treated with dapsone for more than ten years still failed to respond to M. leprae in the lymphocyte transformation test; whereas they responded normally to BCG. Their failure to detect a response to M. leprae could have been
due to their assay being rather insensitive; about 30% of the tuberculoid group were negative.

The antigen MLW 1 has previously been shown to be a particularly potent stimulator in the LST (7). This antigen would therefore seem to be a more sensitive indicator than the integral M. leprae preparations used by previous workers to see whether LL patients regain some degree of responsiveness to a specific antigen after long-term treatment. Our results showed, however, that the failure to respond to the stronger stimulant MLW 1 also remained after long-term treatment. This confirms the findings of Godal, et al. and supports the view that the specific defect in CMI in LL is permanent.

Godal, et al. presumed that no antigen was present after ten years of treatment because no acid-fast bacilli were found in the lesions and concluded that the unresponsiveness of LL patients to M. leprae was caused by a permanent lack of circulating M. leprae-reactive T lymphocytes (11). Subsequently it has been shown that even after long-term treatment, when the body is cleared of viable bacilli, large amounts of fragmented cell wall material of M. leprae are still present in the tissues and may be detected by a prolonged Gomori's-methamine-silver staining technique (10). Since MLW 1 is a cell-wall derived antigen (7), it is conceivable that the observed lack of LST response to this antigen could be due to a continuous antigen driven suppressor mechanism. There is evidence that both T lymphocytes and macrophages (14, 18, 23) exert a functional suppression on the lymphoproliferative responses in leprosy. Recently, a M. leprae-reactive suppressor subpopulation of T lymphocytes has been identified in LL patients (19). Whether the presence of such cells in the circulation will explain the complete and consistent lack of specific cell-mediated immune response in these patients remains to be clarified.

The specificity of the defect in CMI in LL has been widely discussed (9-26). Whereas the strong depression of CMI to M. leprae in LL is firmly established, there is contradictory evidence as to whether there is also a non-specific depression of CMI in these patients. In order to elucidate this problem we included four categories of stimulants in the present study: a) M. leprae antigens, b) non-mycobacterial antigens, c) non-specific mitogens, and d) tuberculin PPD.

A slight but statistically insignificant depression of the LST responses to the four non-mycobacterial antigens was seen in the untreated LL group; whereas the responses to the non-specific mitogens Con-A and PHA were essentially the same as in the HC group which was used as a reference. These findings indicate that if there is a non-specific component in the depression of CMI in untreated LL patients, it is relatively weak. However, it should be noted that the HC group and the patients are not strictly comparable because of the higher socioeconomic status of the HC group. Moreover, there was a clear tendency to higher LST responses to non-mycobacterial antigens in treated than in untreated LL patients, and for candida antigen the difference was statistically significant. This further strengthens the impression that a moderate non-specific suppression of lymphoproliferative responses is present in untreated LL patients. That the suppression was reversed by long-term treatment accords with the previous findings of others (29).

The pattern of LST responses to tuberculin PPD was different from that of the other three types of stimulants and needs to be commented on separately. Previous studies have concluded that while LL patients lack cell-mediated immune responses to M. leprae they respond normally to BCG or tuberculin PPD (11). Although some workers have found little cross-sensitization between M. leprae and BCG (19), others have found evidence to the contrary. Mehra and Bloom (19) reported a strong reactivity to tuberculin PPD in guinea pigs immunized with M. leprae alone, and BCG vaccination has been found to induce LST responsiveness to M. leprae in humans (9).

The present study confirms our earlier observation that the LST response to PPD is depressed in untreated patients with LL (7). It also shows quite clearly that the median response to PPD was more depressed than the median responses to the non-mycobacterial antigens (Table 3). This further supports our hypothesis that the basis of this depression is an antigen specific mechanism; patients with LL lose their ability to respond to those determinants of PPD which
are shared with \textit{M. leprae}. Consequently, the proportion of LL patients with a low response to PPD becomes an indicator of the antigenic relationship between \textit{M. leprae} on the one hand and BCG and \textit{M. tuberculosis} on the other. Moreover, our findings are compatible with the view that there is a similar degree of specificity in the induction and suppression of immunity to mycobacterial antigens.

Whereas the response of long-term treated LL patients remained negative to \textit{M. leprae} antigens, we found a significant increase in their LST response to PPD, compared with untreated LL patients. This could indicate either that the mechanisms responsible for the depression of the PPD responses are no longer operating after long-term treatment or that the lack of response to shared determinants has been compensated for by sensitization to additional determinants of PPD. The latter possibility is supported by the findings of Smelt, \textit{et al.} (28) who showed that in long-term treated LL patients who were skin test negative, a positive reaction to PPD could be detected after immunization with BCG or BCG plus \textit{M. leprae}. When immunized with \textit{M. leprae} alone, no delayed type hypersensitivity reaction to either \textit{M. leprae} or PPD developed. Thus LL patients may become sensitized to PPD despite their failure to respond to \textit{M. leprae}.

Menzel, \textit{et al.} (28) showed that males in the households of active lepromatous leprosy patients had significantly stronger responses to \textit{M. leprae} antigens than females in the LST, while the PHA responses were not significantly different in males and females. Our results are in agreement with their findings. Since the groups of patients and contacts were not matched for sex in our study and since females were found to be lower responders than males to some of the antigens, one could fear that this would interfere with our results. For example, by comparing the LL\textsubscript{UT} and the LL\textsubscript{T} groups an increase in the response in the LL\textsubscript{T} group was expected because there were fewer females in that group. This could, of course, partly explain the relatively strong response to PPD in the LL\textsubscript{T} group. But it cannot be the only reason since the responses in the LL\textsubscript{T} group, although no longer statistically significant, were also higher than in the LL\textsubscript{UT} group when only males were compared. The number of females was the same in both the HC and the LL\textsubscript{UT} groups, while there were two more males in the LL\textsubscript{UT} group. Therefore the marked depression in the PPD responses in the LL\textsubscript{UT} group is not a result of an excess of females in that group. The difference in the female/male ratio could, however, explain our finding of lower responses to MLW 1 in the BT\textsubscript{UT} than in the BT\textsubscript{T} group.

The median response in the BT\textsubscript{UT} group was higher than in the BT\textsubscript{T} group for all the antigens except MLW 1. The enhancement was particularly evident in the response to leishmanial antigen. In this connection it may be relevant to point out the antigenic relationship which has been reported between species of the genera \textit{Mycobacterium} and \textit{Leishmania} (24,29). It is reasonable to assume that the lower responses in the treated BT group are an effect of dapsone. Depression of PHA induced lymphocyte stimulation of peripheral blood lymphocytes has been observed in 15 healthy volunteers after administration of 100 mg DDS daily for seven days (27). On the other hand, Anderson, \textit{et al.} (1) have recently found that dapsone treatment in newly diagnosed LL patients was associated with a progressive recovery of granulocyte motility and increased lymphocyte responsiveness to mitogens.

In both BT groups a surprisingly high number of low-responders to both MLW 1 and PPD were seen. Three possible explanations are: a) that these individuals were generally low-responders in the LST; b) that they were insufficiently sensitized to specific antigens; or c) that they had circulating suppressor cells which were triggered by specific antigen in the test situation. Nath, \textit{et al.} (22) have reported a suppression of Con-A responses by \textit{M. leprae} antigen in patients with tuberculoid leprosy. We observed in the HC group that a low response to MLW 1 was followed by a low response to the four non-mycobacterial antigens. In BT, on the other hand, a low response to MLW 1 was also seen in patients who were high responders to non-mycobacterial antigens. Thus they were not low responders in general. It also seems reasonable to rule out the possibility that these patients responded weakly because of insufficient ex-
posure to antigen. Low responses to MLW I in BT patients who responded strongly to non-mycobacterial antigens therefore seem to support the view that a suppressor mechanism may become activated in some patients with BT leprosy in response to in vitro stimulation with M. leprae antigen. Further evidence is needed to clarify this point, however. Since such patients were found among both treated and untreated patients, we have no indication that a possible suppressor mechanism is related to the activity of the disease.

SUMMARY

Lymphocytes from peripheral blood were isolated from leprosy patients and healthy contacts (HC) of leprosy patients and stimulated in vitro with: Mycobacterium leprae and a M. leprae cell wall antigen, MLW I; tuberculin purified protein derivative (PPD); antigens prepared from Candida albicans, Entamoeba histolytica, Leishmania aethiopica, and parotitis virus; the non-specific mitogens phytohemagglutinin (PHA) and concanavalin A (Con-A). Lymphocytes from patients with untreated lepromatous leprosy failed to respond to the M. leprae antigens, and the median response to PPD was also significantly (p < 0.005) lower than in the HC group. They responded almost as well as the other groups to non-mycobacterial antigens, PHA, and Con-A. In LL patients who had been treated with dapsone for several (median 10) years, the failure to respond to M. leprae antigens remained, but the depression of the PPD response and the slight non-specific depression of the lymphocyte stimulation test (LST) responsiveness had been reversed.

Our results confirm that the major defect in the cell-mediated immune response of LL patients is M. leprae-specific and permanent. The possibility that the defect may be due to a continuous, antigen-induced suppression of the immune response is discussed. That the defect also affected the response to PPD is important since it points to a clear antigenic relationship between M. leprae and BCG/M. tuberculosis. Evidence is presented suggesting that an antigen-induced suppressor mechanism may be operating in vitro with cells from patients with borderline tuberculoid leprosy.

RESUMEN

Se aislaron los linfocitos de la sangre periférica de pacientes con lepra y de contactos sanos (CS) de pacientes con lepra. Los linfocitos se estimularon in vitro con: Mycobacterium leprae y un antígeno de la paréкл celular del M. leprae (MLW I); el derivado protéico purificado de la tuberculina (PPD); antígenos preparados de Candida albicans, Entamoeba histolytica, Leishmania aethiopica, y virus de la parotiditis; los mitógenos no específicos fitohemaglutinina (PHA) y concanavalina A (Con-A). Los linfocitos de los pacientes con lepra lepromatosa sin tratamiento no respondieron a los antígenos del M. leprae y su respuesta al PPD también estuvo disminuida con respecto al grupo CS (p < 0.005). Sin embargo, los linfocitos de los pacientes que habían sido tratados con dapsona por varios años (mediana = 10) persistió la falta de respuesta a los antígenos del M. leprae pero reapareció la reactividad al PPD y desapareció la liger a depresión inespecífica en la prueba de estimulación de linfocitos.

Los resultados confirman que el principal defecto en la respuesta inmune celular de los pacientes LL es específico y permanente para el M. leprae. Se discute la posibilidad de que el defecto pueda deberse a la continua supresión de la respuesta inmune inducida por el antígeno. Es interesante el hecho de que el defecto también afectó la reactividad al PPD ya que esto señala una clara relación antigenica entre M. leprae y BCG/M. tuberculosis. Se presentan evidencias que sugieren que un mecanismo supresor inducido por el antígeno puede operar in vitro en las células de los pacientes con lepra tuberculoi de o intermedia.

RÉSUMÉ

Des lymphocytes du sang périphérique ont été recueillis chez des malades de la lep rae et chez des contacts sains de malades atteints de lèpre. Ces lymphocytes ont été stimulés in vitro avec l'une des préparations suivantes: M. leprae associé à un antigène de paroi cellulaire de M. leprae (MLW I); le dérivé protéique purifié de la tuberculine (PPD); des antitènes préparés à partir de Candida albicans, Entamoeba histolytica, Leishmania aethiopica, ou du virus de la parotidite; des mitogènes non spécifiques, à savoir la phytohémagglutinine (PHA) et la concanavaline A (Con-A). Des lymphocytes provenant de malades souffrant de lèpre lépromateuse non traitée n'ont pas répondu aux antigènes de M. leprae; leur réponse moyenne au PPD était également significativement plus faible que celle relevée dans le groupe témoin (p < 0.005). Ces lymphocytes répondaient presque aussi bien que ceux des autres groupes aux antigènes non mycobactériens, à la PHA, et à la Con-A. Chez les malades LL, qui avaient été traités par la dapsone pendant plusieurs années (en moyenne 10 ans), l'absence de réponse aux antigènes de M. leprae persistait, mais la diminution observée
dans la réponse au PPD et la diminution faible et non spécifique de la réponse à l’épreuve de stimulation lymphocytaire (LST) étaient inversées.

Ces résultats confirment que le défaut majeur qui se produit dans la réponse immunitaire à médiation cellulaire chez les malades LL, est non spécifique pour M. leprae, et permanent. La possibilité qu’un tel défaut soit dû à une suppression continue de la réponse immunitaire, due aux antigènes, est discutée. Le fait que ce défaut porte également sur la réponse au PPD est important, car ceci révèle une relation antigénique nette entre M. leprae et le BCG/M. tuberculosis. Les données présentées suggèrent que le mécanisme de suppression, provoquée par l’antigène, peut intervenir dans les cellules obtenues chez des malades atteints de leprore tuberculoid borderline.

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