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In Vitro Effect of Interleukin-2 on Proliferative Responses of Peripheral Blood T Cells from Leprosy Patients

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Because of the important role played by interleukin-2(IL-2) in T cell growth and differentiation, we investigated the effect of exogenous IL-2 on the proliferative response of peripheral blood mononuclear cells(PBMCs) from 77 leprosy patients. The proliferative responses of PBMCs from lepromatous leprosy(LL) or borderline lepromatous leprosy(BL) patients to M. leprae were significantly lower(cpm 6,051 ± 803 for LL type; 4,951 ± 2,529 for BL type) than those from tuberculoid leprosy(TT) or borderline tuberculoid leprosy(BT) patients (28,853 ± 28,916 for TT type; 15,884 ± 334 for BT type). To investigate the effect of exogenous IL-2, purified IL-2 was added at the start of culture at 100 unit/ml. There was an apparent increase in ^{3}H-thymidine incorporation of M. leprae-stimulated PBMCs(18,723 ± 6,503) in the presence of IL-2 compared to the results without IL-2(6,051 ± 803) in LL patients. Twenty nine out of 33 LL patients belonged to the responders to IL-2 and four patients were nonresponders. Therefore we conclude that the defective cell mediated immune response in LL patients may result from diminished production of IL-2, but we can not exclude the possibility of diminished expression of the IL-2 receptor. And we suggest that the immunologic heterogeneous response of an individual to M. leprae is important to the pathogenesis of clinical disease in the same LL patients.

Key Words: Interleukin-2, leprosy, cell-mediated immunity

Leprosy is a chronic infection caused by *Mycobacterium leprae*. The immunologic basis for susceptibility to infection is not yet understood. Leprosy exhibits a wide range of cell-mediated immune response in disease states ranging from the paucibacilli high resistant form, tuberculoid leprosy (TT), to the multibacilli low resistant form, lepromatous leprosy (LL) (Godal, 1978; Sansonetti and Lagrane, 1981; Nath *et al.* 1984; Kaplan *et al.* 1985). Skin lesions from the TT type reveal an organized granuloma, many CD4+T cells, and good T cell proliferative responses to specific antigen. In contrast, lepromatous leprosy is characterized by a deficient cellular response with a predominance of CD8+T cells in the dermis, the presence of bacilli-laden macrophages, and the absence of T cell proliferative responses (Van Voorlis *et al.* 1982; Modlin *et al.* 1983). So, the selective immune hyporesponsiveness of LL patients to antigen of *M. leprae* appears to be crucial to the pathogenesis of their clinical stage.

Responding to antigens, T cell proliferation is critically dependent on interleukin-2 (IL-2). IL-2 has been reported to promote the proliferation and the generation of cytotoxic T lymphocytes from an alloantigen-stimulated T cell population and to augment antibody synthesis. IL-2 also has been shown to maintain antigen-specific activated helper T cells specific alloreactive cytolytic T cells in long-term culture (Gillis and Smith, 1978).

In human, decreased IL-2 production has been

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shown in patients with systemic lupus erythematosus (Warrington *et al,* 1989), rheumatoid arthritis (Kitas *et al,* 1988) and pulmonary tuberculosis (Toossi *et al,* 1986). Another important lymphokine, γ-interferon was reported to be lower in the production among leprosy patients (Horwitz *et al,* 1984); the production of leukocyte inhibitory factor was also lower (Kim *et al.* 1988). The peripheral blood mononuclear cells (PBMCs) of *Mycobacterium bovis* infected mice showed defective proliferative response; exogenous IL-2 corrected such defective T cell proliferation (Colizzi, 1984). The addition of conditioned medium in the cultures of PBMCs from LL patients enhanced the proliferation of PBMCs to *M. leprae* (Hoffenbach *et al,* 1983; Mohaghepour *et al,* 1984; Mohaghepour *et al.* 1985) and the production of γ-interferon (Nogueria *et al,* 1986; Converse *et al.* 1988). This might be due to the presence of IL-2 present in the conditioned medium.

In this study, we investigated phytohemagglutinin (PHA)- or purified protein derivatives (PPD)-, or *M. leprae* -induced lymphocyte proliferation in leprosy patients. Because of the important role believed to be played by IL-2 in T cell growth and differentiation, we investigated the effect of exogenous IL-2 on the proliferative response of peripheral blood lymphocytes from LL patients.

MATERIALS AND METHODS

Study population

Seventy-seven patients with leprosy from the Catholic Skin Clinic (Taegu, Korea) were studied. Diagnosis was based on clinical features and Wade's skin test and was eventually confirmed by histopathology of biopsy speciments.

Preparation of PBMCs

Twenty ml of heparinized (20 U/ml) blood was obtained from each subject. PBMCs were separated by Ficoll-Hypaque (Pharmacia Fine Chemicals, Pistacavay, NJ) density gradient (Böyum, 1968) centrifugation.

Antigen and reagents

Gamma-irradiated *M. leprae* bacilli was kindly donated by Dr. Patrick J. .Brennan (Colorado State University, U.S.A.) and at a final concentration of 10 μg/ml. PPD (Statens Seruminstitut, Copenhagen, Denmark) was used at a final concentration of 20 μg /ml. PHA (PHA-M, Gibco, Grand Island, NY) was used at a final concentration of 13 μg/ml. Purified IL-2 (Collaborative Research Inc., Badford, MA) was used at 100 unit/ml.

Assay for proliferation of PBMCs

Ficoll-Hypaque separated PBMCs were suspended at 1 x 10^6 cells/ml in RPMI 1640 (Hazleton, Denver, PA) supplemented with ten % vol/vol heat-inactivated pooled human AB serum (Green Cross, Seoul, Korea). One hundred thousand cells were cultured in round bottomed microculture plate (Costar, Cambridge, MA) after adding *M. leprae,* PPD or PHA in triplicate. To evaluate exogenous IL-2 effect, purified IL-2 was added to wells with *M. leprae,* PPD or PHA. Cultures were incubated at 37°C in 5% CO_2 for five days. Eighteen hours before the termination of culture, 1 μCi of ^{3}H-thymidine (sp act 6.7 Ci/mmol, New England Nuclear, Boston, MA) was added per well. After harvesting by aspirating onto glass filter paper, the radioactivity was assessed by liquid scintillation counter (Beckman, Fullerton, CA); the results were expressed as counts per min (cpm).

Statistical analysis

Unpaired Student's t-test was used for intergroup comparisons and paired Student's t-test was used for the evaluation of IL-2 effect in intragroup.

RESULTS

Proliferative responses of PBMCs from leprosy patients to *M. leprae,* PPD or PHA

From peripheral blood of 77 leprosy patients, PBMCs responsiveness to *M. leprae,* PHA or PPD were studied. There was an apparent lymphocyte proliferative response in PBMCs from TT (cpm 28,853 $\pm$ 28,916) or borderline tuberculoid leprosy (BT) (15,884 $\pm$ 334) patients, but not in LL (cpm 6,051 $\pm$ 803) or borderline lepromatous leprosy (BL) (4,951 $\pm$ 2,529) patients to *M. leprae* (Table 1). The responses to PPD or PHA were similar between all groups (Table 2 and Table 3).

Proliferative responses of PBMCs from leprosy patients to exogenous IL-2

The effects of exogenous IL-2 on PBMC responsiveness to *M. leprae,* PHA or PPD were studied. Purified IL-2 was added from the start of culture with PBMC at 100 unit/ml in proliferation assay. There was an apparent increase in ^{3}H-thymidine incorporation of *M. leprae*-stimulated PBMC of

Table 1. Effect of exogenous IL-2 on peripheral blood lymphocyte proliferative response to *M. leprae* among leprosy patients

Group	No.	Lymphocyte proliferation(cpm)[a] mean±SD			
		M. leprae	control	*M. leprae*+*IL-2*	IL-2 control
Lepromatous	40	6,051± 803[b]	648± 98	18,723± 6,503[d]	5,583±5,484
Borderline lepromatous	16	4,951± 2,529[b]	357±194	21,337±12,261[d]	2,649±1,917
Tuberculoid	15	28,853±28,916	1,182±194	35,775±24,934[e]	5,182±9,219
Borderline tuberculoid	3	15,884± 334[c]	125± 38	28,811±11,077[e]	771± 803

[a] PBMCs($1x10^5$/well) were cultured for 5 days with *M. leprae* (10 μg/ml). Eighteen hours before termination, 1 μCi of ^{3}H-thymidine was pulsed. PBMCs were harvested and the radioactivity was counted by scintillation counter. Purified interleukin-2 (100 unit/ml) was added to lymphocyte culture medium at the start of culture.
[b] $p<0.01$ versus tuberculoid group.
[c] $P>0.05$ versus tuberculoid group.
[d] $p<0.01$ versus *M. leprae* only in intragroup.
[e] $p>0.05$ versus *M. leprae* only in intragroup.

Table 2. Effect of exogenous IL-2 on peripheral blood lymphocyte proliferative response to PPD among leprosy patients

Group	No.	Lymphocyte proliferation(cpm)[a] mean±SD			
		PPD	control	PPD+IL-2	IL-2 control
Lepromatous	40	50,721±30,605[b]	648± 98	56,611±32,202[c]	5,583±5,484
Borderline lepromatous	16	39,626±28,825[b]	357±194	46,462±27,082[c]	2,649±1,917
Tuberculoid	15	41,364±22,072[b]	1,182±194	46,744±23,994[e]	5,182±9,219
Borderline tuberculoid	3	64,921±35,071	125± 38	74,020±21,788[e]	771± 803

[a] PBMCs (1 x 10^5/well) were cultured for 5 days with PPD (20 μg/ml). Eighteen hours before termination, 1 μCi of ^{3}H-thymidine was pulsed. PBMCs were harvested and the radioactivity was counted by scintillation counter. Purified interleukin-2 (100 unit/ml) was added to lymphocyte culture medium at the start of culture.
[b] $p>0.05$ versus tuberculoid group.
[c] $p>0.05$ versus PPD only in intragroup.

Table 3. Effect of exogenous IL-2 on peripheral blood lymphocyte proliferative response to PPD among leprosy patients

Group	No.	Lymphocyte proliferation(cpm)[a] mean±SD			
		PHA	control	PHA+IL-2	IL-2 control
Lepromatous	40	112,016±58,142[b]	648± 98	104,027±59,573[c]	5,583±5,84
Borderline lepromatous	16	107,946±77,920[b]	357±194	69,684±52,260[c]	2,649±1,917
Tuberculoid	15	76,713±52,300	1,182±194	104,311±72,478[c]	5,182±9,219
Borderline tuberculoid	3	134,462±49,791[b]	125± 38	131,290±56,645[c]	771± 803

[a] PBMCs (1 x 10^5/well) were cultured for 3 days with PHA (13 μg/ml). Eighteen hours before termination, 1 μCi of ^{3}H-thymidine was pulsed. PBMCs were harvested and the radioactivity was counted by scintillation counter. Purified interleukin-2 (100 unit/ml) was added to lymphocyte culture medium at the start of culture.
[b] $p>0.05$ versus tuberculoid group.
[c] $p>0.05$ versus PHA only in intragroup.

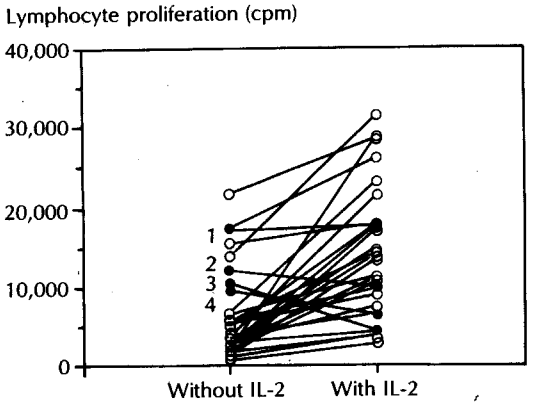

Fig. 1. *Effect of exogenous IL-2 on peripheral blood lymphocyte proliferative response to M. leprae among lepromatous leprosy patients. PBMCs (1 x 10^5/well) were cultured for 5 days with M. leprae (10 μg/ml). Eighteen hours before termination, 1 μCi of [^{3}H] thymidine was pulsed. PBMCs were harvested and the radioactivity was counted by scintillation counter. Purified IL-2 (100 unit/ml) was added to lymphocytes culture medium at the start of culture. Nonresponders to exogenous IL-2 are expressed as closed circles(No. 1, 2, 3 and 4).*

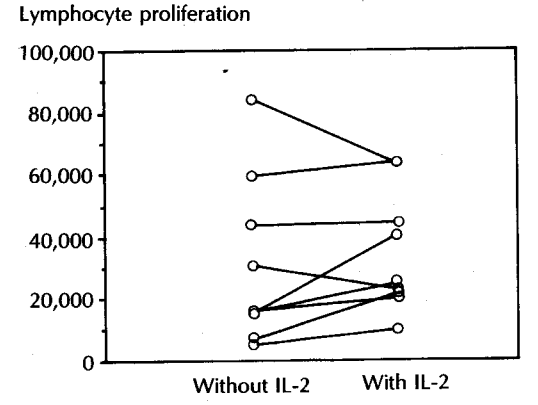

Fig. 2. *Effect of exogenous IL-2 on peripheral blood lymphocyte proliferative response to M. leprae among tuberculoid leprosy patients. PBMCs (1 x 10^5/well) were cultured for 5 days with M.leprae (10 μg/ml). Eighteen hours before termination, 1 μCi of ^{3}H-thymidine was pulsed. PBMCs were harvested and the radioactivity was counted by scintillation counter. Purified IL-2 (100 unit/ml) was added to lymphocytes culture medium at the start of culture.*

LL patients (18,723 ± 6,503) in the presence of exogenous IL-2, when compared to the results without IL-2 (6,501 ± 803). But PBMCs from TT patients (35,775 ± 24,934) did not show an apparent increase in ^{3}H-thymidine incorporation of *M. leprae*-stimulated PBMC in the presence of exogenous IL-2 when compared to the results without IL-2 (28,853 ± 28,916) (Table 1 and Fig. 2).

LL patients were divided to responders or nonresponders arbitrarily: responders showed an increase in cpm by 10% or more when PBMCs were stimulated with *M. leprae* in the presence of exogenous IL-2 compared to that with *M. leprae* alone; and nonresponders showed a decrease or an increase by less than 10% in cpm. Twenty nine out of 33 patients were responders to exogenous IL-2, and the remaining four patients were nonresponders to exogenous IL-2 (Fig. 1; expressed as closed circles, No. 1, 2, 3 and 4).

DISCUSSION

Our data unequivocally show that PBMCs from TT or BT patients responded normally to *M. leprae* bacilli, but that PBMCs from LL patients did not. PBMCs from LL patients show a much lower proliferative response when compared to those from BT or TT patients ($p<0.01$). However, the responses of PBMCs from LL patients to related but distinct antigen PPD, or to the nonspecific mitogen PHA, are similar to the responses of TT patients.

Adding exogenous IL-2, the proliferative response of PBMCs from LL patients to *M. leprae* antigen increased. These data are consistent with existing reports (Haregewoin *et al.*, 1982; Modlin *et al*, 1984). There are supporting reports that *M. leprae* reactive T cells exist in leprosy patients (Laal *et al*, 1985; Chiplunker *et al*, 1986; Launois *et al*, 1987; Desei *et al*, 1988). Exogenous IL-2 had no effect on the PPD- or PHA-induced PBMCs proliferation in LL or TT groups. These data have suggested that the immunologic alteration; selective immune hyporesponsiveness to *M. leprae* antigen, in the majority of LL patients, are associated with a defective production of IL-2 (Table 4).

LL patients included in this study can be divided into two groups on the basis of the responsiveness to exogenous IL-2 arbitary. Twenty-nine out of 33 patients were responders to exogenous IL-2; the remaining four patients were nonresponders to exogenous IL-2 (Fig. 1; No. 1, 2, 3 and 4). These results indicate that it is possible to reverse *in vitro* the

specific unresponsiveness to *M. leprae* in most but not all LL patients. Of the 33 LL patients, 21 showed at least a two-fold increase in ^{3}H-thymidine incorporation in the presence of exogenous IL-2, but 12 patients including four nonresponders showed an increase lower than two-fold. Therefore, we concluded that there exists an apparent heterogeneity in the response to the addition of IL-2 *in vitro* among LL patients. We suggest that the immunologic heterogeneous response of an individual to *M. leprae* is important to the pathogenesis of clinical disease even in the same LL patients. Exogenous IL-2 could not enhance the proliferative response from TT patients to *M. leprae* antigen because the PBMCs from TT patients were fully responsive *M. leprae* antigen (Fig. 2). The proliferative responses to PPD or PHA of PBMCs from LL and TT patients were not enhanced by exogenous IL-2. That result is quite possible because the PBMCs from patients had already responded to PPD or PHA.

Because it has been found that IL-2 can adjust the expression of its own receptors *in vitro* (Reem *et al*, 1985), we wondered if the defects of IL-2 production during the initial activation phase might be responsible for the abnormal IL-2 receptor expression. This is likely to happen in PBMCs of LL patients, and adding exogenous IL-2 *in vitro* at the start of culture with *M. leprae* significantly enhanced IL-2 responsiveness. It still remains, however, to be determined if this enhancement of responsiveness is secondary to increased IL-2 receptor expression.

Therefore, we conclude that the defective cell mediated immune response in LL patients may result from diminished IL-2 and some other lymphokine, such as leukocyte inhibitory factor or γ-interferon. But we still can not exclude the possibility of diminished expression of the IL-2 receptor because there are PBMCs from some LL patients that do not respond to exogenous IL-2.

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