

Increased expression of adhesion molecules (ICAM-1 and LFA-1) on alveolar macrophages from asthmatic patients

Chanez, P, Vignola AM, Lacoste P, Michel FB, Godard P, Bousquet J.
Increased expression of adhesion molecules (ICAM-1 and LFA-1) on alveolar macrophages from asthmatic patients.

Allergy 1993; 48: 576-580. © Munksgaard 1993.

In the airways inflammation observed in asthma, activated macrophages are present in increased numbers. Adhesion molecules are required for the cell:cell contacts between leukocytes and endothelial cells or other leukocytes, and they are induced by inflammatory stimuli. We studied the expression of two adhesion molecules (ICAM-1 and LFA-1) on alveolar macrophages recovered by bronchoalveolar lavage from 11 normal subjects and 13 asthmatic patients by using immunocytochemistry. Two specific monoclonal antibodies were used, and the reaction was revealed by the alkaline phosphatase-antialkaline phosphatase (APAAP) method. The percentage of cells expressing ICAM-1 or LFA-1 was significantly increased in asthmatic patients, as compared with normal subjects ($P < 0.001$ and $P < 0.002$, respectively; Mann-Whitney U test), and there was a significant correlation with the percentage of cells expressing both markers in asthma ($P < 0.03$, Spearman rank test). This study highlights the importance of macrophages in the inflammation of asthma and suggests that macrophage interactions with other cells play a role in this inflammation.

P. Chanez, A. M. Vignola, P. Lacoste,
F. B. Michel, P. Godard, J. Bousquet

Clinique des Maladies Respiratoires and Contrat
Jeune Formation INSERM, Hôpital
Aiguelongue, Montpellier, France; Istituto di
Fisiopatologia Respiratoria, C.N.R., Palermo, Italy

Key words: adhesion molecules; alveolar
macrophages.

Dr Jean Bousquet
Clinique des Maladies Respiratoires
Hôpital Aiguelongue
Centre Hospitalier Universitaire
34059 Montpellier Cedex 1
France

Accepted for publication 21 December 1992

A mixed inflammatory infiltrate of many cell types, including eosinophils, lymphocytes, and macrophages, is present in the airways of asthmatic patients (8). Many studies of alveolar macrophages (AM) have been performed in asthma and suggest their involvement in this disease (13, 22). After *in vivo* (19, 24) or *in vitro* (16) contact with specific allergen or nonspecific stimuli, AM from asthmatic patients have been shown to release lysosomal enzymes, arachidonic acid metabolites (12), and platelet-activating factor (3). They are also able to generate oxygen free radicals after nonspecific stimulation, and Cluzel et al. (6) found a correlation between the severity of asthma, the release of oxygen free radicals by AM, and the degree of eosinophilia in BAL fluids. Moreover, it has been shown that macrophages are present in increased numbers in the bronchi of asthmatic patients (21). However, details of the circulation and recruitment of macrophages within the airways in asthma are still unknown: we do not know whether 1) macrophages are recently derived from blood monocytes, 2) whether they can proliferate *in situ*, or 3) whether they have a prolonged lifespan as a result of the inflammatory processes.

It has been widely recognized that adhesion molecules are important for the recruitment of cells from the peripheral blood to the inflamed tissues as well as for all immunologic and inflammatory processes requiring cell:cell contacts (14, 20, 23). Many adhesion proteins expressed by monocytes and other cells, including endothelial cells, have been characterized. They increase in expression and function in response to mediators and cytokines released by inflamed tissues (20). Intercellular adhesion in monocytes is mediated in part by the interaction of the integrin lymphocyte function-associated antigen-1 (LFA-1) (15) with the intercellular adhesion molecule-1 (ICAM-1) (18), and it has been shown that monocytes express both LFA-1 and ICAM-1.

To characterize better the activation status of macrophages in asthma and to gain insight into the importance of cell:cell contacts, we studied the expression of two adhesion molecules (LFA-1 and ICAM-1) on AM recovered by BAL from 11 normal subjects and 13 asthmatic patients. The expression of LFA-1 and ICAM-1 was examined by immunocytochemistry with specific monoclonal antibodies.

Material and methods

Subjects

Thirteen asthmatic patients aged 23–62 years were selected according to the criteria of the American Thoracic Society (2). All patients had a reversible airways obstruction characterized by an increase of 20% of predicted FEV₁ after inhalation of 200 µg of salbutamol. The clinical severity of asthma was assessed according to the score of Aas (1). None of the subjects were current smokers, and none had smoked within the previous 2 years. No subject had had any bronchial or respiratory tract infection during the month preceding the test. Patients were excluded from the study if they had had a severe exacerbation of asthma requiring hospitalization during the month preceding the study, or if they had taken systemic corticosteroids in any form during the past 2 months, inhaled corticosteroids during the previous month, nedocromil sodium, sodium cromoglycate, or ketotifen during the week before, or theophylline during the previous 48 h.

Eleven sex-matched, normal, healthy volunteers aged 22–54 years were used as a control group. None of them had ever suffered from asthma or had had bronchial or respiratory tract infection during the month preceding the trial. Pulmonary function was within normal range. None of them were current or previous smokers, and their pulmonary functions were normal.

The study was performed after informed consent by the patients, and it fulfilled all criteria set by the ethical committee of the hospital.

Investigations

Bronchoalveolar lavage. Fiberoptic bronchoscopy was performed as previously described (5). The bronchoalveolar lavage (BAL) was performed in one of the subsegmental bronchi of the middle lobe by the injection of several aliquots of sterile saline (up to a total volume of 0.2 l), reaspirated by gentle syringe suction.

Immunostaining of BAL cells. Immediately after BAL, cells were allowed to adhere to a microscopic slide without any fixative method, and after an adherence period of 2 h, they were washed to remove nonmacrophagic cells. LFA-1 and ICAM-1 expression was studied by the APAAP method. Monoclonal antibodies against ICAM-1 (K-562, Immunotech, Luminy, France) (18) and the α -chain of LFA-1 (CD11a, clone MHM24, Dako A/S, Denmark (13)) were used as the first antibodies in an immunostaining assay. The Dako-CD11a reacts with the specific 180-kDa α -chain of the LFA-1 protein. This protein belongs to a family of heterodimeric proteins desig-

nated CD11 in the classification system of human leukocyte differentiation antigens which share a common 95-kDa β -chain. Besides the LFA-1 molecule, this protein family contains two other members, C3bi (CD11b) and p150,95 (CD11c). The specificity of the antibodies was revealed by two-dimensional gel electrophoresis. The optimal concentration of the antibodies had been previously characterized by means of a positive control represented by peripheral blood mononuclear cells stimulated for 36 h with PHA (1 µg/ml) and IFN- γ (300 U/ml) and allowed to adhere on microscopic slides without any fixative method. The nonadherent cell population was not studied.

The APAAP method was used to determine the percentage of cells expressing each molecule (10). A rabbit antimouse IgG (linked mAb, Dako, Versailles, France) and a mouse antirabbit mAb conjugated to the APAAP system were used as secondary and tertiary antibodies, respectively. Staining was revealed by a substrate containing fast red (Sigma, Versailles, France). Slides were stained by the APAAP system, and cells were counted with a phase-contrast microscope (Zeiss Axioskop, Oberkochen, Germany) by two independent observers who were unaware of the clinical details of the subjects. Results obtained by the APAAP method are expressed as percent cells stained.

Statistical analysis

Statistical analysis was performed by nonparametric tests, the Mann-Whitney U test being used for comparisons.

Results

Demographic characteristics

The demographic characteristics of the asthmatic patients are presented in Table 1. They were suffering from asthma of variable severity, and FEV₁ before BAL ranged from 30 to 102% of predicted values. The BAL cell analysis is presented in Table 2. There was no significant difference between normal subjects and asthmatic patients except for eosinophils.

Immunostaining

The results of immunostaining are shown in Fig. 1. ICAM-1 and LFA-1 expression was observed only on mononuclear cells and never on granulocytes. Over 50% of the control subjects had no detectable ICAM-1- or LFA-1-positive cells. On the other hand, all asthmatic patients had cells expressing these two markers. There was a significant difference between asthmatic and control subjects for both

Table 1. Demographic characteristics of asthmatic subjects

	Age	Aas score	FEV ₁ (%pred)	BAL fluid analysis			
				Total cell no.	AM %	PMN %	EOS %
1	30	1	102	144	92	4	0
2	40	2	92	560	81	1	1
3	56	3	65	300	99	0	0
4	62	3	92	170	89	2	0
5	53	4	30	152	85	3	3
6	59	4	55	40	85	4	10
7	55	1	100	59	96	0	0
8	30	1	100	336	97	0	1
9	29	2	70	44	91	0	0
10	59	4	55	208	83	2	10
11	37	2	80	276	86	2	4
12	23	2	64	88	86	0	5
13	26	2	97	125	91	2	5

AM: alveolar macrophages; PMN: neutrophils; EOS: eosinophils; Ly: lymphocytes.
Total cell number ($\times 1000/\text{ml}$).

Table 2. BAL cell analysis

	Normal subjects	Asthmatic subjects	
Total cell number $\times 1000/\text{ml}$	160.8 $\pm$ 81.4	192.5 $\pm$ 146.5	NS
Macrophages %	88.7 $\pm$ 8.2	89.3 $\pm$ 5.6	NS
Lymphocytes %	10.0 $\pm$ 7.0	6.0 $\pm$ 4.8	NS
Eosinophils %	0	3.0 $\pm$ 3.6	$P < 0.05$
Neutrophils %	1.0 $\pm$ 1.5	1.5 $\pm$ 1.5	NS

Statistical analysis by Mann-Whitney U test.

ICAM-1 ($P < 0.0001$, Mann-Whitney U test) and LFA-1 ($P < 0.0002$, Mann-Whitney U test) expression. However, there was no significant correlation between the percentage of cells expressing the markers and the severity of asthma assessed by either the clinical score of Aas (1) or FEV₁.

Although we did not use double staining, there was a significant correlation between the percentage of cells expressing ICAM-1 and LFA-1 in asthmatic patients (Fig. 2).

Discussion

In the present study, we have shown a significant increase in the expression of two adhesion molecules (ICAM-1 and LFA-1) on AM of asthmatic patients, as compared with normal subjects.

The methods used are simple and well characterized. The selection of macrophages was based on their ability to adhere. Over 99% of adherent cells from BAL are macrophages. However, although there is a population of nonadherent macrophages, there is no information on this population in asthmatic patients. The same method has been used for asthmatic and control subjects. Thus, even if it is clear that adherence activates cells, it is unlikely that major differences have occurred in the two popula-

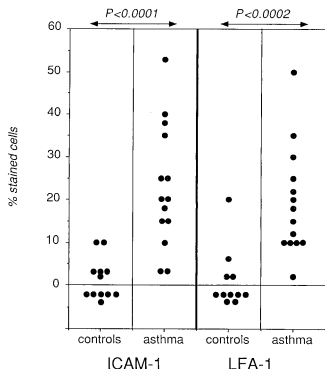


Fig. 1. Percentage of cells expressing ICAM-1 and LFA-1 in bronchoalveolar lavage of asthmatic and control subjects.

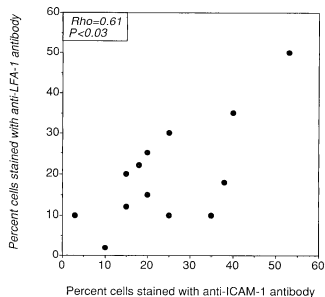


Fig. 2. Correlation between percentage of cells expressing ICAM-1 and LFA-1 in asthmatic subjects (statistical analysis by Spearman rank test).

tions. There are many immunoenzymologic staining methods which can be used to localize antigens. In this study we used the APAAP method, which is an immunoenzymatic staining method utilizing an enzyme-substrate reaction to convert colorless chromogens into red end products. By this method, it is

possible to delineate positive cells with great clarity even if they are few. In addition, cellular structure is optimally preserved and can be visualized simultaneously with the antigen label. Finally, the alkaline phosphatase reaction product is permanent, allowing one to study the labeled slides carefully a long time after the staining (7, 10). The APAAP method is routinely used; its specificity or sensitivity is high when appropriate antibodies are used, and it usually compares favorably with other immunohistochemical methods (11, 17). However, it is not possible with the APAAP method to quantify the expression of adhesion molecules on a single cell, and more sophisticated methods such as confocal microscopy are needed. Thus, our results present only the percentage of stained cells. In this study, the monoclonal antibodies used were highly specific, indicating that the methods used can adequately identify HLA-DR and ICAM-1 molecules on BAL cells.

Macrophages are probably key cells in the bronchial inflammation of asthma and can be studied in either biopsies or BAL fluid. It is clear that cells present in the airways directly participate in the inflammation, whereas those recovered by BAL are in the lumen and may not be totally relevant. However, many studies of asthma performed with AM recovered by BAL have observed that these cells are activated and that their activation stage may be related to the severity of asthma (3, 6, 12, 13, 16, 19, 22, 24). Moreover, it is difficult to identify macrophages in the airways without using monoclonal antibodies, and all macrophages may not be completely characterized whatever antibody is used. Thus, in this study, we have explained AM obtained by BAL.

Most adhesion proteins increase in expression and function in response to mediators and cytokines (4) released by inflamed tissues. They enhance cell:cell interaction, and although it is clear that they are required for leukocyte extravasation (leukocyte:endothelium interactions), it is not known yet whether such expression is a prerequisite for intraepithelial migration of lymphocytes. Moreover, although the interaction of leukocyte integrins with ligand is necessary, it is not sufficient for function (9). In experimental asthma, the importance of ICAM-1 has been put forward (25). Antigen inhalation induced a prolonged airway eosinophilia and an increase in airway responsiveness in the monkey. By means of monoclonal antibodies to ICAM-1, eosinophil adhesion to endothelium was reduced as well as hyperresponsiveness. Adhesion molecules are also of importance in most immunologic and inflammatory processes in which cell contact is required (14, 23). Thus, the presence of an increased expression of adhesion molecules on AM from asthmatic patients is another feature of airways inflammation and may have several explanations. First, there may be an increased

recruitment of macrophages from the peripheral blood, as suggested indirectly by other studies. Poston et al. (21) found that many of the macrophages present in the airways of asthmatic patients bore monocyte markers, suggesting their recent recruitment from the blood, and Chanez et al. (paper submitted) have observed, in both normal and asthmatic subjects, that macrophages recovered from BAL or present in the airways do not replicate. On the other hand, the increased expression of adhesion molecules on AM from asthmatic patients may be related only to the increased capability of these cells to participate in inflammatory processes by interactions with other inflammatory cells.

Thus, this study requires further experiments to demonstrate the presence of adhesion molecules in bronchial wall macrophages and show that there is a link between these molecules and migration of cells in asthma.

References

1. Aas K. Heterogeneity of bronchial asthma. *Allergy* 1981; 36: 3-10.
2. American Thoracic Society. Definitions and classifications of chronic bronchitis, asthma and emphysema. *Am Rev Respir Dis* 1962; 85: 762-8.
3. ARNOUX B, JOSEPH M, SIMOES MH, et al. Antigenic release of PAF-acether and β -glucuronidase from alveolar macrophages of asthmatics. *Bull Eur Physiopathol Respir* 1987; 23: 119-224.
4. BEEKHUIZEN H, CORSEEL VAN TILBURG AJ, BLOKLAND I, VAN FURTH R. Characterization of the adherence of human monocytes to cytokine-stimulated human macrovascular endothelial cells. *Immunology* 1991; 74: 661-9.
5. BOUSQUET J, CHANEZ P, LACOSTE JY, et al. Eosinophilic inflammation in asthma. *N Engl J Med* 1990; 323: 1033-40.
6. CLUZEL M, DAMON M, CHANEZ P, et al. Enhanced alveolar cells luminal dependent chemiluminescence in asthma. *J Allergy Clin Immunol* 1987; 80: 195-201.
7. CORDELL JL, FALINI B, ERBER WN, et al. Immunoenzymatic labelling of monoclonal antibodies using immune complexes of alkaline phosphatase and monoclonal anti-alkaline phosphatase (APAAP) complexes. *J Histochem Cytochem* 1984; 32: 219-29.
8. DJUKANOVIC R, ROCHE WR, WILSON JW, et al. Mucosal inflammation in asthma. *Am Rev Respir Dis* 1990; 142: 434-57.
9. DRANSFIELD I, CABANAS C, BARRETT J, HOGG N. Interaction of leukocyte integrins with ligand is necessary but not sufficient for function. *J Cell Biol* 1992; 116: 1527-35.
10. ERBER WN, MYNHEER LC, MASON DY. APAAP labeling of blood and bone marrow samples for phenotyping leukemia. *Lancet* 1986; 1: 761-5.
11. GLOGHINI A, COZZI M, SULFARO S, VOLPE R, CARBONE A. A combined cytochemical and immunocytochemical method for simultaneous visualization of cytoplasmic enzyme reactivity and cell surface antigens in cell suspensions. *Am J Clin Pathol* 1989; 91: 67-71.
12. GODARD P, CHAINTEUIL J, DAMON M, et al. Functional assessment of alveolar macrophages: comparison of cells from asthmatic and normal subjects. *J Allergy Clin Immunol* 1982; 70: 88-93.
13. GODARD P, DAMON M, VAGO P. Alveolar macrophages in asthma. In: MICHEL FB, BOUSQUET J, GODARD P, eds.

- Highlights in asthmology. Munich: Springer-Verlag, 1987: 186–95.
14. HASKARD DO, LEE TH. The role of leukocyte-endothelial interactions in the accumulation of leukocytes in allergic inflammation. *Am Rev Respir Dis* 1992; 145: S10–13.
15. HILDRETH JEK, GOTCH FM, HILDRETH PD, MC-MICHAEL AJ. A human lymphocyte-associated antigen involved in cell-mediated lympholysis. *Eur J Immunol* 1983; 13: 202–8.
16. JOSEPH M, TONNEL AB, TORPIER G, CAPRON A, AR-NOUX B, BENVENISTE J. The involvement of IgE in the secretory processes of alveolar macrophages from asthmatic patients. *J Clin Invest* 1983; 71: 221–30.
17. LI CY, LAZCANO-VILLAREAL O, PIERRE RV, YAM LT. Immunocytochemical identification of cells in serous effusions. *Am J Clin Pathol* 1987; 88: 696–706.
18. MAKGOBA MW, SANDERS ME, GINTHER LUCE CE, et al. ICAM-1 a ligand for LFA-1-dependent adhesion of B, T and myeloid cells. *Nature* 1988; 331: 86–8.
19. METZGER WJ, ZAVALA D, RICHESON HB, et al. Local allergen challenge and bronchoalveolar lavage of allergic asthmatic lungs. *Am Rev Respir Dis* 1987; 135: 403–40.
20. MONTEFORT S, HOLGATE ST. Adhesion molecules and their role in inflammation. *Respir Med* 1991; 85: 91–9.
21. POSTON R, LITCHFIELD P, CHANEZ P, LACOSTE JY, LEE TK, BOUSQUET J. Immunohistochemical characterization of the cellular infiltration of asthmatic bronchi. *Am Rev Respir Dis* 1992; 145: 918–21.
22. RANKIN JA. The contribution of alveolar macrophages to hyperreactive airway disease. *J Allergy Clin Immunol* 1989; 83: 722–9.
23. SPRINGER TA. Adhesion receptors of the immune system. *Nature* 1990; 346: 425–34.
24. TONNEL AB, GOSSET P, JOSEPH M, FOURNIER E, CAPRON A. Stimulation of alveolar macrophages in asthmatic patients after local provocation test. *Lancet* 1983; 1: 1406–9.
25. WEGNER CD, GUNDEL RH, ROTHLEIN R, LETTS LG. Expression and probable roles of cell adhesion molecules in lung inflammation. *Chest* 1992; 101 (Suppl. 3): 34S–9S.

This document is a scanned copy of a printed document. No warranty is given about the accuracy of the copy. Users should refer to the original published version of the material.