Interleukin-1β Production by Monocytes from Leprosy Patients

In-Hong Choi, Jeon-Soo Shin, Sun-Kyung Park, Sang-Nae Cho, Joo-Deuk Kim and Se-Jong Kim

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 lepromatous 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±167 pg/ml) or lipopolysaccharides (LPS) (lepromatous: 3,479±2,168 pg/ml, tuberculoid: 4,246±2,432 pg/ml). The IL-1 concentration in sera from lepromatous patients (42±30 pg/ml) tended to be higher than those from tuberculoid patients (28±6 pg/ml). And there was no significant difference in IL-1 production between peripheral macrophages from mice sensitized with PGL-I and those from nonsensitized mice. In conclusion, this study suggests that the immunosuppression in lepromatous patients may 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 (Horsitz 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. 1986). 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; Noquiera 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-
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 10 μg/ml. IL-1 was also provided by P. J. Brennan. Lipo polysaccharide (LPS, Gibco, Grand Island, NY) was used at the final concentration of 10 μg/ml.

Preparation of PBMCs: About 20 ml of heparinized blood (200 μl) was obtained from each subject. PBMCs were separated by Ficoll-Hypaque (Pharmacia Fine Chemicals, Piscataway, NJ) density gradient centrifugation (Boyum 1968).

[^1] 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 10 μg/ml. The cultures were set up in triplicate. Cultures were incubated at 37°C in humidified 5% CO₂ incubator for 5 days. Eighteen hours before the termination of culture, 1.0 μCi of H-thymidine (specific activity=6.7 Ci/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 fibronectin-coated 25 cm² tissue culture flask (Corning, Corning, NY) in a 37°C humidified CO₂ incubator. After 45 min, nonadherent cells were discarded and the flask was washed twice with warm RPMI 1640. To harvest adherent monocytes, 10 ml of Ca²⁺ and Mg²⁺ 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 × 10⁶ cells/ml of RPMI 1640 supplemented with 5% human AB serum.
Interleukin-1β Production in Leprosy Patients

One ml of cell suspension per well of a 24-well plate (Costar, Grand Island, N.Y.) was cultured in a 37°C humidified CO₂ 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, N.J.) 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 CO₂ incubator, the nonadherent cells were discarded. The adherent macrophages were harvested by vigorous pipetting with cold Ca²⁺ and Mg²⁺ free Hanks’ balanced salt solution. Peritoneal macrophages were cultured at 2x10⁶/ml in RPMI 1640 media supplemented with 5% fetal calf serum in a 37°C humidified CO₂ 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 (1.5x10⁶/100μl) 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 pulsed for 6 hours with 1μCi of [³H]-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 (1x10⁶/well) were cultured with heat-killed M. leprae (10μg/ml). After 5 days, 1.0μCi of [³H]-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

<table>
<thead>
<tr>
<th>Patients</th>
<th>Number</th>
<th>IL-1 concentration (pg/ml)* mean±S.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lepromatous leprosy</td>
<td>23</td>
<td>3,479±2,188</td>
</tr>
<tr>
<td>Tuberculoid leprosy</td>
<td>10</td>
<td>4,268±2,432</td>
</tr>
</tbody>
</table>

* 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

<table>
<thead>
<tr>
<th>Patients</th>
<th>Number</th>
<th>IL-1 concentration (pg/ml)* mean±S.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lepromatous leprosy</td>
<td>13</td>
<td>42±30</td>
</tr>
<tr>
<td>Tuberculoid leprosy</td>
<td>9</td>
<td>28±69</td>
</tr>
</tbody>
</table>

* 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 PCL-1*⁺* on the production of IL-1 by mouse peritoneal macrophages

* PCL-1 0.1mg was injected intraperitoneally three times to mice preimmunized with M. leprae. One week after the third injection, peritoneal macrophages were harvested. Peritoneal macrophages (2×10⁵/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.
Effect of PGL-1 on IL-1 production in mice: Peritoneal macrophages from mice sensitized with PGL-1 intraperitoneally were stimulated in vitro with LPS to examine the in vivo effect of the PGL-1 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-1 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 ± 3,680 pg/ml (data not shown). So peripheral blood monocytes from 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 possibility 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-1 antigen suppressed lymphocyte proliferation in vitro. Among untreated LL patients, 50% or sera were detectable for PGL-1 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 Krashenbuhl 1988) has indicated that defective macrophage activation is a prominent feature of LL patients. In our study peritoneal macrophages from mice injected with PGL-1 intraperitoneally produced the same level of IL-1 in comparison with PGL-1 nonsensitized mice. These data, therefore, indicated that PGL-1 may not be involved in the suppression of IL-1 production in LL patients. These data ruled out the suppressive effect of PGL-1 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-1.

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* antigen 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 epithelial 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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