The Leukocyte Inhibitory Factor and Circulating Immune Complex in Leprosy Patients

Se Jong Kim¹, In Hong Choi¹, Sang Nae Cho¹, Sung Hwa Kim² and Joo Deuk Kim¹

To investigate leukocyte inhibitory factor (LIF) production and circulating immune complexes (CIC) in leprosy, peripheral blood mononuclear cells (PBMC) from 61 patients and sera from 60 patients were tested. The results indicate that there is a defect in LIF production in the lepromatous (LL) or borderline lepromatous (BL) types compared to the tuberculoid (TT) type (mean migration index - 66.0 ± 16.0 in LL, 61.1 ± 15.3 in BL, 51.9 ± 11.2 in TT) (p < 0.05). The number of patients with positive CIC was higher among the LL patients (30%) than the TT patients (20%). There was also a positive correlation between the bacterial index (BI) and the CIC level (r = 0.46, p < 0.05). The correlation between CIC and LIF in LL patients and the possibility (p = 0.06) that the increase in CIC may account for the decrease in LIF production in LL patients and vice versa are discussed.

Key Words: Leprosy, leukocyte inhibitory factor, circulating immune complexes

Leprosy exhibits a wide range of cell-mediated responses in disease states ranging from the paucibacilli high resistant form, tuberculoid leprosy (TT) to the multibacillary low resistant form, lepromatous leprosy (LL) (Sansonetti and Lagrange 1981). Skin lesions from the TT type reveal an organized granuloma, many CD4+ helper T cells, and good T cell proliferative responses to specific antigens. In contrast, lepromatous leprosy is characterized by a deficient cellular response with a predominance of CD8 + suppressor/cytotoxic cells in the dermis, the presence of bacilli-laden macrophages, and the absence of T cell proliferative responses (Godal 1978; Van Voorhis et al. 1982; Modlin et al. 1983).

Some evidence has implicated the leukocyte inhibitory factor (LIF) as an important and effective tool for estimating cellular immune response in vitro (Clausen 1973). Moreover, LIF is an important lymphokine for microbicidal systems such as phagocytosis (Borish and Rocklin 1987a) and the antibody-dependent cell-mediated cytotoxicity of polymorphonuclear leukocytes (PML) (Borish and Rocklin 1987b).

There are some reports suggesting that circulating immune complexes (CIC) are found more frequently in LL than in TT (Bjorvatin et al. 1976; Park et al. 1984). So the detection of immune complexes may be particularly relevant to the clinical condition of leprosy, especially to the erythema nodosum leprosum (ENL) complication of LL, since immunohistological studies suggest participation of immune complexes in the pathogenesis of ENL.

We now report that peripheral blood lymphocytes from leprosy patients failed to produce LIF upon exposure to specific antigen M. leprae and that the CIC level in serum was higher in LL and that the level of CIC is correlated with the bacterial index (BI).

MATERIALS AND METHODS

Study Populations

Patients were selected from those attending Catholic Skin Clinic, Taegu or World Vision Leprosy Clinic, Seoul. Clinical diagnosis of leprosy was made by BI in skin, skin biopsy and lepromin skin test as
described previously (Ridley and Jopling 1966).

**Preparation of cells**

Twenty ml of heparinized blood was obtained from each subject. Peripheral blood mononuclear cells (PBMC) were separated by centrifugation over Ficoll-Hypaque (Pharmacia Fine Chemicals, Piscaray, Nj.) (Boyum 1968).

**Antigen**

*M. leprae* was kindly provided by Dr. P. J. Brennan, Dept. Microbiology, Colorado State University, Fort Collins, Co., U.S.A. and used at a final concentration of 10 µg/ml.

**LIF production and assay**

PBMC were suspended in a concentration of 1×10⁶/ml in RPMI medium (Hazleton research product, Denver, Pa.) containing 10% heat treated human AB serum (donated by the Mogam Biotechnology Research Institute, Seoul, Korea). 1.6mM L-glutamine, 100 µg/ml streptomycin and 100 IU/ml penicillin. One hundred microliters of the cell suspension were pipetted into the round bottom wells of a microtiter plate (Costar, Cambridge, Ma.) and incubated with *M. leprae* for 5 days at 37°C in a humidified 5% CO₂ incubator. Cell free LIF supernatants were harvested and stored at -20°C until assayed. Control samples consisted for unstimulated PBMC culture supernatants were prepared similarly.

LIF was assayed by the indirect two-stage agarose method (Clausen 1973; Choi et al. 1984). PMN were isolated from fresh normal heparinized peripheral blood by sequential application of Ficoll-Hypaque centrifugation, 5% dextran MW=250,000, Sigma, St Louis, MO.) sedimentation and hypo-osmolar lysis of contaminated erythrocytes (Weisbart et al. 1982). Pooled PMN in a concentration of 3×10⁶/ml from three donors were suspended in 30 µl of LIF containing supernatants or control PMN suspension was pipetted into each well of the agarose plate containing RPMI media, 1% agarose (Bio-Rad Laboratories, Richmond, Ca.), 10% horse serum (Gibco, Grand Island, Ma.), 150 µg/ml streptomycin and 150 IU/ml penicillin.

After 18 hrs in a 37°C, 5% CO₂ incubator, the agarose plates were fixed with 2% glutaraldehyde for 2 hours, washed and stained with 2% Giemsa solutions.

The migration area was measured by Yonsei Anatomy Program 1, a computerized cell measure-

**RESULTS**

**LIF production**

The LIF activity of the PBMC was measured in 61 leprosy patients, including 35 LL patients, 15 borderline lepromatous (BL) patients, 9 TT patients and 2 borderline tuberculoid (BT) patients.

The leukocyte migration index of the LL (60.6±16.0) or BL (61.1±15.3) patients was significantly higher than that of the TT (51.9±11.2) patients. Thus, LL or BL pat-

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**Table 1. LIF activity in leprosy patients**

<table>
<thead>
<tr>
<th>Group</th>
<th>No.</th>
<th>Migration index (mean±S.D.)</th>
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<tbody>
<tr>
<td>LL</td>
<td>35</td>
<td>60.6±16.0*</td>
</tr>
<tr>
<td>BL</td>
<td>15</td>
<td>61.1±15.3*</td>
</tr>
<tr>
<td>TT</td>
<td>9</td>
<td>51.9±11.2</td>
</tr>
<tr>
<td>BT</td>
<td>2</td>
<td>62.8±12.0</td>
</tr>
</tbody>
</table>

* p<0.05; LL vs TT, BL vs TT

**Table 2. LIF activity based on Bi in LL or BL patients**

<table>
<thead>
<tr>
<th>Group</th>
<th>No.</th>
<th>Migration index (mean±S.D.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bi positive</td>
<td>19</td>
<td>61.2±19.4</td>
</tr>
<tr>
<td>Bi negative</td>
<td>34</td>
<td>60.6±14.2</td>
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patients showed a significant decreased in LIF activity as compared to that of the TT patients (p<0.05) (Table 1).

But there was no significant difference between LIF activity and BL in LL or BL patients (Table 2 and Fig. 1). The leukocyte migration indices were 61.2±19.4 in BL positive patients and 60.6±14.2 in BL negative LL or BL patient (Table 2).

**CIC levels in serum**

The levels of CIC were measured by PAT with sera from 60 leprosy patients, 50 lepromatous and 10 tuberculoid leprosy patients. The number of patients exhibiting a positive PAT reaction was higher among the LL patient (30%) than TT patients (20%) (Table 3 and Fig. 2). There was also a positive correlation be-
between the BI and CIC levels of the LL patients (r = 0.46; p < 0.06) (Fig. 3).

The correlation coefficient between the BI and CIC levels in the LL patients was 0.43 with p-value of 0.06 (Table 4).

**DISCUSSION**

Studies on the unresponsiveness of leprosy have been concerned with cellular immune function (Hahn and Kaufman 1981; Closs et al. 1982). LIF is one class of lymphokines produced by stimulated T lymphocytes, and its function is to inhibit the migration of PMN from an inflammatory site (Rocklin 1974). It has been shown that the in vitro productivity of LIF by lymphocytes following stimulation with antigens such as PPD, streptokinase-streptodornase and candida, has been correlated with in vivo delayed hypersensitivity using the same antigen (Astor et al. 1973). Thus, the LIF assay may be a reliable immunological procedure to determine the competence of T cell-mediated immunity.

We reported defective LIF production by PBMC to phytohemmagglutinin in LL patients (Choi et al. 1984; Choi et al. 1986). In this paper, a specific defect in the production of LIF to the M. lepra antigens is described, and this is supported by the recent reports about deficit in other lymphokines such as IL-2 (interleukin-2) (Ottenhoff et al. 1984; Mohaghpour et al. 1985) and gamma-interferon (Nogueira et al. 1986) to M. lepra in LL patients. The preponderance of CD8+T cells in skin lesions might be associated with the lack of IL-2 production and accordingly, the absence of IL-2 might lead to a defective expansion of specifically sensitized T cells and account for the decreased LIF activity.

The increased serum level of CIC in LL patients was reported in our previous paper (Park et al. 1984) as measured by the polyethylene glycol precipitation complement consumption test. The present study confirmed the increase in CIC in LL patients with the PAT and that CIC levels have a positive correlation with BI in LL patients.

There are some reports that immune complexes have been implicated as factors contributing to the inhibition of immunologic reactivity (Nelson and Gette 1978). Although we found PBMC from LL patients also showed defective LIF production in vitro, the possibility that CIC could inhibit lymphocyte functions and vice versa in LL patients still remains.

**REFERENCES**


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