Reduced Suppressor Cell Response to *Mycobacterium leprae* in Lepromatous Leprosy

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We have previously shown that concanavalin A (ConA) induction of suppressor cell activity is impaired in patients with lepromatous leprosy (LL). In this study, we demonstrated that the proportion of cells bearing the Leu8 antigen (associated with suppressor-inducer cells) is low in LL patients and tends to normalize during the erythema nodosum lepromus (ENL) episode. Antigen-induced suppressor cell function was evaluated by a two-stage assay. In the first stage, peripheral blood mononuclear cells (PBMC) were cultured for 5 days either in the presence of gamma-irradiated *Mycobacterium leprae* or in tissue culture medium as a control. In the second stage, mitomycin C-treated suppressor or control cells were added to phytohemagglutinin (PHA)-or ConA-stimulated autologous PBMC. The results indicate that the ability of *M. leprae* to induce suppressor activity was lower in LL patients than in patients with tuberculoid (TT) and intermediate clinical (BB, BL, BT) forms and *Mycobacterium bovis* BCG-immunized normal controls. In ENL patients, the percent suppression was between that of TT and normal individuals. *M. leprae*-induced suppression was more effective on ConA-than on PHA-triggered T-cell proliferation in all groups. In contrast, normal PBMC cultured for 5 days in RPMI 1640 medium (N-C) and cells from patients with leprosy (TT-C and LL-C) had effects of their own on PHA- or ConA-induced proliferation. LL-C depressed the response to ConA and enhanced PHA-induced proliferation of autologous cells. Conversely, TT-C reduced PHA-induced proliferation and increased the ConA response. Suppression of proliferation could not be overcome with exogenous interleukin-2 and was not related to the induction of the Tac antigen. The abilities of LL, TT, ENL, and normal cells to proliferate upon PHA or ConA stimulus were similar, indicating that the defect in the generation of in vitro suppression by *M. leprae* in LL patients occurred during the induction period (step 1 of assay).

Suppressor mechanisms are thought to play an important role in the susceptibility of patients with lepromatous leprosy (LL) to generalized *Mycobacterium leprae* infection (1, 2, 5, 25, 37). However, the specificity and selectivity of the suppressor responses induced by *M. leprae* antigens remain open to discussion. Restricted specificity in the induction of suppression by *M. leprae* may be related to the method of evaluation used.

Most in vitro assays consist of one-stage reactions in which peripheral blood mononuclear cells (PBMC) are simultaneously exposed to *M. leprae* antigens and to other proliferative signals: mitogens (2, 17), bacterial antigens (37), and alloantigens (23). Using this type of assay, Mehra et al. (16–18) reported that PBMC from LL and from borderline lepromatous (BL) patients but not from patients with the tuberculoid polar forms (TT, BT) or normal individuals could suppress concanavalin A (ConA) proliferation when cultured simultaneously with Dharmendra lepromin. These investigators have also tried to recover *M. leprae*-specific T-cell lines from the skin lesions of leprosy patients. T8 clones were obtained from both TT and LL patients, but only 33% of those expanded from LL skin lesions had suppressor activity in their system (20).

Recently, Kaplan et al. (14) have shown that *M. leprae* antigens could suppress the proliferative responses of PBMC from LL patients as well as those of TT patients and of normal individuals that had never been exposed to *M. leprae* or *M. leprae* endemic areas.

A different approach was used by Stoner et al. (33), who developed a two-stage assay to study subclinical infection in healthy individuals with *M. leprae* contacts. The assay consisted of a first culture of PBMC with *M. leprae* (or other microbial antigens) for 7 days followed by a second culture in which antigen-primed cells were cocultured with fresh autologous cells triggered by *M. leprae* as well as by other microbial antigens. These investigators demonstrated strong suppressor responses in healthy individuals in contact with leprosy patients and challenged the hypothesis that *M. leprae*-induced suppression was restricted to the LL group. In addition, LL patients may have a more generalized defect in their ability to mount suppressor responses (1, 24, 30, 31).

The complexity of the suppressor circuit has been recognized, and recently a role for inducer T cells (28, 35, 36), some of them bearing the Leu8 antigen, has been proposed (12, 13).

In this study, we analyzed the frequency of Leu8-positive cells in LL, TT, and normal *Mycobacterium bovis* BCG-immunized subjects and correlated the results with those of a functional two-stage assay for *M. leprae*-induced suppression. We showed that *M. leprae*-primed PBMC from LL patients weakly suppress the proliferative response of autologous cells to subdoses of ConA or phytohemagglutinin (PHA) in comparison with PBMC from TT patients and normal controls. We propose that this defect is associated to the relatively low proportions of lymphocytes recognized by the Leu8 monoclonal antibody (MAb) in this subgroup of patients.

**MATERIALS AND METHODS**

**Patients.** Sixty-two patients (32 males and 30 females) classified by the method of Ridley and Jopling (29) were studied. The patients did not reside in endemic areas, and...
their age ranged from 15 to 69 years (mean age, 40 ± 14 years). Venous blood samples were obtained from 12 TT patients (one before treatment), 4 borderline (BB) patients, 7 borderline tuberculoid (BT) patients, 23 individuals with LL, 7 borderline lepromatous (BL) patients, and 9 LL patients during an erythema nodosum leprosum (ENL) episode. They received treatment according to the recommendations of the World Health Organization: dapsone for TT patients, dapsone and rifampin-clofazimine for LL patients; thalidomide was added to the therapeutic treatment during the ENL episodes.

Twenty-three healthy subjects (laboratory personnel and volunteer donors) (7 males and 16 females aged 25 to 48 years; mean, 34 ± 10 years) were studied as normal controls. They had been immunized with BCG and they did not reside in leprosy endemic areas.

**PBMC.** Mononuclear cells were isolated by centrifugation of heparinized peripheral blood on Ficoll-Hypaque (3). Cells were collected from the interphase and contained 85 to 95% lymphocytes. PBMC were suspended in RPMI 1640 tissue culture medium (GIBCO Laboratories, Grand Island, N.Y.) containing 20% heat-inactivated AB serum, 1-glutamine (2 mM), and gentamicin (50 µg/ml) (RPMI-AB).

**Suppression of lymphocyte proliferation by *M. leprae* antigens.**

(i) Induction stage (first culture). PBMC were cultured at 2 × 10^6/ml in RPMI-AB. A 2-ml sample of this cell suspension was incubated in Falcon 2063 tubes (Becton Dickinson Labware, Oxnard, Calif.) for 5 days at 37°C in a humidified 5% CO_2 atmosphere. The cells were cultured with RPMI-AB (control cells) or different concentrations of *M. leprae* (3.6 × 10^7, 7.8 × 10^7, and 1.8 × 10^8 *M. leprae* per ml) (suppressor cells). The same batch of armadillo-derived, gamma-irradiated *M. leprae* obtained through the IMMELP bank was used throughout the study. After three washes with RPMI (1,000 rpm), the cells were suspended in 0.5 ml of medium and 50 µg of mitomycin C (Sigma Chemical Co., St. Louis, Mo.) per ml was added to both control and suppressor cells to prevent cell division. After 30 min at 37°C, cells were washed three times and the cellular concentration was adjusted to 1 × 10^6 cells per ml in RPMI-AB.

(ii) Proliferation stage (second culture). Autologous PBMC that had been frozen at −80°C with RPMI-AB supplemented with 10% dimethyl sulfoxide were used as indicator cells in the second culture. A 50-µl sample of indicator cells washed and resuspended in RPMI-AB at 10^6/ml were delivered into each well of microtiter plates (Falcon 3072) and were incubated with suppressor or control cells in a total volume of 100 µl. The suppression test was performed at a suppressor (or control)-to-indicator ratio of 1:1. PHA (10 µl of a 1/500 stock dilution; Difco Laboratories, Detroit, Mich.) or ConA (5 µg/ml; Sigma) was used to stimulate indicator cells. Plates were incubated for 72 h (PHA) or 96 h (ConA) at 37°C. The length of the PHA and ConA cultures was selected on the basis of the kinetics of the proliferative response to each mitogen. As a control of proliferation in the absence of suppressor or control cells, indicator cells were incubated alone with the same mitogens. Thymidine incorporation was measured after an 18-h pulse with [3H]thymidine (specific activity, 20 Ci/mM; Dupont, NEN Research Products, Boston, Mass.). The results are expressed as either percent suppression or thymidine uptake. (i) Percent suppression was calculated as follows: percent suppression = ([cpm (indicator + control) − cpm (indicator + suppressor)]/cpm (indicator + control)) × 100. (ii) For determination of thymidine uptake, we analyzed the effect of the addition of control or suppressor cells on the uptake of [3H]thymidine by lectin-stimulated indicator cells (PHA-I or ConA-I). The results are expressed as percent thymidine uptake: percent ^3H uptake = ([cpm (PHA-I or ConA-I) − (control or suppressor)]/cpm (PHA-I or ConA-I)) × 100. Counts per minute (cpm) of PHA-I or ConA-I were taken as 100% of proliferation. In some experiments, interleukin-2 (IL-2) (Electronucleonics) was added to the second culture to achieve a concentration of 10 U/ml.

**Lymphocyte surface membrane markers.** Mononuclear cells from peripheral blood or cultures stimulated with PHA or ConA bearing the CD4, CD8, CD25, or Leu8 antigen were determined by direct reaction with fluorescein-labeled MAb. Anti-CD4 (Leu3a), anti-CD8 (Leu2a), anti-CD25 (Tac, IL-2 receptor [IL-2R]), or anti-Leu8 (Becton Dickinson) was used.

### RESULTS

**Proportion of Leu8+ cells in the different groups of leprosy patients.** Because cells bearing the Leu8 phenotype have been shown to participate in the generation of suppressor activity (11, 13, 22), we investigated whether they were present in similar proportions in LL, TT, and ENL patients compared with normal individuals. The results shown in Table 1 demonstrate that LL patients have less circulating Leu8+ cells than TT and ENL patients and normal controls. The proportion of Leu8+ cells was not significantly altered by culture with *M. leprae*, PHA, or ConA in any of the groups studied.

**M. leprae-induced suppression of T-cell proliferation.** *M. leprae* induced lower suppression of mitogen-triggered T-cell proliferation in the LL group of patients than in patients with
the other clinical forms of the disease and in the normal group (Table 2). Suppression induced by *M. leprae* was different according to the indicator cell system used. When ConA was used as the trigger in the second culture, differences between the responses of LL patients and the other groups were more marked. Addition of IL-2 (10 U/ml) to the cells proliferating in stage 2 did not alter the results. Expression of IL-2R after *M. leprae* induction was higher in both the LL and TT groups than in normal controls (Table 3) and bore no relationship to the degree of functional suppression observed; at different doses of *M. leprae* during the induction of suppression, this result was confirmed (Fig. 1A and B). Recovery of suppressor activity of LL patients undergoing ENL episodes was also observed.

[^3]H[^2] thymidine uptake of PBMC during second culture in the presence of control or *M. leprae*-primed PBMC. The relative effect of *M. leprae*-stimulated (S) or control precultured (C) autologous PBMC on PHA- or ConA-triggered proliferation was examined in detail in LL, ENL, and TT patients. The results shown in Fig. 2 indicate that although there was virtually no effect of normal precultured cells (N-C) on the subsequent PHA- or ConA-induced proliferation of autologous PBMC indicators, *M. leprae*-primed normal cells (N-S) strongly reduced ConA proliferation and, to a lesser extent, PHA proliferation. In the TT patient group, both TT-C and TT-S reduced the [^3]H[^2] thymidine uptake of PHA-induced cultures. TT-S also reduced ConA-induced proliferation, but TT-C enhanced the [^3]H[^2] thymidine uptake of ConA-induced cultures. The behavior of PBMC from LL patients was in striking contrast to that of PBMC from normal controls and TT patients. There were no significant differences between the effects of LL-C and LL-S on mitogen proliferation, but while the ConA response was always depressed by LL-C and LL-S, both LL-C and LL-S enhanced PHA-induced [^3]H[^2] thymidine uptake. During the ENL episode, the effect of ENL-C and ENL-S was intermediate to that of the two polar groups of patients.

**Induction of IL-2 receptors by culture with *M. leprae***

To determine whether the suppressor activity of PBMC from normal controls and patients with LL, TT, and the intermediate forms was related to absorption of IL-2 necessary for

### Table 2. *M. leprae*-induced suppression of T-cell proliferation in a two-stage assay

<table>
<thead>
<tr>
<th>PBMC</th>
<th>PHA</th>
<th>ConA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control cpm</td>
<td>% Suppression</td>
</tr>
<tr>
<td></td>
<td>-IL-2</td>
<td>+IL-2</td>
</tr>
<tr>
<td>LL</td>
<td>15,344 ± 4,532 (23)</td>
<td>8 ± 4 (23)</td>
</tr>
<tr>
<td>ENL</td>
<td>13,170 ± 3,461 (9)</td>
<td>13 ± 10 (9)</td>
</tr>
<tr>
<td>BL</td>
<td>12,888 ± 5,137 (7)</td>
<td>22 ± 11 (7)</td>
</tr>
<tr>
<td>BB</td>
<td>12,263 ± 3,251 (4)</td>
<td>19 ± 8 (4)</td>
</tr>
<tr>
<td>BT</td>
<td>15,550 ± 5,310 (7)</td>
<td>26 ± 10 (7)</td>
</tr>
<tr>
<td>TT</td>
<td>16,657 ± 7,774 (12)</td>
<td>17 ± 6 (12)</td>
</tr>
<tr>
<td>N</td>
<td>18,137 ± 7,800 (23)</td>
<td>13 ± 5 (23)</td>
</tr>
</tbody>
</table>

<sup>a</sup> A two-stage suppression assay was set up as described in Materials and Methods. During the first 5-day culture, PBMC from LL, ENL, BL, BB, BT, and TT patients or normal controls (N) were incubated with 18 x 10⁶ gamma-irradiated *M. leprae*. Abbreviations are used according to the classification of Ridley and Jopling (29). Suppressor cell activity was assayed in a second culture, adding *M. leprae*-primed mitomycin-treated PBMC to autologous frozen PBMC in the presence of either PHA (15,000) or ConA (5 μg/ml) for 72 or 96 h, respectively. Control proliferation of indicator cells in the second culture is given in cpm (mean ± standard error) for each type of PBMC. In some cases, IL-2 (10 U/ml) was added to second cultures set up in parallel. Percent suppression was calculated as described in Materials and Methods. Mean ± standard error is shown. Statistical differences were calculated by the Wilcoxon rank test.

<sup>b</sup> P < 0.001.

<sup>c</sup> P < 0.001.

<sup>d</sup> ND, Not determined.

### Table 3. Induction of IL-2 receptor antigens by culture with *M. leprae*

<table>
<thead>
<tr>
<th>Patients</th>
<th>n</th>
<th>RPMI</th>
<th><em>M. leprae</em> induced</th>
</tr>
</thead>
<tbody>
<tr>
<td>LL</td>
<td>9</td>
<td>6 ± 1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>13 ± 3&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>TT</td>
<td>8</td>
<td>4 ± 2</td>
<td>16 ± 4&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>N</td>
<td>11</td>
<td>0.8 ± 0.4</td>
<td>6 ± 1</td>
</tr>
</tbody>
</table>

<sup>a</sup> The proportion of PBMC from LL or TT patients and from normal BCG-immunized subjects (N) that reacted with anti-IL-2R monoclonal antibody (anti-IL-2R; Becton Dickinson and Co., Paramus, N.J.) was assayed after culture with *M. leprae* (18 x 10⁶) or RPMI for 5 days. Mean ± standard error is given. Statistical differences were determined by the Student t test for patients versus normal subjects.

<sup>b</sup> P < 0.005.

<sup>c</sup> P < 0.05.

FIG. 1. Dose-response curve of *M. leprae*-induced suppression. The two-stage suppression assay was set up with different amounts of *M. leprae* during the first culture. (A) Suppression of PHA-induced proliferation of autologous PBMC. (B) Suppression of ConA-induced proliferation of autologous PBMC. PBMC from LL (C), ENL (●), TT (◆), and normal (□) individuals were used. Details are given in Materials and Methods. Mean ± standard error (SE) of the percent suppression is given. Statistical differences were calculated by the Wilcoxon rank test: LL versus normal, P < 0.001; LL versus TT, P < 0.001 (in all doses).
the proliferation of T cells during the second culture, we assayed the proportion of cells reacting with anti-IL-2R (anti-Tac). The results shown in Table 3 indicate that both LL and TT patients have higher numbers of Tac-positive cells than the controls at the beginning and after the induction of suppression with *M. leprae*. Nevertheless, while LL patients had similar numbers of Tac-positive cells as TT patients and twice as many as normal controls, their suppressor activity was low.

PHA- and ConA-induced proliferation of T-lymphocyte subsets in leprosy patients and controls. Because *M. leprae*-induced suppressor cells differed in their ability to inhibit the proliferation of autologous PBMC that were stimulated by PHA or ConA, the proportion of CD4 and CD8 T-lymphocyte subsets recovered after culture with PHA or ConA was evaluated in the different groups of patients. The results shown in Table 4 indicate that the proportions of T cells bearing the Leu3 or Leu2 surface marker were essentially the same in LL, TT, or normal individuals. After PHA culture, the Leu3 subset predominated in the three groups. Conversely, after ConA culture, Leu2-positive cells increased to a similar extent in all groups.

**DISCUSSION**

Impaired ability of lymphocytes from LL patients to mount suppression after nonspecific stimulation with ConA has been shown previously by us and by others (1, 24, 30). ConA-induced suppressor cell function is the result of a complex process that involves T regulatory cells from different subsets as well as cells from nonlymphoid cell lines (21). Therefore, the low suppressor response of LL patients could be due to an intrinsic defect in one or many of the components of the suppressor circuit. Recently, a subset of cells recognized by the Leu8 MAb has been described as an important component of the suppressor network (8, 13, 22, 28). These cells may be related to the radiosensitive suppressor-inducer cells that belong to the CD4 differentiation group of lymphocytes (12, 35, 36). MAb directed against the CD45R set of antigens (Lp220/2H4) are also thought to recognize suppressor-inducer cells (34), but expression of these antigens appears to be related to the activation stage of the cells rather than to a particular subset of lymphocytes (32). In contrast, the expression of Leu8 (p80) antigens is not linked to cell activation (11). Furthermore, Damle et al. (7) have clearly shown that CD4+ CD45R+ p80/Leu8+ cells are the only cells that can be activated by antigen to generate suppressor-inducer cells that modulate in turn the activity of CD8+ lymphocytes.

In this study, we determined the proportion of Leu8+ cells in LL, ENL, TT, and normal individuals and found strikingly low levels of Leu8+ cells in LL patients when they were compared with the other groups. LL patients undergoing ENL episodes recovered normal values of Leu8 (Table 1), indicating that the level of these cells could be influenced by the inflammatory process. In vitro activation of lymphocytes with lectins or with *M. leprae* antigens did not change significantly the proportion of Leu8-stained cells (Table 1).

There is considerable controversy on the role of suppressor cells in the absence of a proliferative immune response of LL patients to *M. leprae*. Mehra et al. (16–18) proposed that there was high *M. leprae*-specific suppressor activity in LL compared with the other clinical forms of the disease. However, recent work of other groups suggests the contrary (14, 15, 33). In fact, while some researchers suggest that the

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**TABLE 4. Lymphocyte surface markers of PBMC cultures with PHA or ConA**

<table>
<thead>
<tr>
<th>PBMC</th>
<th>MAb</th>
<th>% Positive cells after PHA were incubated with:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>RPMI</td>
</tr>
<tr>
<td>LL</td>
<td>Anti-Leu3</td>
<td>63 ± 8</td>
</tr>
<tr>
<td></td>
<td>Anti-Leu2</td>
<td>37 ± 8</td>
</tr>
<tr>
<td>TT</td>
<td>Anti-Leu3</td>
<td>68 ± 4</td>
</tr>
<tr>
<td></td>
<td>Anti-Leu2</td>
<td>33 ± 4</td>
</tr>
<tr>
<td>N</td>
<td>Anti-Leu3</td>
<td>67 ± 3</td>
</tr>
<tr>
<td></td>
<td>Anti-Leu2</td>
<td>33 ± 3</td>
</tr>
</tbody>
</table>

* PBMC from LL (n = 3) or TT (n = 3) patients and normal controls (N; n = 3) were incubated for 3 days at 37°C with PHA (1/5,000) or ConA (5 μg/ml) in a humidified 5% CO₂ atmosphere. After incubation, the cells were washed three times and incubated with MAb anti-Leu2a or anti-Leu3a as described in Materials and Methods. The percentage of positive cells was calculated, and the results are expressed as mean ± standard error.
inability to mount in vitro proliferative responses is restricted to *M. leprae* antigens in LL (9, 10), others provide evidence for a more generalized status of anergy in LL (4, 6).

In this study, we demonstrated that *M. leprae* can induce dose-dependent suppression in normal individuals, TT leprosy patients, and patients with the intermediate clinical forms (Fig. 1; Table 2). The ability of *M. leprae* to induce suppressor cell activity in LL patients (excluding those undergoing an ENL episode) was low. It has been suggested by others (14, 26) that the frequency of CD8 *M. leprae*-specific responder cells may be low in LL patients, and this could be one of the reasons for the weak suppressor response of LL patients after 5 days of culture of PBMC with *M. leprae*. Leu8-positive cells are found in both the CD4 and CD8 differentiation groups of T cells as well as in other types of lymphocytes (B cells) and leukocytes (13). Therefore, low numbers of one or more of the regulatory subsets bearing this antigen in quiescent LL patients (Table 1) may be the cause of the poor functional PBMC suppressor response.

Studying the lymphoid cells present in skin sections from LL and TT patients, Modlin et al. (19) have demonstrated that TT granulomas are rich in T cells of CD4*+* 4B4*+* 2H4*+* phenotype (helper). In contrast, in lepromatous lesions approximately 50% of CD4*+* cells were 2H4*+* Leu8*+* (suppressor-inducer). However, functional studies are not available. In that report, the proportion of CD4*+* 2H4*+* cells in PBMC was low in both TT and LL patients and Leu8*+* cells were reported to follow the CD4*+* 2H4*+* profile (19). These results are not in agreement with those shown in Table 1 (low levels of Leu8*+* PBMC in LL patients versus normal levels of Leu8*+* PBMC in TT patients), underlining the differences between the Leu8 and the CD45R (2H4*) systems (7).

Suppression was assayed in our experimental design with autologous cells triggered by T-cell mitogens as indicators. It is possible that the conditions of a two-step assay such as the one used by us allow expression of “physiologic” suppression in TT PBMC that were sensitized in vivo to *M. leprae* (33). During an ENL episode, the behavior of control or *M. leprae*-primed LL PBMC approached that of TT PBMC, in coincidence with the recovery of the level of Leu8-positive cells.

It is unlikely that suppression resulted from absorption of IL-2 by the *M. leprae*-primed cells added to the second culture, since addition of exogenous IL-2 did not alter the results (Table 2). Furthermore, the suppressor activity appeared to be unrelated to the proportion of cells bearing the Tac membrane antigen, known to be related to IL-2R (38). As expected from continuous in vivo activation, the proportion of Tac-positive cells was higher both in LL and in TT patients than in normal controls. *M. leprae* stimulation increased their number in the three groups (Table 3), but LL patients that expressed the same proportion of Tac-positive cells as TT patients had a low suppressor response (Table 2). Taken together with those of IL-2 addition during the second culture, these results suggest that *M. leprae*-induced suppression in TT, intermediate forms, ENL, and normal cells is not an in vitro artifact caused by the remotion of IL-2 by activated cells during the coculture (21, 27).

It is also unlikely that the different effects of *M. leprae*-primed cells on PHA-stimulated or ConA-stimulated PBMC (Fig. 1 and 2) were due to a defect in the ability of a given subset of LL-T or TT-T lymphocytes to proliferate after the lectin stimuli. There were no differences in the proportion of CD4 and CD8 cells activated by PHA or ConA in LL or TT patients or normal controls (Table 4). Leu3-positive cells predominated in PHA cultures and Leu2-positive cells were expanded after ConA cultures whether PBMC were obtained from LL, ENL, or TT patients or normal controls.

We analyzed the activity of spontaneous suppressor cells (PBMC-C) obtained after 5 days of culture in the absence of *M. leprae* and found that PBMC-C from leprosy patients behaved differently from PBMC-C from normal individuals (Fig. 2). Thus, TT-C spontaneously suppressed the proliferation of cells that belonged mainly to the CD4 differentiation group (PHA proliferation), while LL-C suppressed ConA-responsive cells (many of them bearing CD8 antigens). Moreover, probably as a consequence of the balance of suppressor versus stimulatory mechanisms, ConA-induced proliferation was greater when TT-C were present in the second culture and PHA proliferation was enhanced by LL-C. These spontaneous suppressor cells (TT-C, LL-C) obtained after 5 day cultures were probably different from suppressor cells present in short-term cultures of LL PBMC (27). After 24-h culture periods, PBMC from LL patients, mainly those undergoing ENL episodes, exerted spontaneous inhibition of PHA-induced proliferation via cyclooxygenase metabolites of arachidonic acid (S. de la Barrera, R. Valdez, L. M. Baliah, and M. del C. Sasiain, Medicina (Buenos Aires) 47:630, 1987).

The results of this study suggest that there is a link between the reduction of Leu8-positive cells and the low functional suppressor response of LL PBMC after *M. leprae* stimulation. We believe that the low frequency of Leu8-positive cells in LL patients is a clue to the inability of LL PBMC to generate specific and nonspecific suppression of T-cell proliferation and may be related to the inability of LL patients to resist *M. leprae*.

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**LITERATURE CITED**


