Effect of Bronchoalveolar Lavage Fluid from Patients with Sarcoidosis or AIDS on Interleukin 1β Release from Alveolar Macrophages*

Zheng-Bo Huang, M.D.; and Edward Eden, M.D.

We studied the effect of concentrated surfactant-depleted BALF from 8 normal subjects, 13 patients with sarcoidosis, and 13 patients with AIDS on IL1β release by human AM. Adherent target AM were exposed to concentrated BALF in the presence or absence of LPS (1μg/ml) for two hours. Control AM were unexposed to BALF. After an additional 24-hour incubation, AM supernatants were collected and measured for IL1β by ELISA. No spontaneous IL1β release occurred from unstimulated AM. One of the sarcoid-BALF and three of the AIDS-BALF samples induced a small amount of IL1β release from unstimulated AM. In LPS-stimulated AM, exposure to normal BALF did not significantly alter IL1β release compared to unexposed AM. Exposure to sarcoid-BALF significantly increased the release of IL1β, while exposure to AIDS-BALF significantly reduced the IL1β level in the AM supernatants. The latter effect was related to the higher mortality induced by AIDS-BALF in AM. These data show that release of IL1β from LPS-stimulated AM is modified by a short exposure to a sample of alveolar fluid from patients with lung disease.

*(Chest 1990; 98:576-580)

AM = alveolar macrophages; BALF = bronchoalveolar lavage fluid; HIV = human immunodeficiency virus; ILβ = interleukin 1β; KS = Kaposi's sarcoma; LPS = lipopolysaccharide; PCP = Pneumocystis carinii pneumonia; IFN = interferon; FCS = fetal calf serum

R

egulation of lung immunity is determined in large part by the cellular effects of released regulatory proteins which act locally to modify cell responses. Extrapolation of in vitro cell responses to the in vivo situation has limitations, particularly as the in vitro response results from the net effect of both stimulatory and inhibitory influences.

Alveolar fluid obtained by BAL samples the mediators present in the environment that bathes the alveolar cells.1 For example, it has been shown that AM from patients with sarcoidosis release more immune IFN and that immune IFN augments the release of IL1 when AM are stimulated by LPS. We would expect that such augmenting factors would be present in the local environment.

In contrast to sarcoidosis, human immunodeficiency virus infection causes a severe cellular immunodeficiency with the lung being a target for opportunistic infections. Although it has been shown that the macrophage function in AIDS is impaired,2,3 the impact of the alveolar milieu on this function is unknown.

This study determines the effect of concentrated BALF from patients with sarcoidosis or AIDS on the release of IL1β from AM. It was found that the in vitro release of IL1β from endotoxin-stimulated AM can be modified by a short exposure to BALF, and that this modulation varies according to the underlying pulmonary disease.

MATERIALS AND METHODS

Experimental Plan

The experimental plan required the measurement of IL1β in 24-hour culture supernatants from either unstimulated or LPS-stimulated adherent AM exposed to concentrated BALF obtained from normal subjects, patients with sarcoidosis, or patients with AIDS. Control AM from the same volunteer were unexposed to BALF.

Subjects

The BALF was obtained by BAL from 8 normal volunteers, 13 patients with sarcoidosis, and 13 patients with AIDS. Two normal volunteers, four sarcoid patients, and five AIDS patients were smokers. All volunteers had a normal physical examination with a recent normal chest roentgenogram and no past medical history of serious illness. The BAL was performed in sarcoid or AIDS patients at the time of the diagnostic bronchoscopy. The diagnosis of sarcoidosis was made clinically and confirmed pathologically in specimens obtained by transtracheal lung biopsy. None was receiving treatment with corticosteroids. AIDS was diagnosed according to the CDC criteria. Six patients were diagnosed with PCP and two patients with KS. No specific pulmonary diagnosis was made in the other five patients.

The AM to be used as target cells were obtained by BAL from two other normal volunteers and from the opposite lung of four patients with primary lung cancer.

Informed consent was obtained from all volunteers and patients. The study was approved by the Institutional Review Board of St Luke's/Roosevelt Medical Center.

Preparation of Concentrated BALF

After local anesthesia of the airways with lidocaine, bronchoscopy
was performed via the nasal route using a fiberoptic bronchoscope. The instrument was wedged into a subsegmental bronchus, usually the right middle lobe, and lavage was performed with 200 to 250 ml of sterile saline instilled in 50-ml aliquots. The first aliquot of the lavage was discarded, and the remaining washings were pooled, strained through surgical gauze, and then centrifuged at 400g for ten minutes.

The cell-free BALF was further centrifuged at 55,000g for 30 minutes to remove the lipid components. The supernatant was concentrated using a nitrogen pressure filtration system with a membrane (10,000 MW exclusion) at 4°C. The samples were concentrated about 40-fold and then dialyzed against 50 volumes of RPMI medium overnight at 4°C, using an 8,000 MW cutoff dialysis membrane. Samples were sterilized through a 0.2 μm filter, and stored at −70°C. Measurement of total protein in concentrated BALF was made by the Lowry method.

**Preparation and Culture of Target AM**

Target AM obtained by BAL were washed twice in HBSS. The cells were then resuspended in RPMI medium supplemented with 10% heat-inactivated fetal calf serum, 2 mM L-glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin, 0.25 μg/ml amphotericin B (Fungizone), and 10 mM HEPES at a concentration of 1 × 10^6 cells/ml. A volume of 0.5 ml cell suspension was dispensed in each well of a 24-well flat-bottom culture plates and incubated for 1.5 hours at 37°C in 5% CO₂. The nonadherent cells were then washed off with warm HBSS. Previous studies have shown that after this treatment, the remaining adherent cells were >95 percent AM. More than 95 percent of the adherent AM were viable as determined by trypan blue exclusion.

Concentrated BALF was added to adherent target AM to give a final 10 percent vol/vol concentration. Some AM were simultaneously stimulated with LPS (E coli 055:BS) at a concentration of 1 μg/ml. In each experiment, both unstimulated and LPS-stimulated AM control specimens were included. The AM thus treated were incubated for 2 hours after which time the AM were washed twice with warm HBSS and the medium replaced. The incubation was then continued for an additional 24 hours. After incubation, the AM supernatants were collected, centrifuged, and stored at −70°C until IL1β assay. Previous experiments had shown that exposure of AM to LPS of 1 μg/ml for 2 hours generated submaximal IL1 release and thus provided a better chance to show changes after treatment with BALF.

The viability of adherent AM was assessed using trypan blue exclusion. The AM mortality was ranked into three levels: low (<10 percent), medium (10 to 50 percent), and high (>50 percent).

**IL1β ELISA**

The IL1β level in AM supernatants and concentrated BALF was measured in duplicate without dilution by enzyme-linked immunosorbant assay using the IL1β ELISA kit which allows the detection of as little as 20 pg/ml of IL1β concentration. For those showing a concentration greater than the range of the standard curve, the assay was repeated by using the diluted sample. There is no cross reactivity with IL1α, TNFα, or IL2.

**Gamma IFN ELISA**

Gamma IFN level in concentrated BALF was measured in duplicate without dilution by ELISA.

**Determination of Endotoxin in BALF**

A measurement of endotoxin was made in concentrated BALF using the amebocyte lyase test. The test detects as little as 0.005 to 0.01 mg/ml of endotoxin.

**Statistical Analysis**

Data are expressed as mean±SEM. The paired two-tailed Student's t-test was used to evaluate the significance of differences between BALF-exposed AM and their control AM. Comparison of groups was made by analysis of variance. Differences in frequency were compared using the chi-square test. Differences were considered significant when p<0.05. Correlation between two variables was evaluated by using Pearson correlation coefficient.

**RESULTS**

Table 1 shows the major characteristics of the BALF from different groups. The cell concentration and proportions of AM and lymphocytes in sarcoid- and AIDS-BALF are significantly different from those in normal-BALF. The protein concentration in sarcoid-BALF is significantly higher than that of normal subjects. Out of the 34 samples of concentrated BALF, only one, from a patient with AIDS, showed IL1β activity (35 pg/ml).

No IL1β was detected in any supernatants from unstimulated control AM unexposed to BALF. In general, concentrated BALF had little effect on inducing IL1β release from unstimulated AM. None of the normal-BALF samples showed this effect. Of the 13 samples derived from sarcoid patients, only one induced a small amount (36 pg/ml) of IL1β release. Similarly, of the 13 samples from patients with AIDS, three showed a stimulating effect on IL1β release from AM (42 pg/ml; 111 pg/ml; and 173 pg/ml,

### Table 1 – Characteristics of Bronchoalveolar Lavage Fluid*

<table>
<thead>
<tr>
<th>Source of BALF</th>
<th>Volume Instilled, ml</th>
<th>Fluid Returned, %</th>
<th>Cell Concentration, 1×10^6/ml</th>
<th>Cell Differential Counts</th>
<th>Protein in Conc BALF, mg/ml</th>
<th>Endotoxin in Conc BALF, ng/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>Mean 238 ± SEM 68</td>
<td>0.11</td>
<td>AM, % 83.3, Lym, % 15.5, PMN, % 1.3</td>
<td>2.1 0.31</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(n = 8)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sarcoid</td>
<td>Mean 237 ± SEM 65</td>
<td>0.29‡</td>
<td>AM, % 50.4, Lym, % 37.9, PMN, % 3.0</td>
<td>22.7 0.30</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(n = 13)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AIDS</td>
<td>Mean 214 ± SEM 56</td>
<td>0.25‡</td>
<td>AM, % 56.4, Lym, % 40.6, PMN, % 2.5</td>
<td>8.6 0.30</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(n = 13)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Abbreviations: AM, macrophages; Lym, lymphocytes; PMN, polymorphonuclear cells; Conc, concentrated.
‡Compared to normal: p<0.05.
§Compared to normal: p<0.01.
respectively). This effect was unrelated to the endotoxin level in concentrated BALF.

The effect of BALF on the IL1β release from LPS-stimulated AM is shown in Figure 1. Several of the BALF samples were tested against the same target AM as shown. In some instances, the same BALF sample was tested in AM from more than one donor and the average results are presented. A paired two-tailed Student’s t-test showed that there was no significant difference between IL1β release from AM exposed to normal-BALF vs unexposed control AM (756 ± 70 pg/ml vs 735 ± 39 pg/ml). The average change was 4 ± 4 percent. However, the IL1β level in supernatants from AM exposed to sarcoid-BALF was significantly greater than their corresponding control (1109 ± 98 pg/ml vs 742 ± 36 pg/ml, p<0.01) with an average increase of 51 ± 12 percent. In contrast, the IL1β in supernatants from AM exposed to AIDS-BALF was significantly lower than from their corresponding control (469 ± 65 pg/ml vs 621 ± 47 pg/ml, p<0.05) with an average 23 ± 9 percent decrease. The difference among the average percentage of change for the three groups is significantly different (p<0.0001).

Six of the condensed BALF samples were tested respectively twice on different target AM. The percentage of change in IL1β release from LPS-stimulated AM exposed to same BALF for the two experiments correlated well (r = 0.93, p<0.01).

In order to exclude a nonspecific nutritional effect from the increased protein concentration in medium after BALF was added, we incubated normal AM for 2 hours with additional FCS with different concentration covering the range of final concentration of protein in medium plus BALF. No significant increase in IL1β was found in supernatants from unstimulated or LPS-stimulated AM (data not shown).

We did not see any difference in response to BALF whether target AM were derived from subjects with or without lung cancer. There was no spontaneous release of IL1β from AM from both sources. The background release of IL1β from normal AM and Ca AM stimulated by LPS were 693 ± 77 pg/ml and 570 ± 96 pg/ml, respectively. The normal BALF-induced percentage of change of IL1β release from LPS-stimulated normal AM and Ca AM were 6.3 ± 5.4 percent vs 2.5 ± 2.6 percent. For sarcoid-BALF, they are 43.8 ± 10.6 percent vs 60.0 ± 20.2 percent and for AIDS-BALF, they are −20.7 ± 10.4 percent vs −28.2 ± 13.3 percent. None of these differences was significant.

The effect of BALF on the viability of either unstimulated or LPS-stimulated AM is shown in Table 2. The BALF derived from patients with AIDS reduced AM viability whether or not they were stimulated by LPS. This is in contrast to the low mortality seen in AM exposed with normal or sarcoid BALF.

The reduction of IL1β release from LPS-stimulated AM exposed to AIDS-BALF is related to the higher mortality in these cells. In this regard, the percentage of decrease of IL1β in supernatants from AM with a high mortality (n = 7) was significantly lower than that from AM with a low mortality (n = 6) (41 ± 12 percent vs 2 ± 9 percent, p<0.05).

### Table 2 — Mortality Ranking For AM Exposed to BALF

<table>
<thead>
<tr>
<th>BALF</th>
<th>Unstimulated AM</th>
<th>LPS-stimulated AM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Low, n</td>
<td>Medium, n</td>
</tr>
<tr>
<td>Normal</td>
<td>8</td>
<td>0</td>
</tr>
<tr>
<td>Sarcoid</td>
<td>13</td>
<td>0</td>
</tr>
<tr>
<td>AIDS*</td>
<td>6</td>
<td>7</td>
</tr>
</tbody>
</table>

*For unstimulated AM: AIDS vs normal: p<0.05; AIDS vs sarcoid: p<0.01. For LPS-stimulated AM: AIDS vs normal: p<0.05; AIDS vs sarcoid: p<0.01.
No gamma IFN was detected in any of the concentrated BALF samples. Furthermore, no significant correlation was found between the percentage of change in IL1β release and the cell concentration, proportion of lymphocytes, endotoxin level, or protein concentration in BALF on an individual basis.

**Discussion**

When activated by a variety of agents, AM release inflammatory mediators such as IL1. Although IL1 is important in mediating the systemic response to inflammation, its role in the pathogenesis of human lung disease is unclear. The release of IL1 from AM during disease is determined by interaction of mediators according to the disease present. Thus, in vitro studies on AM isolated from the pulmonary milieu may not totally reflect in vivo events. Addition of BALF to cultured AM may simulate conditions in the alveoli.

The current work is consistent with our earlier work showing increased IL1 release from sarcoid-AM stimulated with LPS. Although IL1β gene expression is not increased in sarcoidosis, increased IL1β levels in supernatant may be derived from a preformed store of protein. Augmentation of IL1 secretion may occur as a result of in vivo sensitization of AM to locally released lymphokines with subsequent in vitro stimulation by LPS. Neither IL1β nor immune IFN, factors known to increase IL1 release from macrophages, was present in detectable quantities in BALF and is probably not responsible for the augmentation seen.

In contrast, AIDS, characterized by a severe cellular immune deficiency, has been associated with defects in macrophage function in part due to the lack of macrophage activating factors such as gamma IFN. In addition, IL1 release from HIV-infected monocytes and macrophages has been variously reported as being increased or decreased with some of the variability being due to the concomitant release of IL1 inhibitors.

In our experiments with both stimulated and unstimulated target AM, AIDS-BALF induced higher mortality than either normal-BALF or sarcoid-BALF. Furthermore, the high mortality in stimulated AM exposed to AIDS-BALF was associated with a significant reduction of IL1β release. These observations suggest even AM uninfected with HIV may be adversely affected by the alveolar milieu in AIDS possibly from the release of toxic mediators from other cells.

The concentrated BALF preparation used in our experiments differs in several ways from alveolar lining fluid. Surfactant, which was removed to allow more rapid ultrafiltration of lavage fluid, may affect AM function. Concentration of lavage fluid by ultracentrifugation and pressure filtration causes differential loss of protein from the concentrate and may change the proportion of protein components in BALF. In addition, the extent to which alveolar lining fluid was diluted by the saline solution during BAL is unknown, and it is probable that the final concentration of BALF protein in contact with target AM was less than in alveolar lining fluid. Finally, the presence of fetal calf serum in our medium might interact with factors present in BALF.

Notwithstanding the above limitations, we show that a short exposure to BALF from patients with lung disease can alter the release of inflammatory mediators such as IL1β from LPS-stimulated AM. The mechanism is as yet to be clarified.

This model may give a better understanding as to the overall effect of the host environment on cell function and provides evidence that release of mediators from AM is modified by the surrounding alveolar milieu which changes according to the disease.

**References**

13. Matsushima K, Taguchi M, Kovacs EJ, Young HA, Oppenheim JJ. Intracellular localization of human monocyte associated interleukin 1 (IL1) activity and release of biologically active IL1
21 Coonrod JD, Yoneda K. Effect of rat alveolar lining material on macrophage receptors. Immunology 1983; 130:2589-96