

Cellular and biochemical characteristics of bronchoalveolar lavage fluid in symptomatic nonallergic asthma

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We have undertaken cellular and biochemical examination of bronchoalveolar lavage fluid from nonallergic patients with asthma to determine the nature and degree of inflammatory process in symptomatic asthma. Six patients with asthma (mean methacholine provocative concentration causing a 20% fall in FEV₁ was 0.26 mg/ml) and six control subjects underwent fiberoptic bronchoscopy with bronchoalveolar wash. The patients with asthma shed a higher number of epithelial cells into lavage fluid than normal control subjects ($p < 0.05$). Their lavage fluid also contained increased numbers of neutrophils ($p < 0.025$), eosinophils ($p < 0.025$), and basophilic cells ($p < 0.025$), and increased proportion of activated T cells ($p < 0.05$). The basophilic cells were mast cells, as indicated by positive labeling with the monoclonal antibody MCG35. Biochemical analysis of lavage fluid demonstrated exudation of protein molecules in airways of patients with asthma with increased contents of albumin ($p < 0.05$) and fibronectin ($p < 0.05$). In the lavage fluid of patients with asthma, there were also increased amounts of interleukin-1- β (IL-1- β) ($p < 0.025$), interleukin-6 (IL-6) ($p < 0.025$), and granulocyte-macrophage, colony-stimulating factor (GM-CSF) ($p < 0.05$), as compared with lavage fluid of normal control subjects. Immunocytochemical evaluation of lavage cells demonstrated that IL-1- β , IL-6, and GM-CSF were mostly produced by nonciliated epithelial cells and/or monocytes. IL-1, IL-6, and GM-CSF can prime granulocytes to respond to other stimuli and can promote T cell activation. The production of these cytokines by bronchial resident cells may have a role in the perpetuation of the inflammatory responses in asthma. (J ALLERGY CLIN IMMUNOL 1991;87:794-802.)

Several studies have suggested that inflammation may play a role in the pathogenesis of chronic asthma. Histopathologic studies from patients who died during asthma attacks have demonstrated marked inflammation in the airways with infiltration of inflammatory

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Abbreviations used

IL-1- β :	Interleukin-1- β
IL-6:	Interleukin-6
GM-CSF:	Granulocyte-macrophage colony-stimulating factor
IL-2:	Interleukin-2
SDS-PAGE:	Sodium dodecyl sulfate-polyacrylamide gel electrophoresis
BALF:	Bronchoalveolar lavage fluid
PFR:	Peak flow rate

cells, particularly eosinophils, and disruption of airway epithelium.¹⁻⁴ Similar, but less severe, pathologic changes have been described in bronchial biopsy specimens from mildly affected patients.⁵⁻⁷

In an attempt to quantify the magnitude of airway inflammation and relate this to the functional alterations of asthma, other investigators have examined BALF of subjects with asymptomatic bronchial hyperresponsiveness and have also found an increase in

inflammatory cells, particularly eosinophils, basophils/mast cells, and lymphocytes,⁸⁻¹⁰ supporting the experimental evidence that airway inflammation may underlie bronchial hyperresponsiveness.^{11, 12} However, these studies have been performed in subjects with allergic asthma who were asymptomatic at the time of the investigation; therefore, similar results may not apply to nonallergic asthma or to the symptomatic phase of the disease. In fact, no correlation has been recently found between the degree of bronchial hyperresponsiveness and the number of inflammatory cells in BALF on follow-up examination of both symptomatic and asymptomatic patients with asthma.¹³ Since in all the studies reported, inflammation has been quantitated in terms of number of infiltrating cells, it might be possible that the state of activation of the cells is more important than their absolute number for the development of symptoms and increased hyperresponsiveness.

Alveolar macrophages, mast cells, and granulocytes from patients with asthma do release more mediators and toxic products than cells from normal subjects *in vitro*, both spontaneously and on stimulation,^{8, 14-16} and this finding appears to relate to the degree of airway hyperresponsiveness and severity of symptoms.¹⁴⁻¹⁶ Cytokines, like IL-1, IL-6, and GM-CSF, produced by monocytes, lymphocytes, or bronchial epithelial cells,¹⁷⁻²² may be responsible for this priming of inflammatory cells *in loco*, and the mode of interaction between resident cells and cells migrated to the bronchial mucosa may be a peculiar characteristic of asthma that has been poorly investigated at the present time.

MATERIAL AND METHODS

Subjects and study design

Six patients with asthma (four were female; mean age $\pm$ SEM, 35.6 ± 3.5 years) and six control subjects (three were female; mean age $\pm$ SEM, 45.8 ± 6.5 years) were included in the study. All subjects were nonsmokers; they had no history of allergic rhinitis or eczema and demonstrated negative responses to skin prick testing with 12 common allergen extracts.

The patients with asthma were receiving treatment with inhaled bronchodilators alone, on demand, but their symptoms were not controlled because they were easily elicited by nonspecific irritating stimuli, or required large doses of bronchodilators daily, or had disturbed sleep. No patient had received corticosteroids in the last month. The patients did not recall any known recent precipitating event like viral respiratory illness or exposure to fumes and dust.

Patients with asthma were monitored for 8 days before performing bronchoscopy. During that period they measured PFRs at home four times daily and recorded symptoms and treatment. On the last day, they had pulmonary function

measurements and bronchoprovocation tests with methacholine to define their nonspecific bronchial hyperresponsiveness. They all demonstrated a diurnal variation in PFRs $>10\%$ on more than one occasion during the monitoring period²³; their geometric mean provocative concentration of inhaled methacholine inducing a 20% fall in FEV₁ was 0.26 mg/ml (range, 0.05 to 1.10 mg/ml),²⁴ and their mean baseline FEV₁ ($\pm$ SEM) was $72.3\% \pm 7.1\%$ of predicted.

Control subjects had no past history of asthma and no evidence of bronchial hyperresponsiveness.^{23, 24} Their provocative concentration of inhaled methacholine inducing a 20% fall in FEV₁ was >64 mg/ml,²⁴ and their FEV₁ ($\pm$ SEM) was $105.0\% \pm 9.6\%$ of predicted.

Patients with asthma and control subjects underwent bronchoscopy with BAL according to the protocol indicated below.

All participants gave informed consent.

Bronchoscopy

This procedure conformed to the guidelines outlined by the American Thoracic Society.²⁵

Fiberoptic bronchoscopy was performed after premedication with atropine and diazepam, salbutamol via a nebulizer, and local anaesthesia with 2% lidocaine. Sterile isotonic saline solution, warmed to body temperature, was used for lavage. The tip of the bronchoscope was wedged into a segmental bronchus in the middle or lower lobe of one lung. Four aliquots of 30 ml were instilled into a subsegmental bronchus, and the fluid was aspirated through the suction channel of the bronchoscope with a 50 mm Hg negative pressure, collected in chilled suction traps, and held at 4° C.

Soon after bronchoscopy, salbutamol was administered again to the patients with asthma via a nebulizer; patients received 25 mg of prednisone and were then referred to our outpatient clinic to receive adequate treatment.

Sample processing

The lavage fluid recovered from each subject was pooled, the volume was measured, and total cell count and viability were assessed by the trypan blue exclusion method with a Burkner chamber (Cecchinato, Venice, Italy). The fluid was then centrifuged (500 g for 10 minutes). The cell-free supernatant was decanted and immediately stored at -80° C for subsequent evaluation. The cells were resuspended in Hanks' balanced salt solution and finally transferred onto glass slides after collection on polycarbonate membranes (Nucleopore cytodagnostic membranes, Nucleopore Italia Srl, Milan, Italy) by filtration, according to a standard method.²⁶ At least 20 slide preparations from each sample were air-dried and stored at -20° C for subsequent immunocytochemical staining. Duplicate May-Grunwald-Giemsa-stained slides were used for differential counting of at least 200 cells.

For immunocytochemical evaluation, the slide preparations were thawed at room temperature, fixed in analytical grade acetone, and incubated with specific antibodies (Table I). Antibody binding was demonstrated with the use of an alkaline-phosphatase antialkaline-phosphatase sys-

TABLE I. Monoclonal antibodies for immunocytochemical analysis of cells in BALF

Antibody	Cell marker	Reference	Source
TEC-T11	CD2, T-lymphocytes	27	Technogenetics*
OKT4	CD4, helper/inducer T-lymphocytes	28	Ortho†
OKT8	CD8, suppressor/cytotoxic T cells	28	Ortho
OKT26a	CD25, IL-2 receptor	29	Ortho
OKB2	CD22, B-lymphocytes	30	Ortho
OKM14	CD14, monocytes	31	Ortho
MCG35	Mast cell granules	32	Dr. F. E. Rimmer
DAKO-CK1	Cytokeratin, epithelial cells	33	DAKOPATTS

*Technogenetics, Turin, Italy.

†Ortho, Milan, Italy.

tem.³⁴ To identify subsets of lymphocytes, double labeling was accomplished by incubating the cells, first, with the biotin-conjugated TEC-T11 and demonstrating the binding with the streptavidin-biotin-immunoperoxidase-staining system³⁵ (Immustain kit, DPC Co., Los Angeles, Calif.), which stains positive cells brown. Cells were then incubated with purified OKT4, OKT8, or OKT26a, and binding was revealed by the use of the alkaline-phosphatase antialkaline-phosphatase system and an enzyme substrate that labels positive cells blue (substrate kit III, Vector Laboratories, Inc., Burlingame, Calif.). At least 400 nucleated cells were counted on each slide, and the percentage positivity was calculated.

Total protein content in unconcentrated BALF was quantitated with a protein binding-dye assay (Bio-Rad Laboratories, Milan, Italy). Albumin was quantitated with low-level immunodiffusion plates (Behring Diagnostics, San Diego, Calif.).^{36, 37}

Fibronectin was purified from BALF by affinity chromatography²² with gelatin-Sepharose beads (Pharmacia Fine Chemicals, Uppsala, Sweden).³⁸ Then, fibronectin contents in the samples were evaluated by ELISA³⁸ with a polyclonal antibody against fibronectin (DAKOPATTS, Glostrup, Denmark). Immunodetection was obtained by the streptavidin-horseradish-peroxidase system with o-phenylenediamine as substrate. Absorbance was measured at a wavelength of 492 nm with an ELISA reader laboratory system Titertek Uniskan (Flow Laboratories, Milan, Italy), and the concentration of fibronectin in the samples was calculated by comparison to the standard curves obtained with different concentrations of standard fibronectin (Sigma Chemical Co., St. Louis, Mo.). The sensitivity of this assay was 12 ng/ml.

IL-1- β , IL-6, and GM-CSF proteins were isolated from BAL by binding to CNBr-Sepharose 4B beads (Pharmacia Fine Chemicals) coupled to the specific rabbit antisera against human IL-1- β , human IL-6, or human GM-CSF. (All were purchased from Genzyme Laboratories, Boston, Mass.)²² The contents of immunoreactive IL-1- β , IL-6, and GM-CSF in the samples were then evaluated by the two-antibody sandwich ELISA with commercially available kits

(ELISA IL-1 kit, Quantikine, British Bio-Technology Ltd., Oxford, U.K.; IL-6 ELISA kit and factor test, Genzyme).²² Immunodetection was obtained as indicated for fibronectin assay, and the concentration of each cytokine was calculated by comparison to the curve obtained with different concentrations of the standards, included in each kit. The sensitivity of these assays was IL-1- β , 0.05 ng/ml; IL-6, 0.5 ng/ml; and GM-CSF, 0.01 ng/ml.

To evaluate further the characteristics of the BAL fibronectin-, IL-1- β -, IL-6-, and GM-CSF-like substances, fluid samples from each group of subjects tested were pooled, concentrated 50 times with Amicon 8010 standard cells and UM2 membranes (Amicon Corp., Lexington, Mass.), precleared by incubation with protein A-Sepharose beads (Pharmacia Fine Chemicals), and incubated at 4°C overnight with saturating concentrations of the rabbit monospecific antisera against fibronectin, IL-1- β , IL-6, and GM-CSF described above. Purified goat antirabbit antibody coupled to Immunobead matrix (Immunobead second-antibody reagent, Bio-Rad Laboratories) was finally added, and the samples were reincubated for 2 hours at 37°C.²² Immunocomplexes were precipitated by centrifugation (1000 g for 7 minutes). Precipitates were washed with phosphate-buffered saline, resuspended in equal volumes of SDS sample buffer with 2% 2-mercaptoethanol, and boiled for 3 minutes. Beads were removed by centrifugation, and supernatants were loaded onto 7.5% (fibronectin) or 12% (IL-1- β , IL-6, and GM-CSF) SDS polyacrylamide gels for SDS-PAGE.²² Proteins separated by SDS-PAGE were transferred to nitrocellulose and stained with the antisera against fibronectin, IL-1- β , IL-6, and GM-CSF described above, according to standard procedures for Western blotting. For detection of rabbit immunoglobulins, a Vectastain ABC kit (PK-4001, Vector Laboratories) was used. Biotinylated molecular weight markers were obtained from Sigma Chemical Co.

To identify which types of cells produced IL-1, IL-6, and GM-CSF, the presence of these cytokines in the cytoplasm of lavage cells was evaluated with immunocytochemical techniques.³⁹ Each cell type was identified by labeling the cells with the monoclonal antibody against the specific sur-

TABLE II. Comparison of cellular profile in BALF

	Normal subjects	Patients with asthma	<i>p</i>
N	6	6	
% Cell viability	93.6 ± 4.5	91.8 ± 2.4	>0.1
% Fluid recovery	52.9 ± 8.7	48.7 ± 10.8	>0.1
Cells (× 10/ml)			
Nucleated	12.8 ± 8.6	25.8 ± 3.1	<0.05
Macrophages	10.5 ± 0.08	12.0 ± 2.0	>0.1
Epithelial	1.07 ± 0.6	5.9 ± 1.0	<0.05
Lymphocytes	1.05 ± 0.28	1.3 ± 0.9	>0.1
Neutrophils	0.13 ± 0.06	4.4 ± 1.2	<0.025
Eosinophils	0.04 ± 0.02	1.12 ± 0.3	<0.025
Basophilic	0.01 ± 0.005	0.2 ± 0.08	<0.025
CD2 positive	0.75 ± 0.03	1.32 ± 0.02	<0.05
MCG35 positive	0.01 ± 0.006	0.19 ± 0.08	<0.025
CD14 positive	0.28 ± 0.05	1.15 ± 0.43	<0.05
Ratio CD4/CD8 positive cells	1.65 ± 0.09	2.20 ± 0.41	>0.1

Results are presented as mean ± SEM.

face marker (Table I). Then, the cytoplasmic cytokines were labeled with the monospecific antisera indicated above after immobilization of the proteins by fixation, and cell permeabilization by incubation with 0.4% Triton X-100, 50 mmol/L of glycine HCl, and 5% glucose in phosphate-buffered saline.³⁹ Two immunoenzymatic systems were used to reveal positive binding of the two antibodies to the same cell³⁹: the binding of the monoclonal antibody to the specific marker of each cell type was identified by the streptavidin-biotin-immunoperoxidase system and a substrate that stains positive cells brown, whereas the binding of the polyclonal antibodies to each intracellular cytokine was identified by the alkaline-phosphatase antialkaline-phosphatase system and a substrate that stains positive cells blue (substrate kit III, Vector Laboratories). Duplicate slides from each lavage fluid were incubated with unrelated rabbit antisera to control for nonspecific binding of the antisera against each cytokine. At least 1000 nucleated cells were evaluated on coded slides.

Data analysis

The differential cell counts in the lavage fluid were expressed as the numbers of cells per milliliter, calculated by multiplying the total nucleated cell count by the percentage of the specific cell type in the lavage fluid.

For immunocytochemical evaluation of cells containing immunoreactive IL-1-β, IL-6, or GM-CSF, the epithelial cells, monocytes, and T cells labeled with each monospecific antiserum were counted on duplicate slides, and their counts were expressed as percentage of the total cells, respectively, demonstrating keratin filaments or bearing the surface markers of monocytes (CD14) and lymphocytes (CD2).

The differences between the groups with asthma and without asthma in the total and differential cell counts and protein contents in lavage fluid were analyzed by the two-

tailed Mann-Whitney U test.⁴⁰ Differences associated with probability values of $p < 0.05$ were considered to be significant.

In the text, results are expressed as mean ± SEM.

RESULTS

Cellular profile in BALF

The volumes of lavage fluid recovered from patients with asthma and from normal subjects were almost identical (Table II).

The patients with asthma had greater numbers of epithelial cells, eosinophils, neutrophils, basophilic cells, and monocytes than normal subjects (Table II). These differences were statistically significant.

The immunocytochemical staining of the lavage cells demonstrated that 96.6% of lymphocytes from airways of patients with asthma were T-lymphocytes and that an increased number of these T cells were activated, as demonstrated by the augmented expression of the surface marker of activation CD25 (Table II). There was no significant change in the ratio of CD4 to CD8 positive cells in patients with asthma. The number of basophilic cells detected by May-Grunwald-Giemsa stain was very close to the number of MCG35 positive cells, indicating that most of the lavage basophilic cells were mast cells both in patients with asthma and in normal control subjects (Table II).

Biochemical evaluation of BALF

In the airways of patients with asthma, there was exudation of protein molecules, which was reflected in the finding of increased contents of total proteins and albumin in the lavage fluid from those patients as

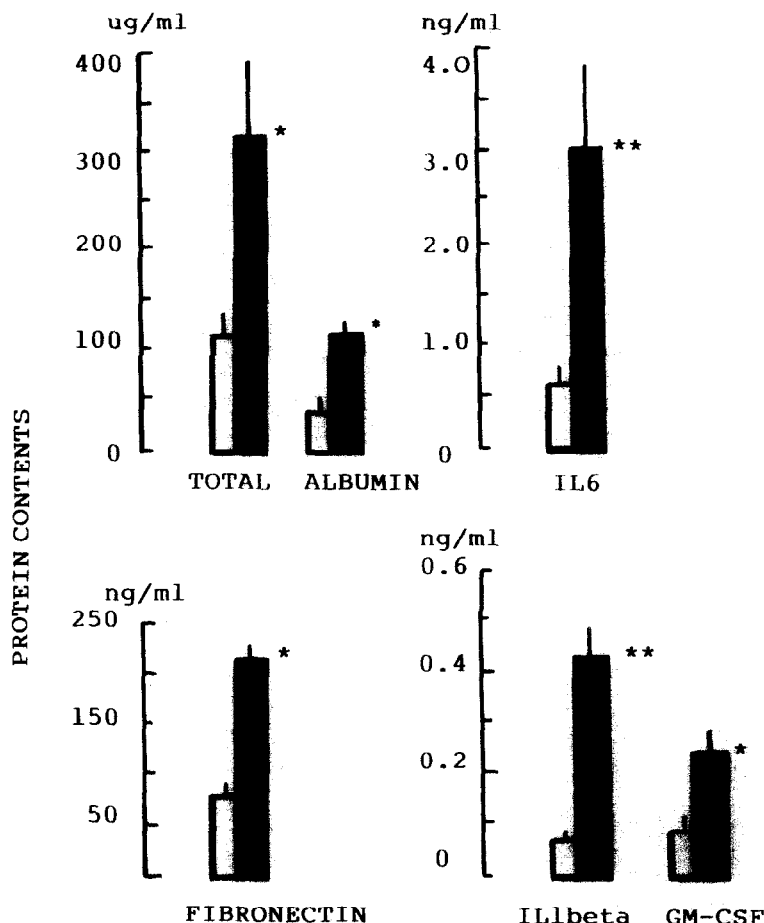


FIG. 1. Analysis of protein contents in BALF from patients with asthma (■) and normal control subjects (□); mean values $\pm$ SEM; * $p < 0.05$; ** $p < 0.025$.

compared to normal lavage fluid (total proteins, 315.6 ± 112.0 versus 103.0 ± 33.1 $\mu\text{g/ml}$, $p < 0.05$; albumin, 104.8 ± 16.2 versus 38.3 ± 12.9 $\mu\text{g/ml}$, $p < 0.05$). The patients with asthma also had increased amounts of lavage fibronectin (219.6 ± 9.8 versus 62.3 ± 8.9 ng/ml , $p < 0.05$), IL-1- β (0.438 ± 0.098 versus 0.065 ± 0.012 ng/ml , $p < 0.025$), IL-6 (3.1 ± 0.85 versus 0.60 ± 0.19 ng/ml , $p < 0.025$), and GM-CSF (0.22 ± 0.098 versus 0.09 ± 0.015 ng/ml , $p < 0.05$) (Fig. 1).

Immunoprecipitation of fibronectin, IL-1- β , IL-6, GM-CSF, and SDS-PAGE, followed by Western blot analysis, resulted in the prominent appearance of bands compatible with the mobility of the authentic cytokines^{20, 41, 42} (Fig. 2). By these methods, two isoforms of IL-6 were obtained (Fig. 2), indicating that the IL-6 in lavage fluid from both patients with asthma and control subjects reveals the heterogeneity demonstrated for IL-6 derived from circulating monocytes,²⁰ keratinocytes,⁴³ and injured airway epithelial cells.²²

In immunocytochemical evaluation of lavage cells, immunoreactive IL-6 and GM-CSF proteins were localized in the cytoplasm of keratin-positive nonciliated epithelial cells and in CD14-positive monocytes (Fig. 3). GM-CSF was also found in the cytoplasm of a small proportion of T cells. The monospecific antiserum against human IL-1- β labeled exclusively CD14-positive cells (Fig. 3). The percentages of epithelial cells, T cells, and monocytes containing IL-1- β , IL-6-, and GM-CSF-like substances were significantly higher in patients with asthma than in normal subjects (Figs. 3 and 4).

DISCUSSION

In this study we have evaluated the cellular and biochemical characteristics of BALF from nonallergic patients with asthma who were symptomatic and had evidence of moderate to severe bronchial hyperresponsiveness, as objectively demonstrated by the degree of the responses to a nonspecific bronchoprovocation tests and by the variability of PFR. Most pre-

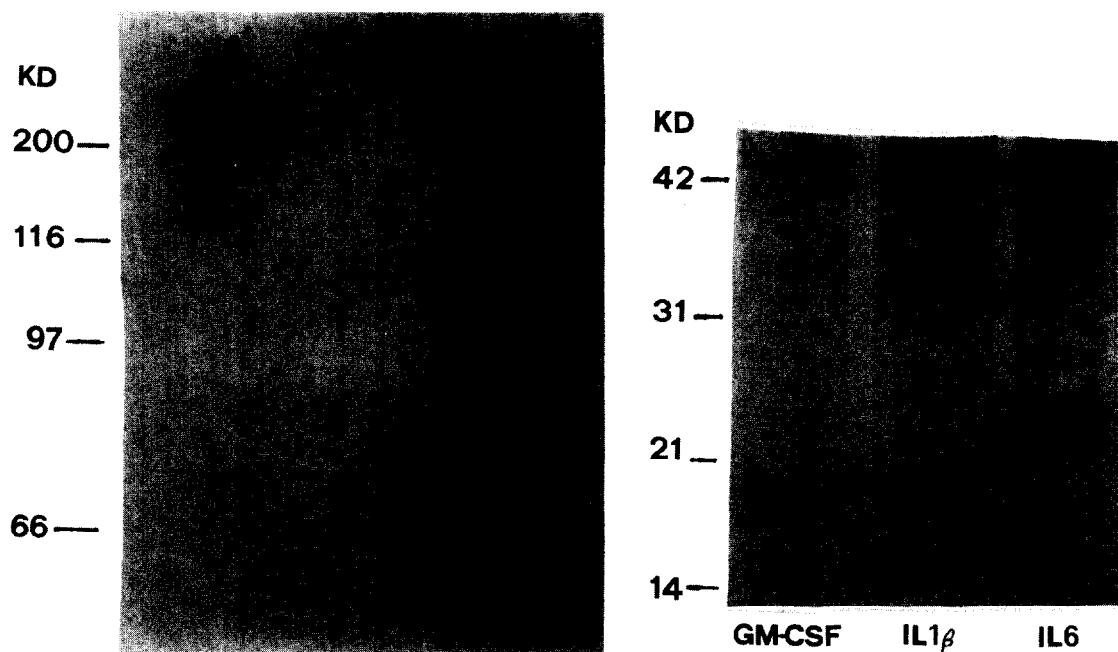


FIG. 2. A, Western blot of fibronectin protein in comparable amounts of pooled BALF from six patients with asthma (A) and six normal control subjects (N). B, Western blot of GM-CSF, IL-1- β , and IL-6 proteins in pooled BALF from six patients with asthma. The position of the protein markers, with the indicated molecular weights, is illustrated on the left.

vious studies have been performed in atopic patients who did have bronchial hyperresponsiveness but were asymptomatic.⁷⁻¹⁰ The atopic status may be a confounder in evaluating the inflammatory responses in the airways of patients with asthma because the alterations can relate to the allergen exposure per se, and specific alterations of asthma may not be easily analyzed when the disease is in the remission phase.

In our study, patients with asthma had varying degrees of inflammatory cell infiltration in the airways, which was similar in characteristics to that previously observed in asymptomatic atopic patients,⁷⁻¹⁰ although the cell infiltration was more severe. In fact, according to previous results, these patients had significantly higher numbers of neutrophils, eosinophils, mast cells, and monocytes in the lavage fluid than control subjects. In addition, patients with asthma demonstrated increased number of lavage T cells, and an increased proportion of these cells was activated, as demonstrated by the augmented expression of the surface marker of activation CD25. There was no parallel increase in the absolute number of lymphocytes in those patients nor did they have any significant change in the ratio of CD4-/CD8-positive lymphocytes. This finding indicates that activation of lymphocytes can occur locally in the airways of patients with asthma who are not sensitized to recognizable allergens. Viral antigens or some still unknown allergen may be re-

sponsible for this activation but also the expression of surface adhesion molecules on endothelial or epithelial cells, and the interaction of these cells with lymphocytes may explain the phenomenon. In fact, adhesion molecules regulate homing of T cells and their migration at the sites of inflammatory responses, and some of these adhesion molecules probably represent the natural ligands for T cell activation via the CD2 receptor.⁴⁴⁻⁴⁶

Patients with asthma shed a significantly higher number of ciliated and nonciliated epithelial cells in the BALF than normal subjects, confirming previous findings^{6, 7, 47} of considerable bronchial epithelial cell disruption in asthma.

The presence of a sustained inflammatory response in the airways of patients with asthma was also demonstrated by the increase in the total protein and albumin contents of their lavage fluid as a result of increased vascular permeability¹² and increased local secretion. This finding is consistent with the data obtained in symptomatic patients with asthma sensitized to occupational agents.¹³

We found increased amounts of fibronectin in the BALF of patients with asthma, and this too might have been the result of both microvascular leakage and increased local production. Fibronectin is known to be produced by a number of different cell types, including alveolar macrophages and bronchial epithe-

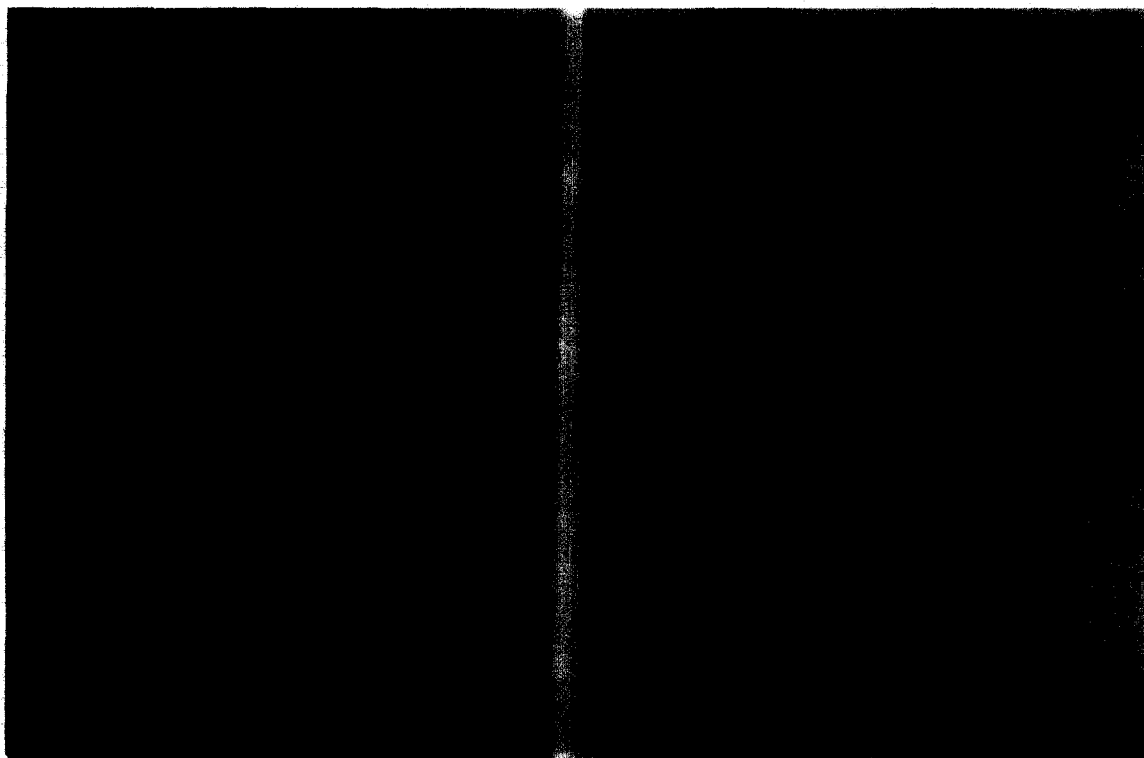


FIG. 3. Identification of cells containing immunoreactive GM-CSF by immunospecific labeling. Lavage cells from one patient with asthma, **A**, and one control subject, **B**, were labeled with the monoclonal antibody against human cytokeratin (DAKO, CK1). Epithelial cells were stained brown (*asterisks*). The cells were then labeled with the antiserum against human GM-CSF. GM-CSF containing cells were stained blue (*arrows*). Thus, the cells blue and brown were epithelial cells containing GM-CSF-like substance. (Original magnification $\times 250$; yellow filter.)

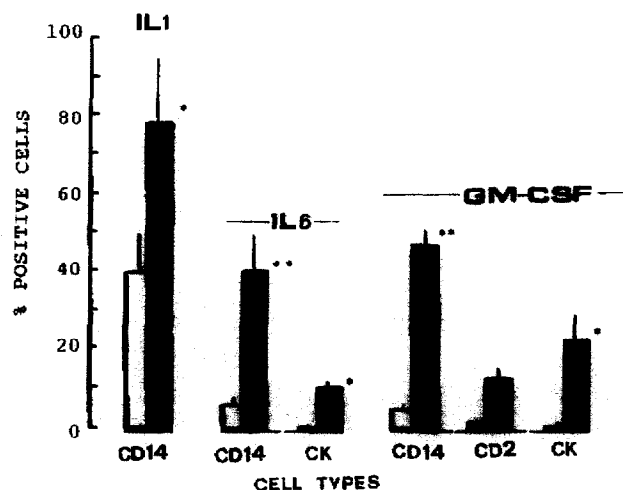


FIG. 4. Analysis of the expression of IL-1- β , IL-6-, and GM-CSF-like proteins by BAL cells from six patients with asthma (■) and six control subjects (□). Results are expressed as mean percent number of epithelial cells (CK-positive cells), monocytes (CD14-positive cells), and T-lymphocytes (CD2-positive cells), which bound the antiserum against each cytokine ($\pm$ SEM); * $p < 0.05$; ** $p < 0.025$.

lial cells,^{41, 48} and it can promote recruitment and local proliferation of fibroblasts.⁴⁸ Increased concentration of plasma fibronectin in the bronchial mucosa and the augmented synthesis and release of fibronectin by bronchial epithelium and monocytes during acute exacerbation of inflammation or repair could be an explanation for the marked subepithelial fibrosis observed in patients with asthma.⁴⁹

Lavage fluid from patients with asthma contained large amounts of immunoreactive IL-1- β , IL-6, and GM-CSF. The IL-1- β -, IL-6-, and GM-CSF-like substances were detected as bands compatible with the mobility of authentic IL-1- β , IL-6,²⁰ and GM-CSF² by SDS-PAGE, and they were labeled by the monospecific antisera on Western blot analysis. All these cytokines are hormone-like substances that are preferentially retained locally, at the site of production, where they modulate cell-to-cell interaction and hematopoiesis; therefore, an increase in the amounts of those substances in the BALF is unlikely due to increased microvascular permeability but rather indicates increased local synthesis and/or release.^{22, 50} Indeed, with the specific antisera, we demonstrated the

presence of immunoreactive IL-6 and GM-CSF into the cytoplasm of nonciliated epithelial cells and monocytes. GM-CSF-like substance was also identified in the cytoplasm of some T cells. The only sources of immunoreactive IL-1- β apparently were monocytes. Patients with asthma had greater numbers of lavage cells containing immunoreactive IL-1- β , IL-6, and GM-CSF, suggesting that the local production of these cytokines by airway resident cells is augmented in asthma.

The ability of monocytes, T cells, and epithelial cells to produce IL-1, IL-6, or GM-CSF, either constitutively or on immunologic and nonimmunologic activation in vitro, is a well-known phenomenon,^{20, 22, 43, 50} but the importance of this phenomenon in asthma has not received much attention previously. In fact, there is just one study about this point,¹⁵ and it has demonstrated that alveolar macrophages of patients with asthma release more IL-1 than normal subjects.

IL-1, IL-6, and GM-CSF are cytokines with potent proinflammatory properties. IL-1 increases the expression of adhesion molecules on endothelial cells, thereby affecting leukocyte migration.⁵¹ GM-CSF can retain circulating neutrophils and eosinophils at the tissue sites and prime them to release mediators and toxic products on minimal stimulation.^{17, 21, 42} IL-1 and IL-6 promote T cell activation and proliferation.^{20, 22} The ability of epithelial cells and monocytes to produce factors that regulate cell-to-cell interaction in airways and promote activation of inflammatory cells may contribute to the persistence of structural and functional alterations in bronchial mucosa of patients with asthma, and this might make the inflammatory response in asthma different from that in other conditions.

However, in our study, no cause-and-effect relationship was established, since the need to test symptomatic nonallergic patients with asthma limited the number of subjects available for the investigation, and it was impossible to consider any correlation between the biochemical findings in lavage fluid and the functional characteristics of the patients. Thus, to clarify the possible role of the proinflammatory cytokines, IL-1- β , IL-6, and GM-CSF, in the pathogenesis of asthma, additional studies should be performed in a larger group of patients, and an attempt should be made to compare the pattern of cytokine production by bronchial resident cells in asthma and in nonasthmatic inflammatory diseases.

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