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Interleukin-1β Production by Monocytes from Leprosy Patients

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The cause responsible for the lack of an efficient cell-mediated immunity or a delayed type hypersensitivity to M. leprae in lepromatous patients is poorly understood. But the resistance to M. leprae infection in humans is likely mediated by the activated macrophages to present M. leprae antigen to T cells for cell-mediated immunity. Phenolic glycolipid-I (PGL-I) is a M. leprae-specific antigen and is supposed to play a significant role in the long lasting unresponsiveness in lepromatous leprosy. In this study, IL-1 activities were tested among leprosy patients to evaluate monocyte function and the role of IL-1 in the immunosuppression in leprosy. We found that peripheral blood mononuclear cells (PBMCs) from tuberculoid patients were strongly reactive to M. leprae (mean cpm; 28,853±28,916), but the proliferative responses of PBMCs from lepromatous patients (mean cpm; 6,051±803) were significantly lower. IL-1 concentration in culture supernatant of monocytes from lepromatous patients was similar to that from tuberculoid patients with stimulation of M. leprae (lepromatous: 1,014±637 pg/ml, tuberculoid: 1,012±167pg/ml) or lipopolysaccharides (LPS) (lepromatous: 3, 479±2,188pg/ml, tuberculoid: 4,246±2,432pg/ml). The IL-1 concentration in sera from lepromatous patients (42±30pg/ml) tended to be higher than those from tuberculoid patients (28±69pg/ml). And there was no significant difference in IL-1 production between peritoneal macrophages from mice sensitized with PGL-I and those from nonsensitized mice. In conclusion, this study suggests that the immunosuppression in lepromatous patients may not be due to the decreased production of IL-1. And the increased IL-1 activity in sera may affect the inflammatory response of lepromatous patients.

Key Words: Leprosy, unresponsiveness, monocytes/macrophages, IL-1

Lepromatous leprosy, a generalized infection caused by *Mycobacterium leprae* is associated with a long lasting antigen specific unresponsiveness (Faber *et al.* 1978; Kaplan *et al.* 1985; Nath *et al.* 1948a). The disease has been characterized by diminished delayed type skin response and an absence of T cell proliferation to lepromin (Godal 1978; Modlin *et al.* 1983).

The cause responsible for the lack of an efficient cell-mediated immunity or a delayed type hypersensitivity to *M. leprae* in lepromatous leprosy (LL) patients is poorly understood, but the defective production of cytokines, such as interferon-γ and interleukin-2 (IL-2), has been reported (Horxitz *et al.* 1984). The addition of conditioned medium in the cultures of peripheral blood mononuclear cells (PBMCs) from lepromatous patients enhanced the proliferation of PBMCs to *M. leprae* and it might be due to the effect of IL-2 present in the medium (Hoffenbach *et al.* 1983; Colizzi *et al.* 1984; Mohaghepour *et al.* 1985; Kim *et al.* 1989). This was also supported by the fact that the presence of exogenous IL-2 in cultures of PBMCs from hyporesponsive leprosy patients enhanced the *M. leprae*-induced proliferation and interferon-γ release (Converse et al. 1988; Kaplan *et al.* 1985; Nath *et al.* 1984b; Nogueira *et al.* 1983).

Resistance or susceptibility to *M. leprae* infection in human is likely mediated by the activated macrophages to present *M. leprae* antigen to T cells for cell-mediated immunity. Some reports suggested that monocytes from LL patients were unable to support the proliferation of *M. leprae* reactive T cells from healthy contacts and from tuberculoid leprosy (TT) pa-

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tients (Chiplunkar *et al.* 1986; Sathish *et al.* 1983). Therefore, such adherent cells may not evoke or even suppress *in vitro* proliferation of PBMCs to *M. leprae* and the production of lymphokines such as IL-2 or interferon-γ.

Interleukin-1 (IL-1) exerts a variety of immunoregulatory effects, among which it has a capacity to act upon T cells in the early G1 phase of the cell cycle preparing them to respond to subsequent signals (Mizel 1980). IL-1 is a polypeptide with a wide spectrum of immunologic and nonimmunologic activities. There are two types, IL-1β and IL-1β, which are products of different genes, but which recognize the same receptor and have same biological activities. IL-1β is membrane-bound and IL-1β is a soluble form. Both of these induce the synthesis and secretion of IL-2 by T cells which is, in turn, essential for the continued proliferation of activated T cells. Thus IL-1 and IL-2 are essential for the amplification of immune responses (Gillis and Smith 1978). IL-1β is the prominent form of IL-1 and the amount of IL-1β mRNA found in activated cells is usually 10-to 50-fold greater than the α form. In addition, culture supernatant and body fluid contain more IL-1β than the α form (Dinarello 1989). So in our study IL-1β contents were measured by ELISA using anti-IL-1β monoclonal antibody.

It is also possible that the lack of immune response to *M. leprae* in LL patients may result from the impairment of IL-1 production by monocytes or macrophages (Wallis *et al.* 1986). Moreover, a proportion of LL patients possess *M. leprae* reactive T cells which can be induced to proliferate in the presence of other HLA-D compatible monocytes (Desei *et al.* 1988; Laal *et al.* 1985; Launois *et al.* 1987;Longley *et al.* 1986).

Phenolic glycolipid-I (PGL-I) is a well known specific antigen of *M. leprae* (Hunter and Brennan 1982). PGL-I has been suggested to play an important role in the pathogenesis of leprosy, because of its abundance on the surface of *M. leprae* and its specificity to *M. leprae* (Cho *et al.* 1984; Cho *et al.* 1988). There was a report that PGL-I suppressed lymphocyte proliferation *in vitro* (Mehra *et al.* 1984). This finding has suggested the possibility that PGL-I may play a significant role in the long lasting *M. leprae* specific unresponsiveness in LL leprosy.

For a better understanding of the mechanisms involved in the unresponsiveness to *M. leprae* in LL patients, we studied *in vitro* production of IL-1 from LL patients and TT patients, and the effect of PGL-I on the production of IL-1.

MATERIALS AND METHODS

Patients: A total of 36 leprosy patients presented at Catholic Skin Clinic (Taegu, Korea) were studied for IL-1 production with peripheral blood monocytes. Diagnosis was based on clinical features and Wade's skin test and was eventually confirmed by histopathology of biopsy specimens (Ridley and Jopling 1966).

For lymphocyte proliferation, 55 patients were studied. They were diagnosed as leprosy by the same criteria described above.

For the assay of IL-1 concentration in sera, a total of 23 sera were collected and stored at −20°C.

Antigen and reagents: Heat-killed *M. leprae*, kindly donated by P. J. Brennan (Colorado State University, Fort Collins, CO) was used at the final concentration of 10μg/ml. PGL-I was also provided by P.J. Brennan. Lipopolysaccharide (LPS, Gibco, Grand Island, NY) was used at the final concentration of 10μg/ml.

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

^{3}H-thymidine incorporation for assay of lymphocyte proliferation: PBMCs were cultured at 1×10^6/ml in RPMI 1640 (Hazleton, Lenexa, KS) supplemented with 10% v/v heat-inactivated pooled human AB serum (Green Cross, Seoul, Korea). One hundred μl of cell suspension was placed in a round bottomed microtiter plate (Costar Co., Cambridge, MA), and *M. leprae* was added at the concentration of 10μg/ml. The cultures were set up in triplicate. Cultures were incubated at 37°C in humidified 5% CO_2 incubator for 5 days. Eighteen hours before the termination of culture, 1.0μCi of ^{3}H-thymidine (specific activity=6.7Ci/mmol, New England Nuclear, Boston, MA) was added per well. Cultures were then harvested and the radioactivities were determined by liquid scintillation counter.

Preparation of monocytes: PBMCs were incubated in a fibronection-coated 25cm² tissue culture flask (Corning, Corning, NY) in a 37°C humidified CO_2 incubator. After 45 min, nonadherent cells were discarded and the flask was washed twice with warm RPMI 1640. To harvest adherent monocytes, 10ml of Ca^{++} and mg^{++} free Hanks' balanced salt solution containing EDTA was added to the flask and detached monocytes were collected.

IL-1 production by monocytes: Monocytes were suspended at the concentration of 2×10^6 cells/ml of RPMI 1640 supplemented with 5% human AB serum.

One ml of cell suspension per well of a 24-well plate (Costar, Grand Island, NJ) was cultured in a 37°C humidified CO_2 incubator with *M. leprae* or LPS. After 24 hours, the culture supernatants were harvested and stored at −20°C until being assayed.

IL-1 assay: IL-1 concentration in culture supernatants and serum was determined by using the ELISA kit (Cistron, Pine Brook, NJ) in comparison with standard recombinant IL-1β. Monoclonal anti IL-1β was used as the primary antibody.

PGL-I effect on IL-1 production of mouse peritoneal cells: C57BL/6 mice were immunized intraperitoneally with *M. leprae* (0.1mg) mixed with an equal volume of incomplete Freund's adjuvant three times with two-week intervals. After that, C57BL/6 mice were given 0.1mg of PGL-I intraperitoneally three times at one week intervals. One week after the last injection, mice were sacrificed and peritoneal exudate cells were harvested by washing the peritoneal cavity with cold RPMI 1640. The harvested peritoneal cells were placed in a 25cm² tissue culture flask in RPMI 1640 media supplemented with 10% fetal calf serum. After incubation for 4 hours in a 37°C humidified CO_2 incubator, the nonadherent cells were discarded. The adherent macrophages were harvested by vigorous pipetting with cold Ca^{++} and Mg^{++} free Hanks' balanced salt solution. Peritoneal macrophages were cultured at 2×10^6/ml in RPMI 1640 media supplemented with 5% fetal calf serum in a 37°C humidified CO_2 incubator with LPS at the concentration of 10μg/ml. After 24 hours, the culture supernatants were harvested and stored at −20°C until being assayed.

Mouse IL-1 activity of the culture supernatant was tested by the thymocyte proliferation test (Waston *et al.* 1984). Briefly, pooled thymocytes (1.5×10^6/100μl) of 4-to 5-week-old C3H/HeJ mice were cultured with serial dilution of test samples for 3 days and thymocyte proliferation was measured by pulsing for 6 hours with 1μCi of ^{3}H-thymidine (specific activity=20Ci/mmol, New England Nuclear, Boston, MA). Test samples were assayed in triplicate.

Statistical analysis: Independent Student's t-tests were used to compare the control and study group.

RESULTS

Proliferative response of PBMCs to *M. leprae*: PBMCs from leprosy patients were stimulated *in vitro* with *M. leprae* and their proliferative responses were measured. As shown in Table 1, PBMCs from TT patients were strongly reactive to *M. leprae* (mean cpm; 28,853±28,916). In contrast, the proliferative responses of PBMCs from LL patients (mean cpm; 6,051±803) to *M. leprae* were significantly lower than those of TT patients.

***In vitro* IL-1 production by monocytes:** Monocytes from leprosy patients were stimulated *in vitro* with *M. leprae* or LPS and IL-1 concentration was determined from the culture supernatants. As shown in Table 2, IL-1 concentration in culture supernatant of *M. leprae*-

Table 1. Proliferative response of peripheral blood lymphocytes to *M. leprae* among leprosy patients

Patients	Number	Lymphocyte proliferation (cpm)* mean±S.D.
Lepromatous leprosy	40	6,051± 803**
Tuberculoid leprosy	15	28,853±28,916

* PBMCs (1×10^5/well) were cultured with heat-killed *M. leprae* (10μg/ml). After 5 days, 1.0μCi of [^{3}H]-thymidine was pulsed for 18 hours and the cells were harvested and counted by liquid scintillation counter.

** $p<0.05$

Table 2. *In vitro* IL-1 production by peripheral blood monocytes stimulated with *M. leprae* among leprosy patients

Patients	Number	IL-1 concentration (pg/ml)* mean ± S.D.
Lepromatous leprosy	23	1,014±637
Tuberculoid leprosy	10	1,012±167

* IL-1 amounts in culture supernatants were tested by ELISA using monoclonal anti IL-1β antibody as the primary antibody.

Table 3. *In vitro* IL-1 production by peripheral blood monocytes stimulated with LPS among leprosy patients

Patients	Number	IL-1 concentration (pg/ml)* mean±S.D.
Lepromatous leprosy	23	3,479±2,188
Tuberculoid leprosy	10	4,268±2,432

* IL-1 amounts in culture supernatants were tested by ELISA using monoclonal anti IL-1β antibody as the primary antibody.

Table 4. IL-1 amounts in sera from leprosy patients

Patients	Number	IL-1 concentration (pg/ml)* mean±S.D.
Lepromatous leprosy	13	42±30
Tuberculoid leprosy	9	28±69

* IL-1 amounts in sera were tested by ELISA using monoclonal anti IL-1β antibody as the primary antibody.

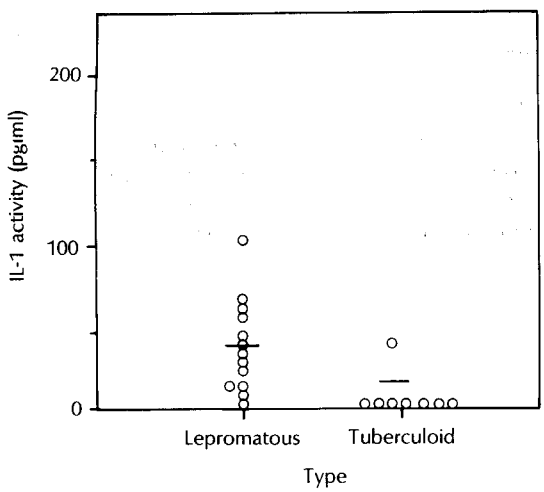

Fig. 1. *IL-1* amounts in sera from leprosy patients*
** IL-1 amounts in sera were tested by ELISA using monoclonal anti IL-1β antibody as the primary antibody.*

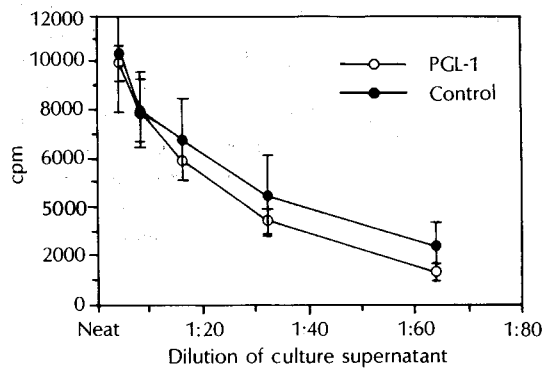

Fig. 2. *In vivo effect of PGL-I* on the production of IL-1 by mouse peritoneal macrophages*
** PGL-I 0.1mg was injected intraperitoneally three times to mice preimmurized with M. leprae. One week after the third injection, peritoneal macrophages were harvested. Peritoneal macrophages (2×10^6/ml) were cultured with LPS 10μg/ml. After 24 hours, the culture supernatants were harvested and the amount of IL-1 was assayed by thymocyte proliferation test.*

stimulated monocytes from LL patients (1,014±637pg/ml) was similar to that from TT patients (1,012±167pg/ml). When the monocytes were cultured with LPS, which is known as a potent IL-1 inducer, no significant difference in IL-1 production was also found between LL (3,479±2,188pg/ml) and TT (4,246±2,432pg/ml) patients (Table 3).

IL-1 levels in sera: To measure the IL-1 activity in sera from leprosy patients, the ELISA method was used with monoclonal anti-IL-1β antibody. The IL-1 concentration of sera from LL patients (42±30pg/ml) tended to be higher than that from TT patients (28±69pg/ml) (Table 4). By the ELISA system, IL-1 activities from seven out of nine tuberculoid patients were not detected and one serum from a TT patients showed 209pg/ml of IL-1 (Fig.1).

Effect of PGL-I on IL-1 production in mice: Peritoneal macrophages from mice sensitized with PGL-I intraperitoneally were stimulated *in vitro* with LPS to examine the *in vivo* effect of the PGL-I antigen on IL-1 production. As shown in Fig. 2, there was no significant difference in IL-1 production between peritoneal macrophages from mice sensitized with PGL-I and those from nonsensitized mice.

DISCUSSION

Monocytes play an essential role in T cell activation both as accessory cells and as regulatory cells, and either enhance or suppress immune reactions. One of the immunoenhancing functions of the monocyte involves the secretion of a soluble product, IL-1, which augments T cell proliferative responses to mitogens or antigens. Therefore, IL-1 has been considered a key mediator of host responses to microbial invasion. Little is known, however, about the IL-1 producing capacity of monocytes in leprosy patients.

In the present study, PBMCs from LL patients showed hyporesponsiveness to *M. leprae*. And we found that the culture supernatants of *M. leprae*-stimulated monocytes from LL patients contained the nearly same amount of IL-1 as those from TT patients. In LPS-stimulated IL-1 production, the IL-1 concentration in culture supernatants from TT patients tended to be higher than that from LL patients, although it was not significant statistically. The concentration of IL-1 produced by monocytes from healthy persons in LPS-stimulated culture supernatant was 6,543±3,680pg/ml (data not shown). So peripheral blood monocytes form leprosy patients produced a lower level of IL-1 than those from healthy persons at stimulation with LPS, indicating a generalized defect in IL-1 production in leprosy patients. In contrast to our findings from leprosy patients, decreased production of IL-1 by monocytes has been observed in LL patients (Watson *et al.*, 1984). Although LPS stimulates monocytes directly to produce IL-1, *M. leprae* might affect monocytes indirectly as a consequence of sensitized T cells contained in the adherent cell fractions.

The PBMCs of LL patients studied here, in fact, showed decreased proliferative responses to *M. leprae*. These results suggest a possiblity that a mechanism other than IL-1 may be involved in the immunosuppression of leprosy.

There was a report that the well known *M. leprae*-specific PGL-I antigen suppressed lymphocyte proliferation *in vitro*. Among untreated LL patients, 50% or sera were detectable for PGL-I antigen. In contrast, PGL-1 was not detectable in sera from treated LL or from TT patients (Cho *et al.*, 1988). So PGL-1 is supposed to have an important role in the pathogenesis and immunosuppression in leprosy.

A functional capacity of macrophages harboring *M. leprae* (Sibley and Krahenbuhl 1988) has indicated that defective macrophage activation is a prominent feature of LL patients. In our study peritoneal macrophages from mice injected with PGL-I intraperitoneally produced the same level of IL-1 in comparison with PGL-I nonsensitized mice. These data, therefore, indicated that PGL-I may not be involved in the suppression of IL-1 production in LL patients. These data ruled out the suppressive effect of PGL-I antigen of *M. leprae* bacilli in the production of IL-1 among LL patients with granulomas which contain numerous bacilli and also many *M. leprae* antigens such as PGL-I.

The relevance of the ability of macrophages occurring at a site distant from the foci of infection to regulate immune response is unclear. But peritoneal macrophages from mice infected by *listeria* systemically were more inhibitory than those from uninfected mice, for both mitogens and antigen-stimulated T cell responses (Petit *et al.* 1985). And we can assume the role of macrophages in LL leprosy through the function of peripheral blood monocytes.

It is interesting that, in this study, the IL-1 activities in sera from LL patients were detected higher than those from TT patients. IL-1 was not detected in sera from healthy persons in our preliminary assay. Although it is not clear whether the increased IL-1 activity is a generalized phenomenon in chronic infection or not, we can assume that in LL patients, a long lasting release of *M. leprae* antigne leads to the activated immune status to produce IL-1. But in the acute infection with Gram-negative bacteria, the IL-1 amounts in sera from patients who died were significantly higher than those from survivors (Girardin *et al.* 1988). It is evident that IL-1 has multiple effects on inflammatory cells. Subcutaneous injection of IL-1 leads to margination of neutrophils (Sauder et al. 1984). IL-1 also enhances collagen type IV production by epidermal cells, induces osteoblasts proliferation (Gowen *et al.* 1984) and stimulates osteoclast to resorb bone (Rifas *et al.* 1984). As a consequence, the increased IL-1 activity in sera may influence the inflammatory reaction in LL patients.

In conclusion, this study suggests that the immunosuppression in LL patients may not be due to the decreased production of IL-1. And the increased IL-1 amounts in sera may affect the inflammatory response of LL patients.

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