Relative Release of Interleukin-1β and Interleukin-1 Receptor Antagonist by Alveolar Macrophages*

A Study in Asbestos-Induced Lung Disease, Sarcoidosis, and Idiopathic Pulmonary Fibrosis

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We examined the influence of untreated interstitial lung disease (ILD) on the *in vitro* release of interleukin-1β (IL-1β) and interleukin-1 receptor antagonist (IL-1ra) from alveolar macrophages (AM); AM were harvested from normal volunteers, ILD patients, and patients with asbestos-related pleural disease but no ILD. AM were cultured for 24 h and assays for IL-1β and IL-1ra were done using sensitive and specific enzyme-linked immunosorbent assay. A greater amount of IL-1β was detected in AM supernatants from asbestosis, sarcoidosis, and IPF patients than in those from normal subjects. The IL-1β:IL-1ra ratio (IL-1β activity index [IL-1AI]) was significantly lower in supernatants of normal macrophages compared with macrophage supernatants from individuals with ILD. The IL-1AI correlated with bronchoalveolar lavage cellularity, a marker of disease activity. Current smoking was associated with lower IL-1β and IL-1ra release in ILD. The IL-1AI is a convenient method for comparison of IL-1β activity between patient populations. *(Chest 1993; 104:47-53)*

IL-1 = interleukin-1; IL-1AI = IL-1β activity index; IL-1β = interleukin-1β; IL-1ra = interleukin-1 receptor antagonist; ILD = interstitial lung disease; IPF = idiopathic pulmonary fibrosis

Asbestosis, sarcoidosis, and idiopathic pulmonary fibrosis (IPF) are all classified as interstitial lung disease (ILD) with different etiologies and clinical features, but they share a potentially similar outcome: end-stage fibrotic lung disease. A common feature of these disorders is the presence of varying degrees of alveolar and interstitial inflammation and fibrosis.[1] Many studies have underscored the importance of alveolar macrophages (AM) and their products, particularly interleukin-1β (IL-1β), for maintaining and modulating active inflammation and fibrosis in the lung.[2-5] Interleukin-1 receptor antagonist (IL-1ra), a recently described member of the pulmonary cytokine family which inhibits interleukin-1 (IL-1),[6,7] also is produced by AM.[8,9] Thus, the total amount of IL-1 activity is related to the relative amounts of both IL-1 and IL-1ra.

We examined the relative amounts of IL-1β and IL-1ra released by AM from patients with asbestosis, asbestos-related pleural disease, sarcoidosis, and IPF and compared these findings to those of normal subjects. We found that AM from all patient groups spontaneously released more IL-1β relative to IL-1ra when compared with macrophages from normal subjects. An increased release of IL-1β relative to IL-1ra was related to parameters of inflammation in bronchoalveolar lavage (BAL) that are associated with active or advanced disease. Smoking caused a decrease in release of both IL-1β and IL-1ra.

Methods and Materials

Normal Subjects

Human AM were obtained from ten normal volunteers with a lifetime history of nonsmoking. At the time of the study, they had no acute or chronic medical illness, were taking no prescribed or over-the-counter medications, and had a normal physical examination. The study protocol was approved by the Committee for Investigations Involving Human Subjects at the University of Iowa. There were no complications or adverse reactions during the study.

Interstitial Lung Disease Patients

All patients with lung disease were followed up as part of our Specialized Center of Research program for Interstitial Lung Disease at the University of Iowa. The study protocol was approved by the Committee for Investigations Involving Human Subjects at the University of Iowa. There were 14 patients with asbestosis, 16 with asbestos-related pleural disease, 15 with sarcoidosis, and 15 with IPF. No patients were treated with corticosteroids for at least one year prior to entering in the study. Patients identified as current smokers did not smoke for 24 h prior to bronchoscopy.

Isolation of Alveolar Macrophages

Samples of human AM were obtained by BAL as previously described.[10] Briefly, the subjects were premedicated with meperi-

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dine and atropine sulfate and pretreated with an inhaled beta agonist (albuterol). The upper airway was anesthetized with 5 percent lidocaine, after which the fiberoptic bronchoscope was inserted transorally into the tracheobronchial tree and advanced into wedge position in a subsegmental bronchus of the lingula or middle lobe. The BAL consisted of six 20-ml aliquots of sterile, warmed saline solution, which were retrieved by low pressure suction. The first 20-ml lavage from each subsegment was discarded. The BAL was repeated in two additional subsegments, including at least one in the opposite lung.

The lavage fluid was filtered through two layers of gauze and centrifuged at 15 g for 5 min. The cell pellet was washed twice in Hanks' balanced salt solution without Ca^{++} or Mg^{++} (HBSS, Cancer Research Center/Tissue Hybridoma Facility, University of Iowa, Iowa City) and suspended in culture medium as described later on. Complete and differential cell counts were determined with a Coulter counter (Coulter Electronics Inc., Hialeah, Fla) and Wright-Giemsa-stained cytocentrifuge preparations, respectively.

Cell Culture

The BAL cells were cultured at a density of 1×10^6 cells/ml in Rosewell Park Memorial Institute tissue culture medium (RPMI 1640) containing 0.3 mg/ml of l-glutamine, and 80 μg/ml of gentamycin. Heat-inactivated endotoxin-free fetal calf serum (FCS, Hyclone Laboratories, Logan, Utah) at a final concentration of 4 percent was added to the medium. Twelve-well flat polystyrene culture dishes (Costar, Cambridge, Mass), containing 1 ml of suspended cells in each well, were incubated in an atmosphere of 95 percent humidified air and 5 percent CO_2 at 37°C. After 24 h, the supernatants were aspirated, centrifuged at 150 g to remove nonadherent cells, and stored at −70°C until analyzed.

Interleukin-1 Receptor Antagonist and Interleukin-1β

The IL-1ra and IL-1β were measured in culture supernatants using solid-phase enzyme-linked immunosorbent assay kits (R&D, Minneapolis). The IL-1ra kit is specific for the presence of IL-1ra and has an accurate range between 50 and 3,000 picogram (pg)/ml. The IL-1β enzyme-linked immunosorbent assay is specific for IL-1β and has an accurate range between 4 and 250 pg/ml. The cell supernatants and standards were diluted in the tissue culture medium described previously.

Statistics

Results are expressed as the mean ± SEM. Significant differences were identified by nonparametric testing, using the Statview II statistical package. The Mann-Whitney U test was used to examine differences between means of unrelated samples. Further analysis of variance was performed using the Kruskal-Wallis test. Probability values less than 0.05 were considered significant.

Results

Bronchoalveolar lavage was performed on 10 normal volunteers, 14 patients with asbestosis, 16 with asbestos-related pleural disease but no ILD, 15 with sarcoidosis, and 15 with IPF (Table 1). The only group whose gender mix was significantly different from the normal volunteers was the asbestos-exposed patients ($p<0.05$). The normal volunteers were significantly younger ($p<0.001$) than each group of patients. Thirty five percent of the asbestos patients were current smokers at the time of the study, which was greater than in any other group ($p<0.05$).

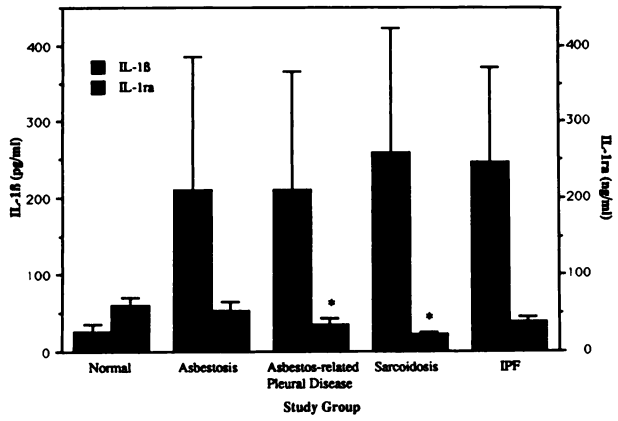
There were no significant differences in the total cell counts between normal volunteers and ILD patients. Sarcoidosis patients had a significantly higher percentage of lymphocytes (26 ± 4 percent) compared with normal control subjects (8 ± 2 percent) and a correspondingly lower macrophage percentage (72 ± 4 percent compared with 92 ± 2 percent, each $p<0.01$). The IPF patients had higher neutrophil (10 ± 3 percent compared with 0.5 ± 0.2 percent, $p<0.001$) and eosinophil percentages (6 ± 2 percent compared with 0.1 ± 0.1 percent, $p<0.01$) and lower macrophage percentages (79 ± 4 percent, $p<0.01$) than normal control subjects.

Only small amounts of IL-1β were spontaneously released from unstimulated macrophages from normal control subjects (25 ± 10 pg/ml). Increased amounts of IL-1β were spontaneously released from ILD patients (asbestosis, 209 ± 177 pg/ml; asbestos-related pleural disease, 208 ± 157 pg/ml; sarcoidosis, 257 ± 165 pg/ml; and IPF, 245 ± 125 pg/ml) with a nearly significant increase in the sarcoidosis ($p = 0.07$) and IPF ($p = 0.08$) groups (Fig 1, *top*). The IL-1ra release from macrophages was detectable in all groups (Fig 1, *top*).

Table 1—*Demographics and BAL Data of Normal Volunteers and Asbestosis, Asbestos-Related Pleural Disease, Sarcoidosis, and IPF Patient Groups**

		Patient Group			
	Normal	Asbestosis	Asbestos-Related Pleural Disease	Sarcoidosis	IPF
Sex, M/F	7/3	14/0	16/0	6/9	10/5
Age, yr	30 ± 6	59 ± 3†	63 ± 2†	52 ± 14†	65 ± 10†
% Smokers	0	35‡	25	7	7
Cell count, $\times 10^3$	28 ± 5	22 ± 6	21 ± 6	16 ± 3	18 ± 6
Macrophage, %	92 ± 2	90 ± 2	84 ± 3%‡	72 ± 4§	79 ± 4§
Lymphocyte, %	8 ± 2	9 ± 2	13 ± 3%	26 ± 4§	6 ± 2
Neutrophil, %	0.5 ± 0.2	1 ± 0.4	2 ± 1%	1 ± 0.3	10 ± 3‡
Eosinophil, %	0.1 ± 0.1	0.2 ± 0.1	1 ± 0.2%	1 ± 0.3	6 ± 2§

*Values expressed as ratio (sex), percentage of total group (% smokers) or mean ± SEM (all other values).
†$p<0.001$, compared with normal control subjects.
‡$p<0.05$, compared with normal control subjects.
§$p<0.01$, compared with normal control subjects.



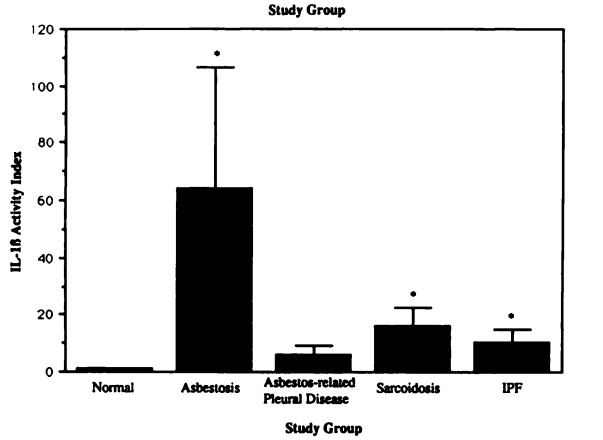


FIGURE 1. *Top,* Spontaneous release of IL-1β (pg/ml) and IL-1ra (ng/ml) from AM, harvested by BAL, cultured for 24 h, obtained from normal volunteers and patients with asbestos exposure, sarcoidosis, or IPF. Each value represents the mean ± SEM of the group. *Bottom,* The IL-1AI calculated by dividing the released IL-1β by the released IL-1ra in each subject, multiplied by 1,000. Each value represents the mean ± SEM of the group; asterisk, $p<0.05$.

Normal macrophages released 60 ± 10 ng/ml of IL-1ra, which was significantly more than was released from macrophages from patients with asbestos-related pleural disease (35 ± 9 ng/ml, $p<0.05$) and sarcoidosis (21 ± 5 ng/ml, $p<0.01$) patients, and substantially more than from IPF (37 ± 8 ng/ml, $p=0.06$) patients.

Since IL-1 activity is a function of the relative amounts of IL-1β and IL-1ra, we compared the ratio of IL-1β to IL-1ra release (interleukin-1β activity index [IL-1AI:IL-1AI = [IL-1β/IL-ra] × 1,000) in the macrophage supernatants from normal volunteers and the three ILD patient populations (Fig 1, *bottom*). Not only did macrophages from each of the patient populations produce more IL-1β, they also produced more IL-1β relative to IL-1ra. Supernatants of BAL cells from normal volunteers had an IL-1AI of 0.73 ± 0.39, supernatants of BAL cells from asbestosis patients had an IL-1AI of 63.7 ± 42.81 ($p<0.01$, vs normal control subjects), supernatants of BAL cells from asbestos-related pleural disease had an IL-1AI of 5.77 ± 3.19, supernatants of BAL cells from sarcoidosis patients had an IL-1AI of 15.63 ± 6.99 ($p<0.05$, vs normal

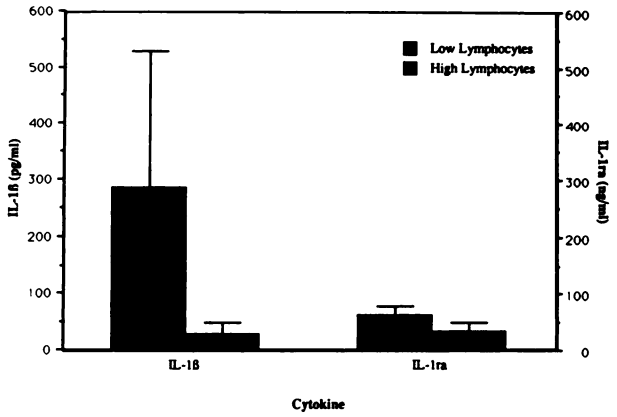


FIGURE 2. Spontaneous release of IL-1β (pg/ml) and IL-1ra (ng/ml) from AM obtained from asbestosis patients grouped by percentage of lymphocytes in the BAL sample. Low lymphocyte percentage is less than 15 percent, high lymphocyte percentage is 15 percent or more. Each value represents the mean ± SEM of the group.

control subjects), and supernatants of BAL cells from IPF patients had an IL-1AI of 9.99 ± 4.78 ($p<0.01$, vs normal control subjects).

For all ILD patients, smoking was associated with a significantly higher BAL cell count (36 ± 10 vs 18 ± 2 × 10^6 ml, $p<0.05$) and lower release of IL-1ra and IL-1β by unstimulated macrophages (data not shown). Normal volunteers were eliminated from this analysis because they were not smokers. Macrophages from current cigarette smokers released 28 ± 21 pg/ml of IL-1β and 12 ± 4 ng/ml of IL-1ra compared with macrophages from ILD patients who were not smokers, which released 300 ± 113 pg/ml of IL-1β ($p<0.05$) and 30 ± 4 ng/ml of IL-1ra ($p=0.07$). We found no significant difference in cytokine release by macrophages from ex-smokers and those who never smoked (data not shown).

The release of IL-1β and IL-1ra further was compared by the types of cells in BAL samples. Asbestosis

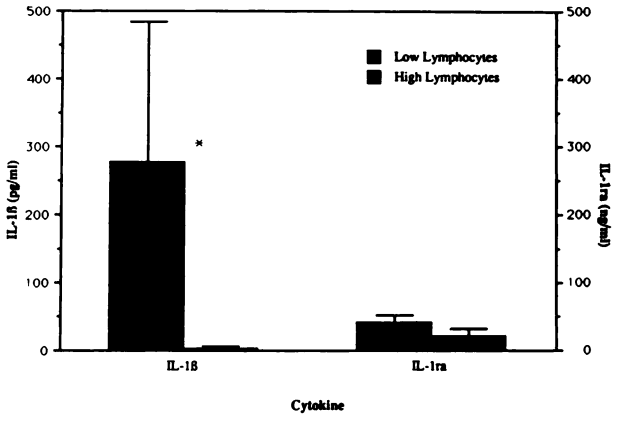


FIGURE 3. Spontaneous release of IL-1β (pg/ml) and IL-1ra (ng/ml) from AM obtained from asbestos-related pleural disease patients grouped by percentage of lymphocytes in the BAL sample. Low lymphocyte percentage is less than 15 percent, high lymphocyte percentage is 15 percent or more. Each value represents the mean ± SEM of the group; asterisk, $p<0.05$.

patients with a relatively high BAL lymphocyte percentage (≥15 percent) may represent a segment of the population with early or mild ILD.[11] The group of asbestosis patients with a higher percentage of lymphocytes released less total IL-β and less IL-1β relative to IL-1ra than the lower lymphocyte percentage group (Fig 2). This relationship also was noted in the group of patients with asbestos-related pleural disease (Fig 3), which had a significantly lower IL-1β release from the high relative to the low lymphocyte percentage (275 ± 207 vs 2.5 ± 2.5 pg/ml, $p<0.05$). Sarcoidosis patients with increased BAL lymphocytes (≥30 percent) are thought to have more active disease than those with lower percentages of lymphocytes.[3] Sarcoidosis patients with a high percentage of lymphocytes spontaneously released more IL-1β (274 ± 208 vs 63 ± 25 pg/ml) and less IL-1ra (20 ± 5 vs 52 ± 9 ng/ml, $p<0.05$) than the low-lymphocyte group (Fig 4).

The IPF patient group contained a substantial number of patients with BAL neutrophilia and eosinophilia (five), each group containing ≥5 percent granulocytes in the BAL samples. These subgroups released IL-1β and IL-1ra in a similar manner. The AM from BAL samples with a high granulocyte percentage released less IL-1β and significantly less IL-1ra (low neutrophils, 52 ± 11 ng/ml IL-1ra vs high neutrophils, 20 ± 7 ng/ml IL-1ra, $p<0.05$; low eosinophils, 52 ± 8 ng/ml IL-1ra vs high eosinophils, 7 ± 2 ng/ml IL-1ra, $p<0.01$) than the low-granulocyte group (data not shown).

The IL-1AI for each disease showed a striking trend toward higher values in more active or severe disease (Fig 5). As described previously, the asbestosis and sarcoidosis patients were analyzed by BAL lymphocyte percentages, and the IPF patients were examined by eosinophil and neutrophil percentages of the BAL samples. High BAL lymphocyte percentages are associated with early ILD in asbestosis,[11] but they are associated with more active alveolitis and fibrosis in sarcoidosis.[3] High BAL granulocyte percentages predict poorer outcome in IPF.[12,13] In each case, analysis of the AM supernatants reveals a higher IL-1AI in the worse-prognosis subset. These ratios were even greater when IL-1β release was corrected for macrophage percentage (data not shown).

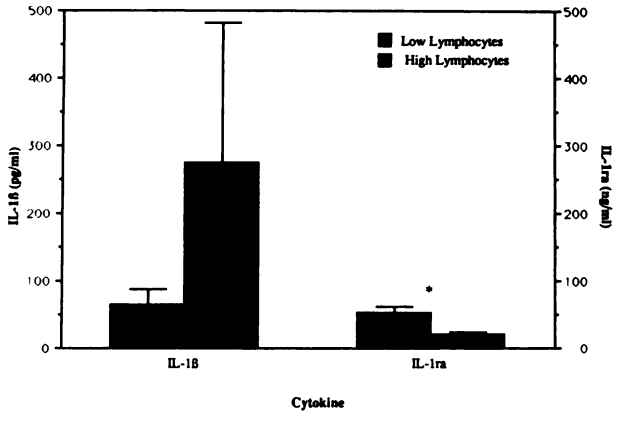


FIGURE 4. Spontaneous release of IL-1β (pg/ml) and IL-1ra (ng/ml) from AM obtained from sarcoidosis patients grouped by percentage of lymphocytes in the BAL sample. Low lymphocyte percentage is less than 30 percent, high lymphocyte percentage is 30 percent or more. Each value represents the mean ± SEM of the group; asterisk, $p<0.05$.

DISCUSSION

In this study, we examined the release of IL-1β and IL-1ra by AM harvested from normal volunteers and patients with asbestos-related lung disease, sarcoidosis, or IPF. We found significant differences in the release of IL-1β and IL-1ra by AM among these conditions. Macrophages from all groups of patients with pulmonary fibrosis spontaneously released more IL-1β relative to IL-1ra when compared with macrophages from normal subjects. Among patients with ILD, subjects who were current smokers released less IL-1β and IL-1ra than nonsmokers. In patients with sarcoidosis and asbestosis, there was a relationship between the relative amounts of IL-1β and IL-1ra released by AM and the percentages of lymphocytes in BAL samples. A similar finding was noted with AM from IPF patients with increased numbers of granulocytes in BAL samples. The IL-1AI was significantly higher in all ILD groups but not in asbestos-related pleural disease, compared with normal control subjects. The IL-1AI demonstrated a consistent trend toward elevation in the patient group BAL-cell-type subsets associated with advanced or active disease.

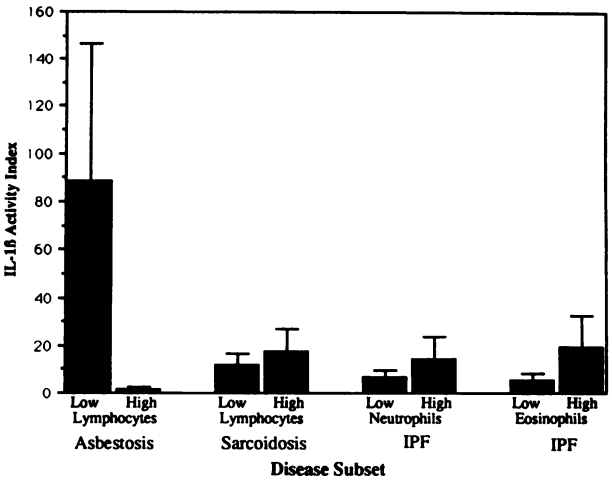


FIGURE 5. The IL-1AI of ILD patient groups. Asbestosis low-lymphocyte group contains less than 15 percent lymphocytes, asbestosis high-lymphocyte group contains 15 percent or more lymphocytes. Sarcoidosis low-lymphocyte group contains less than 30 percent lymphocytes, sarcoidosis high-lymphocyte group contains 30 percent or more lymphocytes; IPF low-neutrophil and eosinophil groups contain less than 5 percent granulocytes, IPF high-neutrophil and eosinophil groups contain 5 percent or more granulocytes.

Previous studies on patients with ILD have demonstrated an association between IL-1 activity and disease activity.[14] The severity of inflammation in sarcoidosis, measured by positivity of gallium scans and lymphocytic alveolitis, correlates with spontaneous or LPS-stimulated IL-1 release from AM.[3,5,15] The IL-1 activity also has been reported to be elevated in AM from IPF patients.[16-18] Animal models of asbestos inhalation have associated elevated AM IL-1 release with formation of fibrosis.[2,4,19] Lemair[20] described development of fibrosis following asbestos inhalation by rats, which was accompanied by an increase in IL-1 release by AM. Oghiso and Kubota[4] similarly demonstrated that silica or asbestos dust caused increased IL-1 release from rat AM, whereas nonfibrogenic (titanium dioxide) dust did not result in increased IL-1 release. These studies support the hypothesis that inhalation of these dusts stimulate AM to release cytokines, including IL-1β, which further act on other cell types such as fibroblasts and lymphocytes to potentiate the inflammatory state within the lung, and ultimately leads to fibrosis.

The release of a substance, from macrophages of ILD patients, with inhibitory activity for IL-1 has been noted previously. Kleinhenz et al[21] described less IL-1 bioactivity from LPS-stimulated macrophages of sarcoidosis patients than normal AM, which correlated with the presence of IL-1 inhibitory activity in the supernatants. Fireman et al[22] reported that supernatants from macrophages obtained from ILD patients significantly suppressed phytohemagglutinin-induced lymphocyte proliferation relative to macrophages of normal control subjects. Subsequently, they evaluated the relative production of IL-1 and PGE_2 (which is known to have inhibitory activity against IL-1) by AM from normal control subjects and sarcoidosis and IPF patients.[15,23] They reported that the inhibitory activity from IPF but not sarcoidosis AM correlated with PGE_2 release, suggesting that other factors may be involved in the IL-1 suppressive activity of sarcoidosis AM. Galve-de Rochmonteix and co-workers[17] also evaluated IL-1 inhibitory activity from patients with ILD and found that AM from IPF patients (and to a lesser extent sarcoidosis patients) released significantly more IL-1 inhibitory activity than those from normal control subjects. Nagai et al[18] demonstrated that the *in vitro* addition of prednisone to AM from sarcoidosis patients resulted in lowered IL-1 activity and enhanced IL-1 inhibitory activity, but that indomethacin did not significantly change either measure, suggesting a mechanism other than the prostaglandin pathway for control of IL-1 inhibition. Using AM from normal volunteers, Monick et al[24] and Iwamoto et al[25] demonstrated that PGE_2 release can be stimulated from AM, but arachidonic acid metabolites do not appear to account for all the diminished IL-1 activity released from AM when compared with monocytes. A major limitation of all of these studies was that IL-1β protein was not directly measured.

Characterization of a specific inhibitor of IL-1 was reported by two groups in 1990[6,7] with the identification of the IL-1ra. This 18- to 25-kilodalton protein, with 19 percent amino acid homology to interleukin-1α and 26 percent to IL-1β,[26] is a specific antagonist of both interleukin-1α and IL-1β. The IL-1ra was first isolated from mononocytes and a monocyte cell line; however, IL-1ra also has been identified in normal human AM.[8,9] Its reported potency as an antagonist to IL-1 varies on the cell type and assay; the concentration required to inhibit 50 percent of the effect of IL-1 ranges between 10 and 1,000 times greater than concentrations of IL-1.[6,27,28] These ratios of IL-1β to IL-1ra are very similar to those observed in macrophage supernatants in this study.

Bronchoalveolar lavage allows investigators to sample the intra-alveolar cell population at the time of the procedure. Although AM are by far the most common BAL cellular constituents in normal subjects, different cell profiles may be found in the BAL samples of ILD patients. These cells may affect the progression of disease or may be only markers for disease activity or severity. Several studies of sarcoidosis patients have found that, in this disease, BAL lymphocytosis strongly correlates with increased disease activity or degree of alveolitis[29-31] and suggests that the T lymphocyte population in the sarcoid alveoli may be responsible for the development or maintenance of pulmonary fibrosis.[1,32,33] Idiopathic pulmonary fibrosis patients have been studied by percentage of granulocytes in the BAL samples; eosinophilia and neutrophilia in the BAL samples appear to be markers of progressive or severe disease in this population.[12,13,34-36] In contrast, lymphocytic alveolitis is associated with an improved prognosis in IPF[12] and possibly asbestosis.[11] These trends are mirrored in the different IL-1AI seen in this study. High BAL lymphocyte percentage correlated with a higher IL-1AI in sarcoidosis but a lower IL-1AI in asbestosis patients. Granulocytosis in BAL samples was associated with a higher IL-1AI in BAL cell culture from IPF patients.

Smoking is associated with altered AM IL-1 activity. Nagai et al[18,37] reported that LPS-stimulated IL-1 activity was greater in AM from smokers than nonsmokers and that IL-1 inhibitory activity was conversely lower. Brown and colleagues[38] demonstrated lower IL-1 protein release from AM of heavy-smokers compared with AM from light smokers and nonsmokers. This appeared to be a specific impairment of release rather than production, since total IL-1 was similar in all groups. Yamaguchi and colleagues[39] reported similar findings and excluded the possibility that cyclooxygenase metabolites were responsible for

diminished IL-1 activity.

The question of whether nonmacrophage BAL cells could be responsible for the secretion of significant quantities of IL-1ra was examined in two ways. First, amounts of IL-1ra were corrected for macrophage counts from the BAL samples; no statistical changes were seen when these were compared with the uncorrected measurements. In addition, peripheral blood neutrophils and lymphocytes were obtained from three normal volunteers and cultured at a density of 5×10^6 cells/ml. Spontaneous IL-1ra release was measured from lymphocytes (1.3 ± 0.28 ng/ml) and from neutrophils (2.0 ± 0.78 ng/ml). These quantities of IL-Ira are not sufficient to explain differences in IL-1ra release between the ILD groups, but it is possible that the cells behave differently in the milieu of the alveolus than *in vitro*.

This study adds a report to the literature that supports a role for cytokines, and IL-1β in particular, in the promotion and maintenance of ILD inflammation. It is likely that IL-1ra plays a prominent role in the control of IL-1 activity, particularly in the diseased lung. The IL-1AI appears to be a useful tool for comparison of IL-β and IL-1ra release among patient groups. Further studies will be important to elucidate the mechanisms by which the balance between inflammation promoters and inhibitors is maintained.

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