IL-1 and IL-1 Inhibitory Activity in the Culture Supernatants of Alveolar Macrophages from Patients with Interstitial Lung Diseases

Sonoko Nagai, M.D.; Htin Aung, M.B.; Minoru Takeuchi, Ph.D.; Kaoru Kusume, M.D.; and Takateru Izumi, M.D.

Under normal conditions, the release of interleukin 1 (IL-1) and IL-1 inhibitors play a role in tissue homeostasis. We have already reported an increase in IL-1 activity and a decrease in IL-1 inhibitory activity (IHA) in the supernatants of alveolar macrophages from healthy long-term smokers as compared with healthy nonsmokers. In this study, we report an alteration in the release of IL-1 and IL-1 IHA from alveolar macrophages in patients with interstitial lung diseases (sarcoidosis and idiopathic pulmonary fibrosis [IPF]). IL-1 activity released from alveolar macrophages stimulated by lipopolysaccharide was increased in patients with active sarcoidosis (mean ± SD, 2.52 ± 1.33 U/ml [n = 6] vs 1.38 ± 0.62 U/ml [n = 15] for healthy noncurrent smokers [HNS], p < 0.05). IL-1 IHA released from alveolar macrophages was significantly different among the groups examined: a decrease of IL-1 IHA occurred in patients with active sarcoidosis (61.4 ± 19.2 [n = 6] vs 85.9 ± 13.9 percent[HNS]; p < 0.05) and IPF (64.7 ± 18.5 [n = 9]; p < 0.05). Prednisolone in the culture medium at physiologic concentrations suppressed the release of IL-1 and enhanced the release of IL-1 IHA. IL-1 IHA inhibited not only mouse thymocyte proliferation but also human fibroblast proliferation in the presence of IL-1.

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IL-1 = interleukin 1; BALF = bronchoalveolar lavage fluid; HS = healthy smokers; HNS = healthy noncurrent smokers; ILD = interstitial lung disease; IPF = idiopathic pulmonary fibrosis; IHA = inhibitory activity; NS = nonsmokers; SACE = serum angiotensin converting enzyme; FCS = fetal calf serum; LPS = lipopolysaccharide; PGE = prostaglandin E

Interleukin 1 (IL-1) is a basic mediator of intercellular communication both within the immune system and between the immune system and all other organ systems. Under normal conditions, the release of IL-1 and IL-1 inhibitors play a role in tissue homeostasis. Normal and inflammatory lung cells are exposed to a variety of antigenic materials. Lung macrophages process these materials and release various mediators. Many reports have noted the concurrent production and release of IL-1 by the same cells as those releasing the inhibitor. Such a situation may not only occur with IL-1 and IL-1 inhibitors, but may also occur with the production and release of other cytokines.

We have previously reported the release of IL-1 from bronchoalveolar lavage fluid (BALF) macrophages in healthy smokers (HS). No such release was observed in healthy noncurrent smokers (HNS). At the same time, we demonstrated the presence of IL-1 inhibitory activity (IHA) in BALF macrophage culture supernatants and a decrease of their activity in HS compared with HNS. Our results suggested that long-term smoking, which gives rise to low-grade inflammation, may disturb the balance between IL-1 production and IL-1 IHA production, which enhances the low-grade inflammatory response in lungs.

Chronic interstitial lung diseases (ILD), including sarcoidosis and idiopathic pulmonary fibrosis (IPF), can be characterized as uncontrolled low-grade inflammatory diseases: in patients with sarcoidosis, it is difficult to evaluate the prognosis and disease activity because the clinical course tends to wax and wane. In patients with IPF, we can find long chronic stable phases clinically, but the overall clinical outcomes are bad with irreversible fibrosis in the lungs. Study of BALF has revealed that the total number of BALF cells and cell differentials are altered in patients with ILD. The accumulation of inflammatory cells may reflect the results of unknown etiologic mechanisms in ILD. However, inflammatory cells may themselves enhance the inflammatory and injurious processes in the lungs of patients with ILD.

IL-1 and its regulators may be involved in the processes leading to T-cell proliferation, T-cell chemotaxtants, the augmentation of B-cell functions, and the stimulation of fibroblasts in ILD.

In this study, we focused on whether altered release of IL-1 and IL-1 IHA could be detected in patients with chronic ILD (sarcoidosis and IPF). We further examined the in vitro effect of corticosteroids on the production of IL-1 and IL-1 IHA.
### Table 1—Profiles of the Examined Cases

<table>
<thead>
<tr>
<th>Cases</th>
<th>Age, yr</th>
<th>M:F</th>
<th>SACE†</th>
<th>Chest Roentgenogram†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sarcoidosis (NS)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Active (8)</td>
<td>30.1±7.5</td>
<td>4:4</td>
<td>26.7±4.8</td>
<td>I(8)</td>
</tr>
<tr>
<td>Chronic (8)</td>
<td>40.4±7.3</td>
<td>2:6</td>
<td>26.1±8.2</td>
<td>I(3), I(2), III(3)</td>
</tr>
<tr>
<td>Inactive (9)</td>
<td>32.1±8.1</td>
<td>4:5</td>
<td>14.9±3.4</td>
<td>I(7), II(2)</td>
</tr>
<tr>
<td>IPF (NS) (9)</td>
<td>59.4±6.8‡</td>
<td>7:2</td>
<td>ND</td>
<td>Reticulonodular Fibrocystic</td>
</tr>
<tr>
<td>Healthy</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NS (6)</td>
<td>39.0±8.5</td>
<td>4:2</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>S (15)</td>
<td>45.8±10.0</td>
<td>14:1</td>
<td>ND</td>
<td></td>
</tr>
</tbody>
</table>

*Data are expressed as mean ± SD. Numbers in parentheses refer to the number of cases examined. M = male; F = female; ND = not done; BHL = bilateral hilar adenopathy. SACE = serum angiotensin converting enzyme; IPF = idiopathic pulmonary fibrosis; S = smokers; NS = nonsmokers.
†Normal range (8.3 to 21.4 IU/L/37°C)
‡Significant (p<0.05), compared with HNS.
§1 = BHL; II = BHL + parenchymal lesion; III = parenchymal lesion.

### MATERIALS AND METHODS

#### Study Population

We studied 25 cases of pulmonary sarcoidosis, 10 cases of IPF, 15 HNS, and 15 HS (Table 1). The diseased groups were all nonsmokers (NS). None was treated with corticosteroids. All cases of IPF were diagnosed by open lung biopsy. Diagnosis of pulmonary sarcoidosis was made by transbronchial lung biopsy.

We divided the patients with sarcoidosis into three groups: active, chronic, and inactive. Active cases were defined as patients in whom abnormal findings on chest roentgenograms and elevated serum angiotensin converting enzyme (SACE) levels had been detected at least six months prior to the lavage. Chronic cases were defined as those patients in whom both abnormalities had been present for more than five years prior to the lavage. Inactive cases were defined as those patients in whom both abnormalities showed clinical improvement at the time of the lavage.

None of the healthy subjects had any signs or symptoms of lung disease. Chest roentgenograms, results of pulmonary function tests, and results of routine laboratory tests were normal.

#### Preparation of BALF and Blood Mononuclear Cells

The BAL was performed as previously described with slight modification.\(^3\) Briefly, the upper airways were anesthetized with 4 percent lidocaine (Xylocaine). The fiberoptic bronchoscope was inserted into the tracheobronchial tree and wedged in the right middle lobe. A lavage was performed using warm (37°C) saline solution (50 ml × 6 times). Recovered BALF was filtered through gauze and centrifuged at 1,200 rpm for 10 minutes at 4°C. The cell pellet was resuspended in Eagle’s MEM medium and centrifuged again to adjust total cell count. A differential cell count was performed in cytoplasmic smears after May-Grünwald-Giemsa staining. Blood mononuclear cells, obtained from heparinized blood by Ficoll-Hypaque centrifugation, were suspended in Eagle’s MEM medium solution at a concentration of 5 × 10^6/ml.

#### Purification of BALF Macrophages and Blood Monocytes

Both BALF and blood mononuclear cell fractions were separated by rosetting with neuraminidase-treated sheep red blood cells at 4°C to obtain non-rosette-forming cell fractions (E4 cells). These fractions were further purified by plastic adhesion at 37°C for 60 minutes. Most (>95 percent) of the non-rosette-forming adherent cells were positive with respect to nonspecific esterase activity and will be referred to as the BALF macrophage and blood monocyte fractions.

#### Preparation of Culture Supernatants with IL-1 Activity and IL-1 IHA

The BALF macrophages and blood monocytes were washed, resuspended in RPMI-1640 medium supplemented with 15 percent fetal calf serum (FCS), and placed into 24-well tissue culture plates at a density of 1 × 10^6/ml, with or without 10 mg/L lipopolysaccharide B (LPS B E coli 055:B5). Cultures were incubated for 24 hours at 37°C in 5 percent CO2 atmosphere. Supernatants were then harvested and stored at -70°C until later use.

#### IL-1 Assay

The IL-1 activity was measured by a standard murine thymocyte proliferation assay. Thymocytes were prepared from 4- to 6-week-old C3H/HeJ mice and cultured at 1 × 10^6 cells/ml. An IL-1 standard

### Table 2—BALF Cell Findings of the Examined Cases

<table>
<thead>
<tr>
<th>Cases</th>
<th>BALF Total Cells (× 10^6/ml)</th>
<th>BALF Cell Differentials(%)</th>
<th>BALF CD4/CD8</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MA</td>
<td>Ly</td>
<td>Neu</td>
</tr>
<tr>
<td>Sarcoidosis (NS)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Active (8)</td>
<td>1.66±0.56†</td>
<td>40.8±16.8†</td>
<td>58.5±16.6†</td>
</tr>
<tr>
<td>Chronic (8)</td>
<td>1.61±1.07‡</td>
<td>74.1±18.3</td>
<td>25.5±18.0</td>
</tr>
<tr>
<td>Inactive (9)</td>
<td>0.73±0.28‡</td>
<td>81.6±17.2</td>
<td>17.8±17.1</td>
</tr>
<tr>
<td>IPF (NS) (9)</td>
<td>1.87±1.21†</td>
<td>90.9±4.9</td>
<td>5.3±3.9†</td>
</tr>
<tr>
<td>Healthy</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NS (15)</td>
<td>0.46±0.10</td>
<td>83.1±7.0</td>
<td>16.0±7.3</td>
</tr>
<tr>
<td>S (12)</td>
<td>1.84±1.01‡</td>
<td>89.3±10.6</td>
<td>9.3±10.1</td>
</tr>
</tbody>
</table>

*Data are expressed as mean ± SD. Numbers in parentheses refer to the number of cases examined. MA = macrophages; Ly = lymphocytes; Neu = neutrophils; Eos = eosinophils; BALF = bronchoalveolar lavage fluid; IPF = idiopathic pulmonary fibrosis; S = smokers; NS = noncurrent smokers.
and the experimental supernatants were serially diluted in RPMI supplemented with 10 percent FCS and penicillin/streptomycin. Aliquots of supernatants were added to thymocytes with triplicate wells set for each variable. The thymocytes were cultured with 5 mg/L of PHA-F H A16 at 37°C in 5 percent CO2 for three days, 3H-thymidine (0.5 μCi) was added 12 hours prior to the termination of the cell culture. The culture was harvested onto filter-paper disks and the 3H-thymidine uptake was assayed by liquid scintillation counter. One unit of the IL-1 activity in the supernatant was defined as the amount showing twofold increase of 3H-thymidine uptake into thymocytes in the presence of PHA alone.

IL-1 Inhibitory Assay

We examined the serial suppression of thymocyte proliferation in the response to standard human IL-1. The IL-1 HIA (percent) was expressed as follows:

\[
\text{IL-1 HIA (percent) = } \frac{1 - \text{standard IL-1 (cpm) + supernatant (cpm)}}{\text{standard IL-1 (cpm)}} \times 100
\]

The Effect of the Culture Supernatant on the Proliferation of Fibroblasts

We examined the effect of IL-1 HIA on another target cell, a cultured human fibroblast cell line (CCD-13Lu). (This cell line, which is induced to proliferate by IL-1, was purchased from the American Type Culture Collection [ATCC, Rockville, Md].) The cell suspension was adjusted to a final concentration of 1 × 10³ cells/ml by Eagle's MEM supplemented with 10 percent FCS; 100 μl of this cell suspension was placed in flat bottom microwells and incubated overnight at 37°C in 5 percent CO2. On the next day, the medium was aspirated and culture supernatant and standard IL-1 were added to the remaining attached fibroblasts. After 48 hours of incubation at 37°C in 5 percent CO2, the culture was pulsed with 0.5 μCi of 3H-thymidine for the final 24 hours. After 72 hours, the medium was gently aspirated from each well and replaced with 100 μl of trypsin (0.5 mg/ml)-EDTA (0.2 mg/ml) solution, followed by incubation at 37°C for 5 minutes. The fibroblasts were harvested using an automated harvester, and the radioactivity was counted by a liquid scintillation counter.

The Effect of Prednisolone Succinate on IL-1 Activity and IL-1 HIA Released from BALF Macrophages

Prednisolone succinate (Shionogi, Osaka) (1.25 to 10.0 μg/ml) was added to the macrophage cultures, and the supernatants were examined for IL-1 and IL-1 HIA.

Statistical Evaluation

All results were expressed as means ± SD. The significance of differences among the six study groups was evaluated using one-way analysis of variance. The unpaired t test was used to assess significant differences in each study group.

Results

BALF Cell Findings in the Examined Populations

The BALF cell findings of the examined populations are summarized in Table 2. Compared with HNS, significant increases in total cell counts were found in HS, the three categories of sarcoidosis, and the IPF group.

A contribution of BALF lymphocytes to the increase in total cell numbers was shown in patients with active sarcoidosis. BALF macrophages were significantly increased in patients with IPF. Neither mean neutrophil nor eosinophil percentages were increased in the diseased or HS groups compared with HNS, but some patients with IPF showed a substantial increase in granulocytes. An increase of CD4⁺ to CD8⁺ ratio was shown in patients with chronic sarcoidosis. On the contrary, a decrease in the ratio was found in HS compared with HNS.

The Effect of IL-1 and IL-1 HIA on the Proliferation of Human Fibroblasts

BALF macrophage culture supernatants suppressed the proliferative response of CCD-13Lu fibroblasts to purified IL-1; no influence was detected if IL-1 was not added (Fig 1).

IL-1 Activity in the Culture Supernatant of BALF Macrophages or Blood Monocytes

IL-1 activity in the culture supernatant of BALF macrophages or blood monocytes with or without LPS stimulation is shown in Table 3. Activity of spontaneously released IL-1 was much lower for BALF macrophages than for blood monocytes. There was no significant difference of IL-1 activity between HNS and other examined groups. IL-1 activity released from LPS-stimulated BALF macrophages or blood monocytes was clearly higher in patients with active sarcoidosis and HS than in HNS. Some patients with IPF showed increased activity of IL-1 released from BALF macrophages, although the mean values were
not statistically significant compared with HNS.

**IL-1 IHA in the Culture Supernatant of BALF Macrophages with or without LPS Stimulation**

We examined the appearance of IL-1 IHA in tenfold dilutions of BALF macrophage culture supernatants with or without LPS stimulation (Fig 2). The mean values of IL-1 IHA were significantly different (p<0.05) among the six groups examined. IL-1 IHA was 85.9 ± 13.9 percent (unstimulated) and 83.3 ± 16.1 percent (LPS stimulated) in HNS. The mean values for HS were significantly decreased (52.3 ± 18.9 percent, unstimulated; 55.8 ± 23.2 percent, LPS stimulated) in comparison with those for HNS. Significant decreases of IL-1 IHA without stimulation were found in patients with active sarcoidosis (61.4 ± 19.2 percent)
and with IPF (64.7 ± 18.5 percent). Although not statistically significant, there was a tendency to a decrease of unstimulated IL-1 IHA in patients with both chronic and inactive sarcoidosis.

**IL-1 IHA in the Culture Supernatant of Blood Monocytes with or without LPS Stimulation**

The IL-1 IHA in the culture supernatant of blood monocytes with or without LPS stimulation is shown in Figure 3. The IL-1 IHA in the culture supernatant was much lower for blood monocytes than for BALF macrophages (Fig 2). The mean values of IL-1 IHA from blood monocytes without or with LPS stimulation showed no difference among the six groups examined. Although statistically not significant, IL-1 IHA without stimulation was lower for HS and the patients with active sarcoidosis than for HNS.

**The Effect of Prednisolone on IL-1 Activity and the IL-1 IHA in the Culture Supernatant of BALF Macrophages**

The IL-1 release from BALF macrophages was significantly suppressed by prednisolone in physiologic concentrations, whereas the release of IL-1 IHA was stimulated under the same conditions, as shown in Figure 4.

**DISCUSSION**

In this study, we have demonstrated the altered release of IL-1 and IL-1 IHA from BALF macrophages in patients with chronic ILD: sarcoidosis and IPF. The amounts of change were similar to those in HS. After LPS stimulation, IL-1 activity in the culture supernatants of BALF macrophages and blood monocytes was higher in the group with active sarcoidosis than in HNS. On the other hand, there was a decrease of IL-1 IHA in the culture supernatants of BALF macrophages from patients with active sarcoidosis and IPF compared with macrophages from HNS.

We have shown in Figure 2 that IL-1 IHA was significantly lower in smokers than nonsmokers, with LPS stimulation having no effect. IL-1 IHA in the unstimulated state was lower for both the active sarcoidosis and the IPF groups than for the HNS control subjects, but was increased by LPS stimulation. Macrophages from HS failed to release IL-1 IHA either in the unstimulated state or after LPS stimulation. Although it has been reported that LPS-stimulated alveolar macrophages release large amounts of prostaglandin E₂ (PGE₂), a contribution of PGE₂ to IL-1 IHA seems unlikely in the unstimulated state. Furthermore, we examined the amount of PGE₂ in the culture supernatants and found only a small amount in the concentrated fraction containing IL-1 IHA (unpublished data).

There was no significant difference among the six groups in terms of IL-1 IHA released from blood monocytes as shown in Figure 3. Compared with the

![Figure 3. IL-1 IHA in the culture supernatants of blood monocytes with or without LPS stimulation. The IL-1 IHA (percent) in the culture supernatants of blood monocytes was measured by using the IL-1 inhibitory assay as shown in Figure 2. The ordinate expresses the IL-1 IHA (percent). Open and closed circles express the IL-1 IHA without LPS and with LPS stimulation, respectively. Each bar shows the mean ± SD. The asterisks indicate the statistical significance (p<0.01) of IL-1 IHA compared with those released from BALF macrophages.](http://journal.publications.chestnet.org/pdfaccess.ashx?url=/data/journals/chest/21625/)
IL-1 IHA released from BALF macrophages, the IHA released from blood monocytes was decreased in all instances. Although not statistically significant, the IL-1 IHA is lower for smokers and those with active sarcoidosis in the unstimulated state, than for non-smoking controls, whereas the IL-1 IHA is increased in all the diseased groups by LPS stimulation.

We routinely tested the culture supernatants for LPS contamination with the Limulus gelatin test, prior to LPS stimulation, and the results were always negative.

As to IL-1 IHA, some reports have claimed that the factor which inhibits mouse thymocyte proliferation enhances the proliferation of fibroblasts. We examined the inhibitory effect of our IL-1 IHA on the proliferative responses of human fibroblasts and mouse thymocytes to IL-1. Our results showed that IL-1 IHA could inhibit the proliferative responses of both cell types to IL-1.

IL-1 may have a variety of effects on many target cells in chronic ILD. Firstly, IL-1 may be a second signal for activation and proliferation of T cells in patients with sarcoidosis. Secondly, IL-1 may be a T-cell chemoattractant and contribute to accumulation of T cells in the lungs. Additionally, IL-1 could play an important role in the formation of the epithelioid cell granuloma.

The study of experimental granuloma has shown the occurrence of IL-1 in the granulomatous extracts. However, it remains to be elucidated if IL-1 also occurs in such extracts from patients with sarcoidosis.

Considering the enhancing effect of IL-1 and the inhibitory effect of IL-1 IHA on the proliferative response of human fibroblasts to IL-1, the alteration of IL-1 and IL-1 IHA release from BALF macrophages may be a factor in the progressive and uncontrolled fibrosis in lungs of patients with IPF. Additionally, IL-1 could augment B-cell functions in patients with IPF. We have noticed a significant increase of y-globulin levels in blood and the appearance of some autoantibodies in patients with IPF.

The appearance of IL-1 IHA was significantly higher in BALF macrophages than in blood monocytes. The lower levels of IL-1 activities in BALF macrophages

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**Figure 4.** The effect of prednisolone on IL-1 activity and IL-1 IHA of BALF macrophages. Prednisolone succinate (Shionogi, Osaka) in the concentration of 1.25 to 10.0 μg/dl was added into the BALF macrophage culture, and the supernatants were examined for IL-1 activity and IL-1 IHA. A: The IL-1 activity found with addition of prednisolone was expressed as the percentages of the inhibition of IL-1 activity (ordinate), when the IL-1 activity found without addition of prednisolone was defined as 100 percent. B: The IL-1 IHA (percent) is shown in the ordinate. Abscissa shows the various concentrations of prednisolone added to the culture. Each bar expresses the mean ± SD.
are considered to be due to a contribution of IL-1 IHA. The ability of BALF macrophages to produce a substantial amount of IL-1 IHA may reflect that they can keep homeostasis under the exposure to various antigenic materials via the airways. Therefore, a slight alteration in IL-1 activity and IL-1 IHA released from alveolar macrophages may have a possible role in advancing the low-grade inflammatory response in lungs.

We have used corticosteroids, which are considered to be anti-inflammatory or immunosuppressive drugs, for the treatment of I LD. Corticosteroids are known to suppress IL-2 production in active sarcoidosis, and the addition of cortisol to cultures of human skin fibroblasts rapidly reduces the amount of type I procollagen mRNAs.

We examined the effect of prednisolone on the release of IL-1 and IL-1 IHA from BALF macrophages. Prednisolone completely suppressed IL-1 release from BALF macrophages of HS. Also, prednisolone enhanced the release of IL-1 IHA from BALF macrophages of HS; the IL-1 IHA percentage reached similar levels as in HNS. These results support the anti-inflammatory effect of corticosteroids in vitro.

The occurrence of various IL-1 inhibitory factors has been reported from urine, monocyte cell lines, or virus-infected macrophages, having various molecular weights. Many reports have noted the concurrent production of IL-1 by the cells releasing the inhibitor, suggesting that IL-1 antagonists are released along with IL-1. In some cases, the antagonistic molecules appear to be direct receptor antagonists of IL-1; in other cases, they may bind to other receptor sites that interfere with the activity of IL-1. The mechanism by which our IL-1 IHF exerts its inhibitory effect on the release of IL-1 remains to be elucidated.

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