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The Leukocyte Inhibitory Factor and Circulating Immune Complex in Leprosy Patients

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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 tubrculoid (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 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 Lagrane 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

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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.) (Boyüm 1968).

Antigen

M. leprae was kindly provided by Dr. P. J. Brennan, Dept. Microbiology, Collorado State University, Fort Collins, Co., U.S.A. and used at a final concentration of 10 $\mu g/m\ell$.

LIF production and assay

PMBC were suspended in a concentration of $1\times10^6/m\ell$ in PRMI 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 $\mu g/m\ell$ streptomycin and 100 IU/$m\ell$ 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% CO_2 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 $3\times10^6/m\ell$ from three donors were suspended in 30 $\mu\ell$ 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 $\mu g/m\ell$ streptomycin and 150 IU/$m\ell$ penicillin.

After 18 hrs in a 37°C, 5% CO_2 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 measurement system under a light microscope (16×). LIF activity was expressed as a migration index.

$$\text{Migration index} = \frac{\text{mean migration area for test sample}}{\text{mean migration area for control sample}} \times 100$$

Detection of CIC

CIC in the serum were detected by the platelet aggregation test (PAT) (Myllyla *et al.* 1971). The platelets were collected from fresh human peripheral blood by differential centrifugation, washed twice with saline and suspended in a concentration of 2×10^6 cells/$m\ell$ in PBS containing 0.018% glucose (pH=7.8). Test samples (25 $\mu\ell$) were diluted in a round bottom microtiter plate and 50 $\mu\ell$ of the platelet suspension were added to each well. After 18 hours of incubation at 4°C, platelet aggregation was examined macroscopically.

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-

Table 1. LIF activity in leprosy patients

Group	No.	Migration index (mean±S.D.)
LL	35	60.6±16.0*
BL	15	61.1±15.3*
TT	9	51.9±11.2
BT	2	62.8±12.0

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

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

Group	No.	Migration index (mean±S.D.)
BI positive	19	61.2±19.4
BI negative	34	60.6±14.2

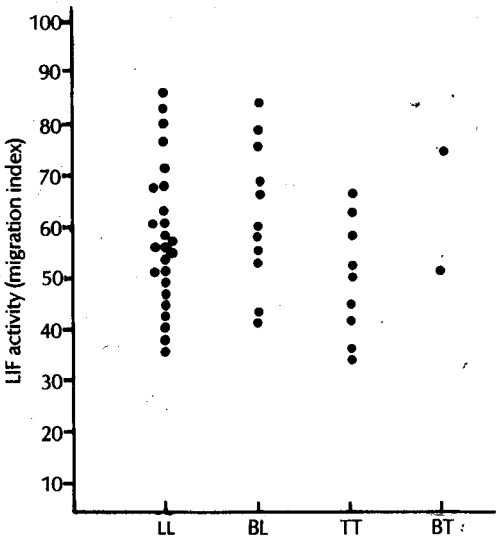

Fig. 1. *LIF activity in leprosy patients.*

Table 3. CIC levels in serum

Group	No.	Titer≥1:8 by PAT
LL	50	15 (30%)
TT	10	2 (20%)

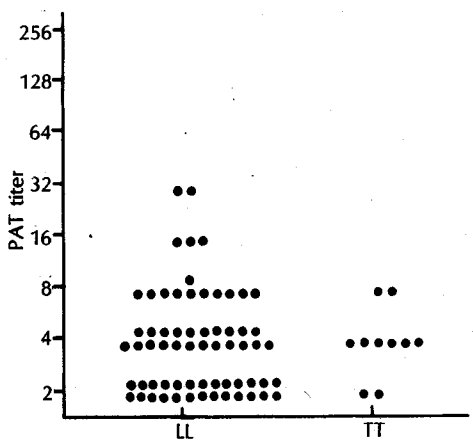

Fig. 2. *CIC levels in leprosy patients.*

tients 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 BI in LL or BL patients (Table 2 and

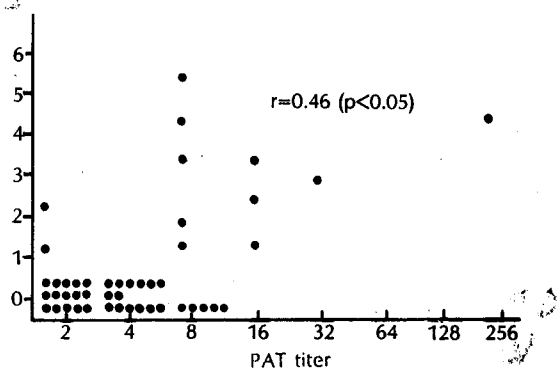

Fig. 3. *CIC levels based in BI.*

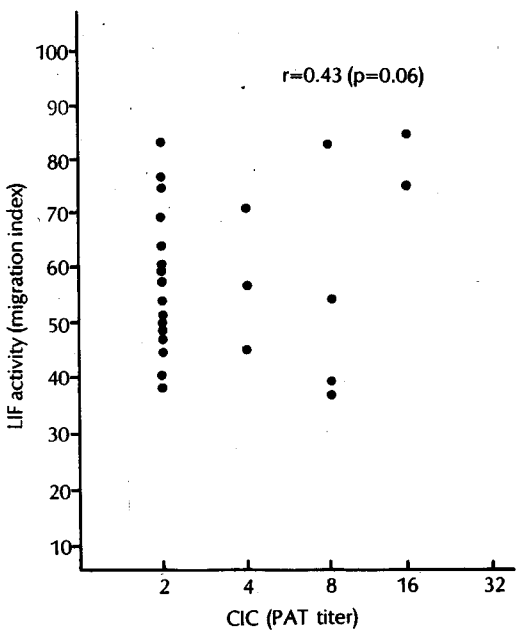

Fig. 4. *Correlation between LIF and CIC.*

Fig. 1). The leukocyte migration indices were 61.2±19.4 in BI positive patients and 60.6±14.2 in BI 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-

tween 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 immunlogical procedure to determine the competence of T cell-medicated 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. leprae* antigen 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; Mohaghhpour *et al.* 1985) and gamma-interferon (Nogueira *et al.* 1986) to *M. leprae* in LL patients. The prepondeance 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 polyethyleneglycol 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.

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