Monocyte-Derived SolubleSuppressor Factor(s) in Patients with Lepromatous Leprosy

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Peripheral blood monocytes from polar lepromatous leprosy (LL) patients were unable to support Mycobacterium leprae-induced in vitro lymphoproliferation of HLA-D-matched T cells from tuberculoid leprosy subjects, whereas those from responder individuals were able to do so. Monocyte-rich adherent cells from untreated LL patients released de novo soluble factors which inhibited antigen-induced lymphoproliferation to a greater extent and mitogenic responses to a lesser extent. Suppressive activity varied in different LL patients. However, the degree of suppression was similar in soluble factors obtained de novo and after treatment of adherent cells with heat-killed and freshly extracted, cryopreserved M. lepra. Treated patients showed less inhibition with de novo released soluble factors (27 ± 7.7%) as compared to parallel soluble factors obtained after antigen treatment (44 ± 4.8%) or with de novo soluble factors from untreated LL patients (62 ± 14.2%). Similar supernatants from tuberculoid individuals showed no or insignificant effects on antigen-induced lymphoproliferation. The suppressive activity of LL soluble factors was produced for up to 72 h, was heat stable at 56°C for 30 min, was indomethacin resistant, and resided in the >25,000 molecular weight fraction.

Adherent cells with macrophage/monocyte characteristics have been implicated in the immunological unresponsiveness associated with a wide variety of infections. Macrophages alone or in combination with T cells have been shown to suppress antibody synthesis and proliferative responses to specific antigens, mitogens, and alloantigens in experimental animals infected with African trypanosomiasis (9), Mycobacterium lepraemurium (4, 36), and Mycobacterium bovis (12). Peripheral blood-derived monocytes from patients with tuberculosis (7), sarcoïdosis (11), schistosomiasis (21), disseminated fungal infection (29), and diffuse cutaneous leishmaniasis (23) were also reported to inhibit antigen- or mitogen-induced lymphoproliferation. There is growing evidence that mycobacteria may cause suppression of immune responses through the release of soluble factors from suppressor lymphocytes and macrophages (31, 35).

It is well established that Mycobacterium lepra-induced infection leading to disseminated lepromatous leprosy is associated with poor T cell functions. These patients have been consistently shown to have poor delayed hypersensitivity reactions and reduced blastogenic and antigenic responses in vitro (16, 32). In contrast, tuberculoid leprosy individuals show optimal responses in similar assays. The mechanisms underlying immunological unresponsiveness in lepromatous leprosy have received recent attention. Having observed repeatedly that suppressor T cell activity was not a conspicuous feature in most untreated lepromatous individuals studied by us (17–19, 28) as well as others (5, 27, 30), we thought it pertinent to investigate the role of peripheral blood monocytes in this disease, using a lymphoproliferative assay with enriched T cell populations.

The present study confirms the earlier findings (19) and indicates that whereas monocytes from tuberculoid leprosy individuals supported M. leprae-induced responses of HLA-D identical responder T-enriched cells, those from lepromatous patients were unable to do so. Moreover, monocytes from lepromatous subjects released soluble factors which inhibited antigen- and mitogen-induced lymphoproliferation. The monocyte suppressive factors were released for up to 3 days of the culture period, were heat stable, and were not affected by indomethacin treatment.

MATERIALS AND METHODS

Patients. A total of 63 polar lepromatous and tuberculoid leprosy patients were studied. Of these, eight patients from an endemic area attending the clinics of Gandhi Memorial Leprosy Foundation, Wardha, In-
dia, were selected on the basis of HLA-D identity between siblings or paired individuals with concordant and discordant leprosy (Table 1). The data on the HLA status of these patients was kindly provided by J. J. van Rood, Department of Immunohematology, Academisch Zeichenhuis, Leiden, The Netherlands, and N. K. Mehra, Department of Anatomy, All-India Institute of Medical Sciences, New Delhi, and formed part of earlier reports of De Vries et al. (6). These patients had received treatment with 100 mg of dapsone daily for 3 to 5 years. Lepromatous patients had residual bacilli in the skin.

The other patients were drawn from the Hansen's clinics of the All-India Institute of Medical Sciences and Safdarjang Hospital, New Delhi, and were 28 untreated lepromatous leprosy patients (27 male and 1 female) who were bacillary positive (bacillary index [24] ranging from 4+ to 6+), 22 untreated tuberculoid patients (20 male and 2 female), and 5 male lepromatous patients treated for 5 to 7 years with dapsone as described above who had a bacillary index of 1+. All patients were graded on the basis of Ridley-Jopling classification (25), bacillary smears, histopathology of skin lesions, treatment status, and the level of lymphocyte transformation to M. leprae. Percent stimulation was calculated as the mean counts per minute of cultures with M. leprae divided by the mean counts per minute of cultures alone, with the quotient multiplied by 100. Peripheral lymphocytes from tuberculoid patients and three healthy volunteers included in the study had 356 to 1,820% antigen-induced stimulation. Stimulation levels of ≤100% were considered negative and were consistently observed in all lepromatous patients.

Characterization of cells. T cells were identified by indirect immunofluorescence using monoclonal antibody OKT3 (Orthoclone, Raritan, N.J.). Lymphocytes (10⁶) were treated with 50 μl of a 1:50 dilution of OKT3 for 45 min at 4°C, washed three times, incubated with antimouse fluorescein isothiocyanate-conjugated antibody F(ab')₂ fraction (New England Nuclear Corp., Boston, Mass.), washed three more times, and suspended in 10% glycerol-phosphate-buffered saline (pH 7.2). Surface immunoglobulin-positive cells were identified similarly by direct immunofluorescence with 1:40 fluorescein isothiocyanate-conjugated rabbit anti-human immunoglobulin (immunoglobulin M plus immunoglobulin G plus immunoglobulin A; Cappel Laboratories, Cochranville, Pa.). Fluorescence was visualized by epillumination with an HBO 50 lamp on a Zeiss standard immunofluorescence microscope (Carl Zeiss, Oberkochen, West Germany). Monocytes were identified by nonspecific esterase staining with α-naphthyl acetate as the substrate by the method of Yam et al. (37). Phagocytosis of latex particles (0.3-μm diameter; Sigma Chemical Co., St. Louis, Mo.) was studied after 10⁶ lymphocytes were incubated with 50 μl of a 1% latex suspension at 37°C for 30 min.

Stimulants. Stimulants were used at optimal concentrations as assessed by earlier studies on lymphoproliferation. Armadillo-derived soluble and integral M. leprae antigens (AB40) were used at a concentration of 5 μg of protein per ml and 5 × 10⁶ bacilli per ml and were a kind gift of R. J. W. Rees, National Institute for Medical Research, Mill Hill, London, England.

Human-derived M. leprae used for the production of monocyte factors was isolated from human lepromas by glass homogenization and used fresh frozen (−20°C for up to 6 months) and after autoclaving at 15 lb/in² for 15 min at a concentration of 1.25 × 10⁶ organisms per 1 × 10⁶ mononuclear cells. Concanavalin A (Pharmacia Fine Chemicals, Uppsala, Sweden) and pokeweed mitogen (GIBCO-Biocult, Irvine, Scotland) were used at concentrations of 100 μg/ml and 1:250, respectively.

Isolation of subpopulations of PBMC. Peripheral blood mononuclear cells (PBMC) were isolated from heparinized (10 to 20 U/ml; preservative-free heparin; Upjohn and Co., Kalamazoo, Mich.) venous blood of patients by Ficoll-Hypaque (Pharmacia Fine Chemicals) centrifugation (3). PBMC were washed three times in Hanks balanced salt solution (GIBCO-Biocult, Boston, Mass.) and suspended in RPMI 1640 medium with antibiotics and sodium pyruvate (GIBCO-Biocult, Irvine, Scotland).

### Table 1. HLA haplotypes of PBMC of cell donors in monocyte and T cell cultures

<table>
<thead>
<tr>
<th>Expt</th>
<th>Diagnosis</th>
<th>Cells</th>
<th>HLA haplotypes</th>
</tr>
</thead>
<tbody>
<tr>
<td>1a</td>
<td>TT</td>
<td>T cells</td>
<td>AW30, B13, W6, C6, DRW7/A2, BW40.2 W6, DRW2</td>
</tr>
<tr>
<td>1b</td>
<td>TT</td>
<td>Monocyte</td>
<td>A3, BW5, W6, C3, DR2/A11, BW51, W4, DRW4</td>
</tr>
<tr>
<td>1c</td>
<td>TT</td>
<td>T cells</td>
<td>A3, BW5, W6, C3, DRW2/A11, BW51, W4, DRW4</td>
</tr>
<tr>
<td>1d</td>
<td>TT</td>
<td>Monocytes</td>
<td>A3, BW5, W6, C3, DRW7/A2, BW40.2 W6, DRW2</td>
</tr>
<tr>
<td>1e</td>
<td>TT</td>
<td>T cells</td>
<td>A3, BW5, W6, C3, DRW2/A11, BW51, W4, DRW4</td>
</tr>
<tr>
<td>2f</td>
<td>TT</td>
<td>T cell</td>
<td>A2, B40, DRW2/A28, B5, DR2</td>
</tr>
<tr>
<td>2g</td>
<td>TT</td>
<td>Monocytes</td>
<td>A2, B40, DRW2/A28, B7, DRW8</td>
</tr>
<tr>
<td>2h</td>
<td>TT</td>
<td>T cells</td>
<td>A2, B40, DRW2/A28, B7, DRW8</td>
</tr>
</tbody>
</table>

a Numbers refer to those in Fig. 1 and 2.
b TT, Tuberculoid; LL, lepromatous.
control) and suspended in RPMI 1640 (GIBCO-Biocult) buffered with 20 mM HEPES (N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid) (GIBCO-Biocult) and supplemented with 100 U of penicillin per ml, 100 μg of streptomycin per ml, and 10% pooled AB serum (complete medium).

(i) Adherent cells. Adherent cells were isolated from PBMC on 24-well tissue culture plates (Flow Laboratories, Irvine, Scotland) which had been precoated with fetal calf serum (GIBCO-Biocult). 10⁶ PBMC per well were incubated for 2 h at 37°C in an atmosphere of 5% CO₂ and air. The nonadherent cells were removed by washing five times with warm Hank's balanced salt solution. The adherent cells were removed by further incubating the monolayers with 12 mM xylocaine (Astra-IDL., Bangalore, India) in RPMI 1640 plus 10% fetal calf serum for 45 min at 37°C, washed two times, and suspended in complete medium.

(ii) Nylon wool column-purified cells. Two-milliliter syringes were packed with 40 mg of nylon wool (Leuko-pak; Fenwall Laboratories, Morton Grove, Ill.), and 10 × 10⁶ nonadherent cells were added to prewashed, prewarmed columns. The columns were incubated for a further 1 h at 37°C, and the cells were removed by dropwise elution with 4 × 1 ml of RPMI 1640 plus 10% fetal calf serum.

MoF(s). Adherent cells obtained from 1 × 10⁶ PBMC per ml per well were overlaid with 1.25 × 10⁶ M. leprae and incubated at 37°C for 24 h in a humidified atmosphere containing 5% CO₂ and air. Subsequently, supernatants were collected, centrifuged at 400 × g for 5 min, filtered through 0.45-μm membranes (Millipore Corp., Bedford, Mass.), and stored at −20°C. In the experiments involving time kinetics, 24 h before collection of monocyte factor(s) [MoF(s)] adherent cells were washed and replaced with fresh medium.

Lymphoproliferation assay. (i) HLA-D defined cocultures. Quadruplicate of 10⁶ PBMC and 10⁵ T-enriched cells in 100 μl of complete medium plus 25 μl of 5, 10, and 20% monocyte-rich adherent cells, respectively, were set up in round-bottomed microculture plates (Nunc-intermed, Roskilde, Denmark). They were cultured with and without 25 μg of the respective antigens for 6 days at 37°C in 5% CO₂ and air. The cultures were harvested on glass fiber disks by a semiautomatic cell harvester (Iacon, Tonbridge England) 16 h after the addition of 0.5 μCi of methyl-tritiated thymidine ([³H]thymidine; specific activity, 2 Ci/mmol; Ameesham International, England). The radioactivity was counted in an LKB Rackbeta II 1215 scintillation counter.

(ii) MoF(s). Quadruplicate cultures containing 10⁵ PBMC in 50 μl of complete medium and 50 μl of MoF(s) with and without 25 μl of soluble AB40-pokeweed mitogen-concanavalin A were set up in microculture plates. Mitogen- and antigen-stimulated cultures were harvested on days 3 and 6, respectively, as described above.

Characterization of factor(s). Indomethacin (Sigma) was dissolved in 0.1 M Na₂CO₃ as 20 mg/ml and diluted in RPMI 1640 to 100 μg/ml. Ten micrograms of indomethacin was added to each well containing adherent cells derived from 10⁶ PBMC. The cultures were treated with and without antigen, and supernatants were collected as described above.

MoF(s) collected without serum were centrifuged in Amicon ultrafiltration membrane cones (CFT 25; Amicon Corp., Lexington, Mass.) which retained constituents with a molecular weight above 25,000. The separa-

![Graph](https://via.placeholder.com/150)

FIG. 1. Reconstitution of in vitro antigen-induced lymphoproliferative responses of five cocultures in which 10⁵ T-enriched cells from tuberculoid leprosy patients were combined with 5, 10, and 20% monocytes (Mo) from HLA-D-defined other tuberculoid leprosy individuals and stimulated with integral and soluble M. leprae antigens. Shaded bars indicate PBMC responses of the T cell donor, with mean counts per minute given inside the bars. The SD was <20%. Open bars indicate the percent response of the cocultures at the monocyte concentration shown on the abscissa [percent response = (mean cpm of cultures with antigen/mean cpm of cultures alone) × 100]. Responses above 100% indicate stimulation in the presence of antigen as compared to control cultures without antigen. The HLA-D haplotypes of monocytes and T cells for each set of cocultures are indicated at the tops of the figures.
FIG. 2. Absence of antigen-induced lymphoproliferation in three cocultures in which 10⁵ T-enriched cells from tuberculoid leprosy individuals were combined with 5, 10, and 20% monocytes (Mo) from lepromatous individuals, two of whom shared partial (f and g) and one shared full (h) identity for HLA-D haplotypes, as indicated at the tops of the figures. Shaded bars indicate PBMC responses of the tuberculoid individuals, with mean counts per minute given inside the bars. The SD was <20%. Open bars indicate percent responses (same as in the legend to Fig. 1) of cocultures at the monocyte concentrations shown on the abscissa. Responses above 100% (---) indicate stimulation in the culture with antigen as compared to cultures without antigen.

Expression of results and statistical analysis. (i) HLA-D cocultures. The counts per minute ± standard deviation (SD) of replicate cultures was calculated, and percent response was derived as

\[
\text{percent response} = \left( \frac{\text{mean cpm of cultures with antigen}}{\text{mean cpm of cultures without antigen}} \times 100 \right)
\]

Statistical analysis was done by using Mann-Whitney's U test (1).

FIG. 3. Dose-related suppressive activity of 10, 25, and 50 µl of MoF(s) derived from two lepromatous patients on soluble M. leprae antigen-induced lymphoproliferative responses of two tuberculoid leprosy patients. Mean counts per minute (-----) ± SD (---) of control cultures without addition of factors is indicated. The inset shows the absence of suppression (bars) on the basal lymphoproliferation (---) of unstimulated PBMC. Lymphoproliferation in the presence of factors released de novo (□□□□), from monocytes (Mo) treated with heat-killed M. leprae (□□□□), and from cryopreserved M. leprae (□□□□) is shown.
### RESULTS

*M. leprae*-induced proliferation of T-enriched cells in the presence of HLA-D-defined monocytes from leprosy patients. To minimize the super-added effects of allogeneic cell combinations, HLA-D-identical cells were used wherever possible. Nylon wool-purified cells and 2-h adherent cells derived from the peripheral blood of different HLA-D-defined leprosy patients were cocultivated in the presence of soluble and integral *M. leprae* antigens, with \(^{3}H\)thymidine uptake as an indicator of proliferation. Nylon wool-purified cells enriched for T cells consisted of 85 to 87% OKT3-positive (pan T) cells, \(<2\%\) surface immunoglobulin-positive cells, and \(<1\%\) esterase-positive phagocytic cells. The adherent cell population was enriched for monocytes and contained 85 to 88% esterase-positive phagocytic cells, 5 to 7% OKT3-positive cells, and \(<3\%\) surface immunoglobulin-positive cells. The yield and nature of cells were similar from tuberculoid and lepromatous subjects. T cells from tuberculoid leprosy patients and healthy responder individuals alone or in combination with 1 and 2% autologous monocytes gave no or insignificant lymphoproliferation with *M. leprae* antigens (data not shown). Cocultures with 5, 10, and 20% monocytes showed antigen-induced proliferation similar to or higher than the paired PBMC of the T cell donor.

(i) **Stimulation of lymphoproliferation in the presence of HLA-D-identical monocytes from tuberculoid leprosy patients.** Five cocultures were undertaken with T cells and monocytes derived from paired tuberculoid individuals (Fig. 1). In four cultures, 5% monocytes supported lymphoproliferation to levels similar to or twofold higher than the values obtained with unfractionated PBMC. In general, soluble antigen gave higher responses than integral antigen. Significant proliferation was still observable in one coculture in which the monocyte concentration was 20% (Fig. 1a). Proliferation was observable even when T cells and monocytes differed in one HLA haplotype (Fig. 1c and Table 1).

(ii) **Lack of lymphoproliferation in the presence of HLA-D-defined monocytes from lepromatous leprosy patients.** Of the three cocultures, one shared full and two shared partial HLA-D identity for T cells and monocytes (Table 1). The T cells and monocytes were derived from paired responder tuberculoid leprosy and nonresponder lepromatous leprosy individuals, respectively. It may be noted from Fig. 2 that under the conditions as described above, monocytes from lepromatous individuals failed to induce T cell proliferation to soluble and integral *M. leprae* antigens. In view of the absence of proliferation at any concentration of monocytes, it was not possible to assess whether lepro-

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**TABLE 2. Overall effects of MoFs derived from leprosy patients on soluble *M. leprae* (AB40)-induced lymphoproliferative responses of tuberculoid leprosy patients**

<table>
<thead>
<tr>
<th>PBMC alone</th>
<th>+ MoFs from:</th>
<th>Heat-killed <em>M. leprae</em></th>
<th>Cryopreserved <em>M. leprae</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean ± SD antigen-stimulated PBMC</td>
<td>de novo</td>
<td>de novo</td>
<td>de novo</td>
</tr>
<tr>
<td>PBMC from 6 tuberculoid patients</td>
<td>2.77 ± 1.369</td>
<td>901 ± 696</td>
<td>791 ± 575</td>
</tr>
<tr>
<td>PBMC from 8 healthy controls</td>
<td>4.718 ± 3.455</td>
<td>3.455 ± 5.793</td>
<td>6.320 ± 2.609</td>
</tr>
</tbody>
</table>

\( *P < 0.005\)

---

**ND** Not done.

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\( ^{2} \) PBMC from 14 tuberculoid patients were tested with MoFs from 23 leprosy patients.

\( ^{3} \) PBMC from 14 tuberculoid patients were tested with MoFs from 23 leprosy patients.

\( ^{4} \) PBMC from 14 tuberculoid patients were tested with MoFs from 23 leprosy patients.

\( ^{5} \) PBMC from 14 tuberculoid patients were tested with MoFs from 23 leprosy patients.

\( ^{6} \) PBMC from 14 tuberculoid patients were tested with MoFs from 23 leprosy patients.

\( ^{7} \) PBMC from 14 tuberculoid patients were tested with MoFs from 23 leprosy patients.

\( ^{8} \) PBMC from 14 tuberculoid patients were tested with MoFs from 23 leprosy patients.

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\( ^{2} \) PBMC from 14 tuberculoid patients were tested with MoFs from 23 leprosy patients.

\( ^{3} \) PBMC from 14 tuberculoid patients were tested with MoFs from 23 leprosy patients.

\( ^{4} \) PBMC from 14 tuberculoid patients were tested with MoFs from 23 leprosy patients.

\( ^{5} \) PBMC from 14 tuberculoid patients were tested with MoFs from 23 leprosy patients.

\( ^{6} \) PBMC from 14 tuberculoid patients were tested with MoFs from 23 leprosy patients.

\( ^{7} \) PBMC from 14 tuberculoid patients were tested with MoFs from 23 leprosy patients.

\( ^{8} \) PBMC from 14 tuberculoid patients were tested with MoFs from 23 leprosy patients.
MOONMMON DERIVED SUPPRESSOR FACTOR IN LEPROSY

FIG. 4. Overall activity of MoF(s) derived from 12 untreated and 5 treated lepromatous patients and 6 untreated tuberculoid patients on the soluble M. leprae antigen-induced lymphoproliferative responses of tuberculoid individuals.

\[
\% \text{ suppression} = \left\{ \frac{\text{mean cpm of cultures with factor and antigen}}{\text{mean cpm of cultures with antigen alone}} \right\} \times 100
\]

Negative values on the ordinate indicate enhancement. Mean percent suppression in the presence of factors released de novo (○), from monocytes treated with heat-killed M. leprae (○), and cryopreserved M. leprae (△) is shown. Mean percent suppression ± SD for each group is indicated as †.

tous monocytes were incapable of antigen presentation or exerted positive suppression.

Effect of MoF(s) on lymphoproliferation. To explore the possibility that the lack of proliferation may be due to suppressive MoF(s), adherent cells were left alone or pretreated for 24 h with heat-killed M. leprae, as well as freshly extracted bacilli stored at -20°C to preserve labile antigens. Supernatants from these three types of cultures were centrifuged to remove any nonadherent cells, filtered through 0.45-μm Millipore membranes to remove bacilli, and stored at -20°C. Preliminary studies on the numbers of esterase-positive adherent cells recovered from 10⁶ PBMC of nine lepromatous and six tuberculoid patients were similar; the mean ± SD of recovered cells was 8.64 ± 1.02 x 10⁶ and 8.79 ± 1.2 x 10⁶, respectively. Therefore, the MoF(s) obtained from adherent cells of 10⁶ PBMC per well from all leprosy patients were used at the same concentration for evaluation of suppressive activity. In total, MoF(s) from 28 untreated, 5 treated lepromatous, and 6 tuberculoid patients were assayed on lymphoproliferative responses of 3 responder healthy individuals and 16 tuberculoid patients.

(i) Dose response. Figure 3 shows dose-related inhibition of antigen-induced lymphoproliferation of PBMC in cultures containing 10, 25, and 50 μl of factors from two lepromatous patients. The addition of such factors to PBMC alone had no or an insignificant effect on basal DNA synthesis (Fig. 3, inset). Suppressive activity was not observable at 1 and 2 μl. In subsequent assays 50 μl of MoF(s) was used.

(ii) Antigen-induced lymphoproliferation. Monocytes from lepromatous patients consistently released suppressive factors de novo (Table 2 and Fig. 4). Significant suppression of antigen-induced lymphoproliferation ranging from 37 to 87% was observed in cultures of PBMC from tuberculoid patients in the presence of MoF(s) derived from 12 untreated lepromatous patients. The addition of either type of antigens did not significantly enhance the suppressive effect. The percentage of suppression varied from individual to individual but was within the same range for all three types of factors derived from the same patient. Suppression was observed regardless of the type of M. leprae antigen used for lymphoproliferation (data not shown). Interestingly, MoF(s) from five treated lepromatous leprosy patients showed lower de novo suppressive effects (27 ± 7.7%) as compared to parallel supernatants from antigen-treated cells (44 ± 4.8%) and de novo factors from untreated lepromatous leprosy patients (62 ± 14.2%) (Fig. 4).

In contrast, similar supernatants derived from tuberculoid patients showed no suppressive effects. In some instances such factors had mild stimulatory effects on antigen-stimulated PBMC of tuberculoid individuals (Fig. 4 and Table 2) but none on T cells or nonadherent cells of lepromatous subjects (data not shown).

Mitogen-induced lymphoproliferation. MoF(s) derived from nine lepromatous patients also showed significant suppression of concanavalin A- and pokeweed mitogen-induced blastogenesis of PBMC from healthy individuals (Table 3). The degree of inhibition was, however, of a lower order compared to the suppression noted in antigen-stimulated lymphocyte cultures.
TABLE 3. Suppression of mitogenic responses of PBMC of three normal healthy individuals in the presence of monocyte factors [MoF(s)] from nine untreated lepromatous patients

<table>
<thead>
<tr>
<th>Stimulant</th>
<th>No. of MoF(s)</th>
<th>Mean cpm ± SD of mitogen-stimulated PBMC + MoF(s)* (% suppression)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Nil</td>
</tr>
<tr>
<td>Concanavalin A</td>
<td>1</td>
<td>32,581 ± 3,252</td>
</tr>
<tr>
<td>Concanavalin A</td>
<td>2</td>
<td>32,581 ± 3,252</td>
</tr>
<tr>
<td>Concanavalin A</td>
<td>3</td>
<td>32,581 ± 3,252</td>
</tr>
<tr>
<td>Pokeweed mitogen</td>
<td>4</td>
<td>8,338 ± 279</td>
</tr>
<tr>
<td>Pokeweed mitogen</td>
<td>5</td>
<td>8,338 ± 279</td>
</tr>
<tr>
<td>Pokeweed mitogen</td>
<td>6</td>
<td>8,338 ± 279</td>
</tr>
<tr>
<td>Pokeweed mitogen</td>
<td>7</td>
<td>10,605 ± 1,353</td>
</tr>
<tr>
<td>Pokeweed mitogen</td>
<td>8</td>
<td>10,605 ± 1,353</td>
</tr>
<tr>
<td>Pokeweed mitogen</td>
<td>9</td>
<td>10,605 ± 1,353</td>
</tr>
</tbody>
</table>

*MoF(s) released de novo or after treatment of monocytes with M. leprae.

**P < 0.005 as compared to nil.

ND, Not done.

Characterization of monocyte suppressive factors from lepromatous patients. (i) Time kinetics. It was consistently observed that the 0- to 24-h supernatants were most suppressive, although inhibitory effects were detectable in supernatants removed from monocytes at 24 to 48 and 48 to 72 h. Factors derived from 15-day-old macrophage monolayers failed to produce significant suppression (Table 4).

(ii) Heat stability. Suppressive activity was heat stable at 56°C for 30 min (Table 5) and on immersion in boiling water for 1 min (data not shown).

(iii) Molecular size. Twenty-four-hour de novo released MoF(s) from serum-depleted cultures derived from five lepromatous patients were centrifuged in ultrafiltration membrane cones which excluded particles of molecular weight <25,000 (antigen-treated supernatants were avoided to preclude the effects, if any, of soluble antigens leached from prefreeze or heat-killed M. leprae). It may be observed from Fig. 5 that the fraction with molecular weight >25,000 showed inhibition of lymphoproliferation to levels observed with unfractionated MoF(s). Fractions below 25,000 were nonsuppressive and permitted lymphoproliferation to the same order as that obtained in control antigen-stimulated PBMC cultures without MoF(s).

(iv) Effect of indomethacin. The addition of indomethacin, a potent inhibitor of prostaglandin synthesis, failed to abrogate the suppressive effects of MoF(s) from four lepromatous patients (Fig. 6). Unstimulated control PBMC, when incubated with similar supernatants, did not show any alteration in basal DNA synthesis (Fig. 6, inset).

DISCUSSION

Earlier studies from various laboratories (5, 27, 30), including ours (17-19, 28), had failed to find a causal relationship between suppressor T cell activity and the immunological unresponsiveness observed in lepromatous leprosy. In the present study, using cocultures of T-enriched and monocyte-rich cell fractions from HLA-D-defined paired individuals, it was found that monocytes from lepromatous patients were unable to support M. leprae-induced lymphoproliferative responses of a tuberculoid leprosy patient.

TABLE 4. Time kinetics of the release of MoF(s) from a lepromatous patient on the soluble M. leprae antigen-induced lymphoproliferative responses of a tuberculoid leprosy patient

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>Mean cpm ± SD of antigen-stimulated PBMC + MoF(s) (% suppression)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0–24</td>
<td>5,958 ± 632</td>
</tr>
<tr>
<td>24–48</td>
<td>1,241 ± 198 (80)</td>
</tr>
<tr>
<td>48–72</td>
<td>2,641 ± 228 (56)</td>
</tr>
<tr>
<td>72 hrs–15 days</td>
<td>5,729 ± 707 (4)</td>
</tr>
</tbody>
</table>

*Twenty-four hours before collection of MoF(s) monocytes were washed and replaced with fresh RPMI 1640, and MoF(s) were collected de novo or after treatment of monocytes with M. leprae. Supernatants collected on day 15 refer to cultures left uninterrupted after a change of medium at 72 h.
proliferation of responder individuals, whereas similar cells from tuberculoid individuals were able to do so. These results confirm those of our earlier studies in which HLA-D-identical nonadherent cell and adherent cell populations from siblings with discordant leprosy were investigated (19). However, neither of these studies discriminated between a defect in antigen-presenting capacity and the suppressive ability of monocytes from lepromatous patients, as no stimulation was obtained in any of the cultures. Antigen-related membrane defects (2) and a failure in antigen presentation of macrophages have been postulated by some (10) and refuted by others (30).

Evidence from our study (15) and other studies (14, 33) has indicated that macrophages in lepromatous dermal granulomas possess abundant Ia antigens, one of the prerequisites considered to be important for antigen presentation. Thus, further experiments were designed to evaluate the suppressive effects of monocytes. Adherent cells were treated with heat-killed M. leprae as well as bacilli which had been kept frozen after fresh extraction to preserve any labile antigens. Our results indicate that monocytes from lepromatous patients spontaneously release soluble factors which suppress antigen-induced lymphoproliferation of tuberculoid patients. Such supernatants inhibited in vitro mitogenic responses of healthy individuals to a lesser degree. In untreated lepromatous leprosy the suppression exerted by de novo released factors was of the same order as that exerted by the

**TABLE 5. Effect of temperature on MoF(s) released de novo from lepromatous leprosy patients**

<table>
<thead>
<tr>
<th>No. of MoF(s)</th>
<th>Nil cpm ± SD</th>
<th>Untreated cpm ± SD</th>
<th>Heated at 56°C for 30 min cpm ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5,465 ± 178</td>
<td>1,045 ± 63 (81)</td>
<td>1,321 ± 50 (76)</td>
</tr>
<tr>
<td>2</td>
<td>5,465 ± 178</td>
<td>1,211 ± 116 (78)</td>
<td>1,074 ± 84 (80)</td>
</tr>
<tr>
<td>3</td>
<td>5,465 ± 178</td>
<td>1,409 ± 403 (74)</td>
<td>1,595 ± 200 (71)</td>
</tr>
<tr>
<td>4</td>
<td>7,687 ± 435</td>
<td>2,869 ± 616 (63)</td>
<td>2,661 ± 907 (65)</td>
</tr>
<tr>
<td>5</td>
<td>7,687 ± 435</td>
<td>2,752 ± 255 (64)</td>
<td>2,645 ± 201 (66)</td>
</tr>
<tr>
<td>6</td>
<td>7,687 ± 435</td>
<td>2,740 ± 407 (64)</td>
<td>2,967 ± 269 (61)</td>
</tr>
</tbody>
</table>

* The antigen used was soluble armadillo-derived *M. leprae* (AB40).

FIG. 5. Five individual experiments depicting the suppressive activity of MoF(s) in the ultrafiltration fraction of >25,000 molecular weight. Mean counts per minute (---) ± SD (---) of the soluble *M. leprae* antigen-induced lymphoproliferation of control PBMC without addition of factors is indicated. Mean counts per minute ± SD of antigen-stimulated PBMC cultures in the presence of unfractionated (□) and <25,000 molecular weight (△) and >25,000 molecular weight (□) factors is given as bars.
antigen-treated monocyte supernatants. The possibility of intracellular bacilli in the circulation of these patients influencing the production of suppressive factors cannot be excluded. However, patients treated for 5 to 7 years who harbored decreased numbers of residual bacilli in the skin showed lower suppressive effects, particularly in the de novo supernatants. Since all the factors had been filtered through 0.45-μm Millipore membranes, particulate antigens were not considered to be contributing to the suppressive activity. Monocytes from tuberculoid leprosy patients treated in a similar manner showed a conspicuous absence of suppressive factors. The suppressive activity of the MoF(s) from lepromatous patients had a molecular weight of >25,000 and was heat stable, indomethacin resistant, and continuously produced for up to 72 h.

In recent years, MoF(s) have been reported in guinea pigs and mice infected with Mycobacterium tuberculosis (34) and BCG (31). In the former experimental model, spontaneous release of suppressive factors was observed, a feature similar to ours. Such factors associated with human adherent cells treated with many species of mycobacteria, including M. leprae, have also been shown to inhibit blastogenesis, protein synthesis, and lymphokine production of lymphocytes (26, 35).

Monocyte/macrophage factors rich in thymidine (20), arginase (13), interferon (22), and prostaglandins (8) have been reported to have suppressive effects on lymphoproliferative responses. The presence of thymidine and other low-molecular-weight compounds can be ruled out in our system, in which suppression was observed exclusively in the molecular weight ranges above 25,000. However, our present studies cannot conclusively rule out either interferon- or prostaglandin-related suppression. Although indomethacin treatment failed to abrogate the suppressive effects, the observed de novo production of suppressive factors could suggest the possibility of an in vivo induction of prostaglandin whose effects would not be abrogated by the later in vitro addition of indomethacin. Similarly, although prostaglandins are low-molecular-weight compounds, their presence in micellar form cannot be excluded.

The present results suggest the possibility that suppressive factors released from the peripheral blood monocytes of lepromatous patients play a significant role in the immunological unresponsiveness associated with this disease. Whether the release of these factors is influenced by T cells, and conversely, whether these factors function through other suppressive cells requires further elucidation. It would be of considerable importance to determine whether the suppressive factors produced by monocytes/macrophages in the lesions also release such suppressive factors and whether this suppression could be abrogated by immunological manipulation.

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