Reduced Interleukin-18 Levels in BAL Specimens From Patients With Asthma Compared to Patients With Sarcoidosis and Healthy Control Subjects*

Ling-Pei Ho, MD; Margaret Davis; Alan Denison, MD; Fraser T. Wood, MD; and Andrew P. Greening, MD

Study objectives: To investigate whether differing airway interleukin (IL)-18 levels may be implicated in the pathogenesis of asthma and sarcoidosis.

Setting: University teaching hospital.

Patients and methods: IL-18 levels were measured in BAL fluid and in the supernatant of lipopolysaccharide (LPS)-stimulated alveolar macrophages obtained by BAL from 15 patients with sarcoidosis, 11 patients with asthma, and 13 healthy subjects. We also examined the relationship between IL-18 levels and macrophage and lymphocyte concentrations in BAL fluid. IL-18 was measured using an in-house enzyme-linked immunosorbent assay.

Results: IL-18 levels were significantly lower in BAL fluid from patients with asthma (median, 0.0 pg/mL; interquartile range, 0.0 to 0.0 pg/mL) compared to patients with sarcoidosis (median, 222.0 pg/10^6; interquartile range, 110 to 340 pg/mL; p = 0.009, Mann Whitney rank-sum test) and healthy control subjects (median, 162 pg/mL; interquartile range, 38 to 203 pg/mL; p = 0.025, Mann Whitney rank-sum test). Individual analyses comparing IL-18 levels with BAL macrophage counts, and IL-18 with lymphocyte counts in the three groups showed no correlation between these indexes. The mean levels of IL-18 in unstimulated macrophage supernatants were 410 pg/10^6 cells for patients with asthma, 723.4 pg/10^6 cells for patients with sarcoidosis, and 734.8 pg/10^6 cells for healthy control subjects (p > 0.05). Stimulated macrophages from patients with sarcoidosis responded with increasing amounts of IL-18 at lower doses of LPS than macrophages from healthy control subjects or patients with asthma.

Conclusion: Our findings suggest that inherently low levels of IL-18 may be associated with the pathogenesis of asthmatic airway inflammation.

(CHEST 2002; 121:1421–1426)

Key words: asthma; BAL; interleukin-18; macrophage stimulation; sarcoidosis; T-helper type 1/T-helper type 2 lymphocytes

Abbreviations: ELISA = enzyme-linked immunosorbent assay; IFN = interferon; IL = interleukin; LPS = lipopolysaccharide; Th1 = T-helper type 1; Th2 = T-helper type 2

Interleukin (IL)-18 is a cytokine with potent interferon (IFN)-γ-inducing activity.1 It is predominantly produced by activated macrophages and synergizes with IL-12 to induce IFN-γ synthesis from T lymphocytes, promoting differentiation of T cells to the T-helper type 1 (Th1) subset.2,3 The functional heterogeneity in CD4+ T lymphocytes (in the form of Th1 and T-helper type 2 [Th2] subsets) is now established, and it is thought that the balance between these two subsets of T cells may affect the phenotypes and progression of some clinical diseases.4,5 Indeed, several studies6,7 suggest that Th2 polarization in airway inflammation promotes production of IL-4 and IL-5 and hence a predominantly eosinophilic cell influx in asthma. In sarcoidosis, the converse is apparent.8,9 It seems that the imbalance in T-helper cell subsets, toward IFN-γ-producing and IL-2-producing Th1 cells, accompanies the granulomatous response in this disorder. The precise mechanisms for divergence in CD4 response in these diseases are not known. Since IL-18 is known to act early in the cascade of events...
leading to T-helper cell differentiation, we were interested in investigating whether IL-18 may be implicated in the pathogenesis of these diseases. We hypothesized that if this were true, then IL-18 levels would be low in patients with asthma and increased in patients with sarcoidosis.

**Materials and Methods**

**Study Protocol**

IL-18 levels were measured in BAL fluid and in the supernatant of stimulated alveolar macrophages obtained from BAL of patients with sarcoidosis, patients with asthma, and healthy control subjects. In addition to this, we also analyzed the relationship between IL-18 levels and macrophage and lymphocyte cell concentrations in BAL fluid.

**Subjects**

Fifteen patients with sarcoidosis, 11 patients with asthma, and 13 healthy control subjects were recruited. All patients with sarcoidosis had classical clinical presentations of the disease and, with the exception of two patients, had transbronchial biopsy findings of characteristic noncaseating granuloma. In the two patients who did not have tissue diagnosis, serum angiotensin-converting enzyme levels were elevated and a lymphocytic alveolitis was present. All patients had typical radiographic changes, ranging from stages 1 to 4 of Sitzbach criteria. One patient was receiving oral steroids at the time of bronchoscopy. Asthma was diagnosed in accord with American Thoracic Society guidelines. All patients with asthma had moderate-to-severe persistent asthma (classified according to Global Initiative for Asthma guidelines) and were receiving therapy with inhaled corticosteroids, 500 to 2,000 mg/d; four patients with asthma also were receiving oral steroids, 5 to 20 mg/d. The group of healthy control subjects was composed of healthy staff (n = 6) who volunteered for the study and nonsmoking subjects who underwent bronchoscopy for investigation of a single episode of hemoptysis (n = 7). In the latter group, the subjects were otherwise symptomatically well and were healthy at review after 3 months.

Smokers and patients with intercurrent infections were excluded from the study. Demographic data of the subjects are shown in Table 1. The study was approved by the local hospital ethics committee.

**BAL Procedure**

BAL was performed using an Olympus KeyMed fiberoptic bronchoscope (Olympus; Tokyo, Japan). Subjects were sedated with fentanyl, 50 μg, and midazolam, 2 to 5 mg, and received 5 L/min of oxygen via nasal prongs throughout the procedure. Four to 12 mL of 1% lidocaine was administered to the lower bronchial tree during the procedure.

The tip of the bronchoscope was wedged in a segmental or subsegmental bronchus of the middle lobe or the lingula. Eight aliquots of 30 mL of sterile normal saline solution at 37°C were instilled, aspirated into a collection trap, and placed immediately on ice. BAL fluid was then filtered through sterile gauze and centrifuged at 200g for 10 min at 4°C. The cell pellet was then resuspended, and total harvested cells were counted. Differential cell counts were obtained from smears stained with hematoxylin-eosin with at least 500 cells counted. The cell free BAL fluid was stored, in aliquots, at −80°C until batch analysis for IL-18.

**Stimulated Alveolar Macrophage Cultures**

Cells from the cell pellet derived from centrifuged BAL fluid were resuspended in 1640 RPMI (GIBCO; Paisley, UK), supplemented with glutamine and penicillin/streptomycin. Macrophages were plated at 1 × 10⁶ cells/mL on multiwell plastic plates and adhered for 1 h at 37°C. The plates were then washed to leave only adherent cells. Lipopolysaccharide (LPS) [Sigma; Poole, UK] in 1640 RPMI was added to give final concentrations of 0.1, 1, 10, and 100 ng/mL. The cells were then incubated at 37°C for 20 h, and culture supernatants were collected and stored at −80°C until batch analysis.

**IL-18 Assay**

IL-18 was measured by a blinded investigator (M.D.) using an in-house enzyme-linked immunosorbent assay (ELISA). Briefly, ELISA plates were coated with mouse antihuman IL-18 antibody (R&D Systems; Abingdon, UK) for 3 h at 37°C. The plates were then washed with distilled water containing 0.05% Tween 20 and blocked with 3% bovine serum albumin in commercially available blocking buffer (Dynex; Middlesex, UK). One hundred microliters of sample were added and left overnight (18 to 20 h) at 4°C. A biotin-conjugated polycononal rabbit antihuman IL-18 antibody (R&D Systems) was added with agitation on an orbital shaker for 2 h. After further washing, an antirabbit alkaline phosphatase conjugate (Jackson Immunoresearch; Dundee, UK) was applied and left for 2 h. The substrate, p-nitrophenyl phosphate (Sigma) in diethanolamine buffer (BDH; Poole, UK), was added for 30 min at room temperature. The plates were read using an ELISA plate reader at 405 nm. Standard curves were prepared in 0.9% saline solution for the BAL fluids and 1640 RPMI for macrophage supernatant using commercially available recombinant human IL-18 cytokine (R&D Systems).

A standard curve for IL-18 concentrations between 15.6 pg/mL and 1,000 pg/mL was generated for each sample batch. The lower limit of detection of our assay was 15.6 pg/mL.

**Results**

**BAL Fluid**

The volumes of fluid recovered from the 240 mL of saline solution instilled ranged from 80 to 180 mL (mean, 143 mL). Volumes of BAL fluid recovered did not differ significantly in the three groups.

**BAL Total and Differential Cell Counts**

The mean macrophage cell counts for all three groups were not significantly different from each

![Table 1—Demographic Data of Recruits*](http://journal.publications.chestnet.org/pdfaccess.ashx?url=/data/journals/chest/21977/ on 04/09/2017)
other. However, patients with sarcoidosis had significantly increased lymphocytes compared with healthy control subjects and patients with asthma (Table 2).

**IL-18 Levels in BAL Fluid**

IL-18 levels were significantly lower in BAL for patients with asthma (median, 0.0 pg/mL; interquartile range, 0.0 to 0.0 pg/mL) compared with patients with sarcoidosis (222.0 pg/mL; interquartile range, 110 to 340 pg/mL; \( p = 0.009 \), Mann-Whitney rank-sum test) and healthy control subjects (median, 162 pg/mL; interquartile range, 38 to 203 pg/mL; \( p = 0.025 \), Mann-Whitney rank-sum test) [Fig 1]. Analyses comparing IL-18 levels with BAL macrophage counts and IL-18 levels with lymphocyte counts in the three study groups showed no definite correlations, although there was a trend for IL-18 levels to increase with a decrease in lymphocyte counts in samples from patients with sarcoidosis and healthy subjects.

**IL-18 Levels in Stimulated Macrophage Supernatants**

The mean levels of IL-18 in unstimulated macrophage supernatants were 410 pg/10^6 cells for patients with asthma, 723.4 pg/10^6 cells for patients with sarcoidosis, and 734.8 pg/10^6 cells for healthy control subjects. These differences did not reach statistical significance (\( p = 0.415 \), one-way analysis of variance). When stimulated with LPS, macrophages from patients with sarcoidosis but not from healthy control subjects or patients with asthma showed a trend of increasing IL-18 production at lower concentrations of LPS (Fig 2). The shape of the dose response curves to LPS suggested the following: (1) the sarcoid macrophages responded to 10 ng/mL, and the response was still rising at the maximal dose of 100 ng/mL; (2) macrophages from healthy control subjects responded to stimulation with 100 ng/mL, but not 10 ng/mL, and the response may still have been rising at the maximal dose of 100 ng/mL; and (3) macrophages from patients with asthma had a limited response to 10 ng/mL, with a plateau at that level and no further rise in response to 100 ng/mL.

![Figure 1. IL-18 levels in BAL fluid from patients with asthma compared to patients with sarcoidosis (sarcoid) and healthy control subjects (normals). Levels in patients with asthma were significantly lower than in patients with sarcoidosis and healthy control subjects. Levels represented are median values with interquartile range.](image1)

![Figure 2. Mean levels of IL-18 in supernatants from macrophage cultures with no stimulation and with increasing levels of LPS stimulation (all measured after 20 h) for patients with asthma, patients with sarcoidosis, and healthy control subjects. Levels quoted as amount per 10^6 cells (error bars = SEM).](image2)

**Table 2—Differential Cell Counts in BAL Fluid From the Three Groups**

<table>
<thead>
<tr>
<th>Participants</th>
<th>Total Cell Count, ( \times 10^6 )</th>
<th>Macrophages, ( \times 10^6 )</th>
<th>Lymphocytes, ( \times 10^6 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal subjects</td>
<td>12.0 ± 8.2</td>
<td>9.7 ± 6.9</td>
<td>2.2 ± 2.9</td>
</tr>
<tr>
<td>Asthma patients</td>
<td>5.3 ± 4.4</td>
<td>4.5 ± 4.3</td>
<td>2.2 ± 1.5</td>
</tr>
<tr>
<td>Sarcoidosis patients</td>
<td>14.0 ± 6.2</td>
<td>8.0 ± 3.4</td>
<td>5.6 ± 4.4</td>
</tr>
</tbody>
</table>

*Data are presented as mean ± SD. Lymphocyte counts were significantly higher in patients with sarcoidosis compared to patients with asthma and normal subjects (\( p = 0.001 \) and \( p = 0.004 \), respectively, with Student t test). No statistically significant difference was found for the other parameters.
Discussion

This study is one of the first to examine IL-18 levels in vivo in the airway compartment of patients with lung diseases that may be modulated by that cytokine. Like many patient-based clinical studies, there are constraints placed by the heterogeneity of the patient population. However, within these constraints, the data show that IL-18 levels in the airway lavage of patients with asthma are significantly lower than those of patients with sarcoidosis and healthy control subjects. The levels did not correlate with total numbers of cells or of macrophages in the BAL fluid in any of the subject groups. The LPS macrophage stimulation data suggest that the ability of macrophages to produce IL-18, compared with healthy control subjects, may be increased in patients with sarcoidosis and reduced in patients with asthma. The limitations of the LPS stimulation data are discussed below.

The low IL-18 levels in the asthmatic airways appears marked. One explanation to consider for this finding is that all of these patients were receiving inhaled corticosteroids. The effect of corticosteroids on IL-18 is currently uncertain. However, we found that all patients except one had equally and markedly reduced levels of IL-18, irrespective of the dose of inhaled steroids that they were receiving. One would, therefore, have to hypothesize that IL-18 is highly sensitive to corticosteroids such that even the lowest dose inhibited IL-18 maximally. However, the sample with the highest level of IL-18 (0.5 ng/mL) came from a patient with asthma who was receiving 7 mg of prednisolone continuously (in addition to 800 µg/d of inhaled budesonide). Thus, in that instance, high doses of inhaled and systemic corticosteroid did not cause any hypothesised corticosteroid suppression of IL-18 synthesis. Further, Cameron et al14 found that in situ hybridization expression of IL-18 in airway epithelium of patients with asthma not receiving corticosteroids was decreased compared with patients with sarcoidosis and healthy control subjects. Thus, it appears likely that our observations are real rather than an artifact of inhaled steroid therapy. The observations relate only to a single time point and cannot distinguish cause from effect. Nevertheless, existing in vitro data lend support to the hypothesis that low levels of IL-18 may be an important causative factor in the genesis of asthmatic inflammation.

A number of animal and in vitro studies have implicated IL-18 in the divergence of Th1 and Th2 immune response.15–20 Two strands of message are apparent from these data. First, IL-18 has an important role in Th1 differentiation and therefore effective cellular immunity. This is seen most clearly in containment of mycobacterial and cryptococcus infection in mice.21–23 Second, IL-18 deficiency appears to promote allergic inflammation characterized by eosinophilia.16,24 The latter data, however, are not unchallenged. Some studies25–27 have also shown that IL-18 can promote IL-4 and serum IgE production and induce airway eosinophilia. Hence IL-18 is likely to have different functions, possibly dependent on where and when it is produced.28 Monteforte and colleagues21 demonstrated that IL-18 deficient mice could eventually clear Leishmania major infection, although the response was significantly delayed when compared with wild-type mice. It would seem that there is a nonessential interdependence between IL-4, IL-12, and IL-18, so that the deficiency of one of these cytokines will stimulate the production of another.21 Our finding of almost uniformly low IL-18 levels in patients with asthma, irrespective of disease severity, suggests that IL-18 may be implicated at an early stage of the pathogenesis of the disease, and the downstream effect of this deficiency interacts with other mediators to modulate disease severity. This is concordant with recently published data on IL-18 deficient mice, which suggest that IL-18 enhances eosinophil inflammation by acting on eosinophil recruitment.24 Indeed, there is evidence that the role of IL-18 in development of a critical Th1 response occurs early in this response.10

We had expected to find increased levels of IL-18 in patients with sarcoidosis, in keeping with the hypothesis that IL-18 is involved in T-helper cell differentiation. We did not find such raised levels in the BAL fluid, although the macrophage activities suggested the possibility of an IL-18 response at lower levels of LPS stimulation. The state of T-helper cell differentiation in sarcoidosis has not been as fully investigated as in asthma, and differentiation of T-helper cells at different stages of sarcoidosis is unclear. We have previously observed different patterns of CD4+ and CD8+ lymphocytes in BAL of patients with varying degrees of disease chronicity.29 It is possible that patients with differing disease severity may demonstrate different levels of IL-18 in their lungs. There have been three articles30–32 that have shown increased levels of IL-18 in sarcoid lungs; the levels reported in these studies were actually lower than the levels we found in our patients with sarcoidosis (5 to 70 pg/mL compared with a mean of 222 pg/mL). There were differences in methodology between studies, which could account for differences in absolute values reported. For example, Shigehara and colleagues32 concentrated their BAL fluid 10-fold before assay. However, the implication is that patient and control selection may be important in the finding of signifi-
significant elevation of IL-18, rather than our data indicating that the hypothesis associating IL-18 and sarcoidosis is incorrect.

What our BAL fluid data do suggest is that there is a large difference between the levels of IL-18 in patients with sarcoidosis and patients with asthma. Our macrophage stimulation data would suggest that macrophages derived from sarcoid airways have a greater ability to produce IL-18. Although the differences between the disease groups and healthy control subjects did not reach statistical significance, the pattern suggests that there may be alterations between healthy control subjects and both the sarcoidosis and asthma groups in the pattern of the dose response. The response to LPS in sarcoidosis is sharp, commencing at low doses, and may not have reached the top of the dose-response curve at 100 ng/mL. The healthy control subjects seem not to respond to the lower doses of LPS, but they do respond to 100 ng/mL, and the top limit of the dose response is not clear. However, asthmatic alveolar macrophages appear to have completed the dose response by 10 ng/mL and show a much more flat response. These interpretations are, of course, made cautiously because the maximum stimulation dose was 100 ng/mL. It would have been valuable to have examined other LPS doses (eg, 50, 500, and 1,000 ng/mL), and perhaps future studies should be more comprehensive. In conclusion, our findings, in conjunction with animal data, suggest that inherently low levels of IL-18 may be implicated in airway inflammation of patients with asthma.

References

17. Yoshimoto T, Takeda K, Tanaka T, et al. IL-12 up-regulates IL-18 receptor expression on T cells, Th1 cells, and B cells: synergism with IL-18 for IFN-γ production. J Immunol 1999; 161:3400–3407
26. Kumanu K, Nakao A, Nakajima H, et al. Interleukin-18 enhances antigen-induced eosinophil recruitment into the...
27 Yoshimoto T, Tsutsui H, Tominaga K, et al. IL-18, although antiallergic when administered with IL-12, stimulates IL-4 and histamine release by basophils. Proc Natl Acad Sci U S A 1999; 96:13962–13966
31 Shigehara K, Shijubo N, Ohmichi M, et al. IL-12 and IL-18 are increased and stimulate IFN-γ production in sarcoid lungs. J Immunol 2001; 166:642–649