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Integrin expression on neutrophils and mononuclear cells in blood and induced sputum in stable asthma

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Key words: adhesion molecules; airway inflammation; cytology.

Background: We speculated that the expression of integrins in the airway lumen of asthmatic subjects might be altered compared with normal subjects during cell recruitment from circulation.

Methods: To test this hypothesis, we investigated the expression of integrin α -chains (CD11a, CD11b, and CD11c) in hypertonic saline-induced sputum and peripheral blood leukocytes in mild to moderate stable asthmatic and control subjects. Immunoreactivity for integrin α -chains was assessed by immunocytology on cytospin preparations of sputum and blood.

Results: In comparison of the percentages of CD11a⁺, CD11b⁺ and CD11c⁺ mononuclear cells in sputum with their blood counterparts, no significant differences were observed in control subjects, whereas CD11a and CD11b in asthmatic subjects were less expressed on sputum cells. In both control and asthmatic subjects, sputum neutrophils tended to decrease their expression of integrin α -chains compared with circulating neutrophils.

Conclusions: We showed that the sputum of asthmatics, unlike normal subjects, is characterized by decreased expression of integrins on mononuclear cells compared with their blood counterparts. The results suggest that downregulation of integrins occurs in asthmatic airways after cell recruitment from circulation.

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β_2 -integrins are a subgroup of cell adhesion molecules expressed by leukocytes and involved in cell accumulation and activation at the site of inflammation. They consist of three receptors with a common β -chain (CD18) and three different α -chains (CD11a, CD11b, and CD11c).

Cell differentiation of phagocytic mononuclear cells is accompanied by a decrease in CD11a and CD11b expression

(1, 2), while a reduced function of CD11c/CD18 was observed in adherent neutrophils compared with neutrophils in suspension (2). This suggests that expression of integrins on leukocytes after their migration from circulation to the tissue may be reduced. However, there are several stimuli potentially active in asthmatic inflammation that upregulate these molecules *in vitro*, including chemoattractants, arachidonic acid metabolites, and cytokines (3).

We speculated that the expression of integrins on leukocytes could be modified during cell recruitment from circulation and that this phenomenon might differ in asthmatic subjects from normal subjects. To test this hypothesis, we investigated the expression of integrin α -chains on leukocytes in induced sputum and in peripheral blood in a group of stable asthmatic subjects and in a group of nonasthmatic controls.

Material and methods

Subjects

We examined 17 subjects with bronchial asthma and 10 control subjects. Asthma was defined according to international guidelines (4). Control subjects had no history of asthma or chronic bronchitis and normal lung-function tests (5).

None of the subjects had had acute upper respiratory tract infections or received oral glucocorticoids or antibiotics within the preceding month. The study conformed to the Declaration of Helsinki, and each subject gave written informed consent.

Induction and analysis of sputum

Induction of sputum was performed as already described (6). Briefly, 10 min after inhalation of 200 μ g of salbutamol, hypertonic saline (3% and 4%) was nebulized with an ultrasonic nebulizer (UltraNeb 2000; De Vilbiss Health Care, Inc., Somerset, PA, USA) for 5-min periods, up to 20 min. Every 5 min, subjects were asked to rinse their mouths and throats and then try to cough sputum into a Petri dish. Forced expiratory volume in 1 s (FEV₁) was measured before and after each 5-min inhalation of hypertonic saline to detect whether bronchospasm occurred.

Sputum plugs were selected from saliva, and mixed mechanically by aspiration in and out with 1 ml of dithiothreitol 0.1% (Sigma, Milan, Italy). Cell viability was determined by trypan blue exclusion. Cytospin

preparations of the cells were kept frozen at -20°C until they were analyzed. The counts of leukocytes and squamous epithelial cells were performed on May-Grünwald-Giemsa-stained slides. The results of differential counts were expressed as a percentage of nucleated cells excluding squamous cells.

Immunostaining was performed in acetone-fixed slides using monoclonal antibodies to CD11a, CD11b, and CD11c (Dakopatts, Copenhagen, Denmark), as previously described (7). Counterstain with 0.2% fast green (Merck, Darmstadt, Germany) was used to distinguish eosinophils from neutrophils. On immunostained slides, neutrophils and mononuclear cells were identified by morphologic analysis of the nuclei, and positively stained cells within each population were counted (7). The results were expressed as percentage of positive cells within each leukocyte population.

Peripheral blood analysis

Heparinized peripheral blood was taken from asthmatic and control subjects before induction of sputum. Leukocytes were separated by the dextran sedimentation method and incubated with dithiothreitol 0.1% for 10 min. Contaminating red blood cells were then eliminated by hypotonic lysis. The cells were washed in PBS and cytocentrifuged. The slides were fixed and stained by the same procedure as used for sputum.

Data analysis

Data are presented as mean $\pm$ SEM or median as appropriate. Comparison of data was done with the Mann-Whitney *U*-test or the Wilcoxon signed rank test. *P* values of <0.05 were considered significant.

The intraclass correlation coefficients for at least three repeated measurements on immunostained slides were between 0.78 and 0.62 for the counts of granulocytes and between 0.81 and 0.36 for the counts of mononuclear cells, while they were between 0.80 and 0.70 for the differential cell counts.

Results

The characteristics of control and asthmatic subjects are reported in Table 1.

Four of the 17 asthmatics were classified as moderate at the time of the study (4). The other asthmatic subjects were classified as mild and treated with inhaled β_2 -agonists as

required. Total cells in sputum samples were similar in asthmatics ($8.7 \pm 1.8 \times 10^3$ cells/mg) and controls ($8.1 \pm 1.7 \times 10^3$ cells/mg). Squamous cell contamination of sputum cytopins was generally low ($6 \pm 2\%$). The viability of sputum cells was $75 \pm 4\%$.

The percentage of eosinophils was higher in induced sputum from the asthmatics ($13.5 \pm 5.3\%$) than that in control subjects ($0.5 \pm 0.2\%$; $P < 0.05$), whereas no differences were observed in peripheral blood between the two groups. No

significant differences between the two groups of subjects were observed for other cell types in sputum or blood.

The quality of cytopins was not adequate for immunostaining of sputum cells with anti-CD11a in three cases, and for immunostaining of blood cells with anti-CD11a in seven cases, with anti-CD11c in six cases, and with anti-CD11b in one case.

Fig. 1a compares the percentages of CD11a-, CD11b-, and CD11c-positive mononuclear cells in sputum with the

Table 1. Characteristics of subjects

Subject no.	Age (years)	Sex (M/F)	Smoking habit (NS, CS, Ex)§	Atopy (+/-)	FEV ₁ (% pred)
Controls					
1	31	M	NS	+	101
2	32	M	NS	+	111
3	22	F	NS	-	119
4	41	M	NS	+	96
5	31	M	NS	+	109
6	30	F	NS	-	90
7	33	M	NS	-	106
8	28	F	NS	-	124
9	25	M	NS	-	85
10	25	M	NS	-	116
Mean	30				106
SEM	2				4
Asthmatics					
11	56	M	NS	+	102
12	55	M	Ex (>8 years)	-	98
13	44	M	NS	-	81
14	19	M	NS	+	90
15	55	M	Ex (>14 years)	-	108
16	50	F*	NS	-	74
17	21	F	NS	-	110
18	56	M*	Ex (>6 years)	+	82
19	55	M	Ex (>12 years)	-	148
20	54	M	NS	-	106
21	30	F	CS	+	124
22	22	F	NS	-	113
23	43	F	NS	-	86
24	45	M*	Ex (>9 years)	+	55
25	53	M*	Ex (>20 years)	-	96
26	23	M	NS	+	98
27	60	F	NS	-	100
Mean	44‡				98
SEM	4				5

§: NS: nonsmoker; CS: current smoker; Ex: ex-smoker (no. of years of cessation).
 * On inhaled steroids treatment.
 ‡ $P < 0.05$ compared with controls.

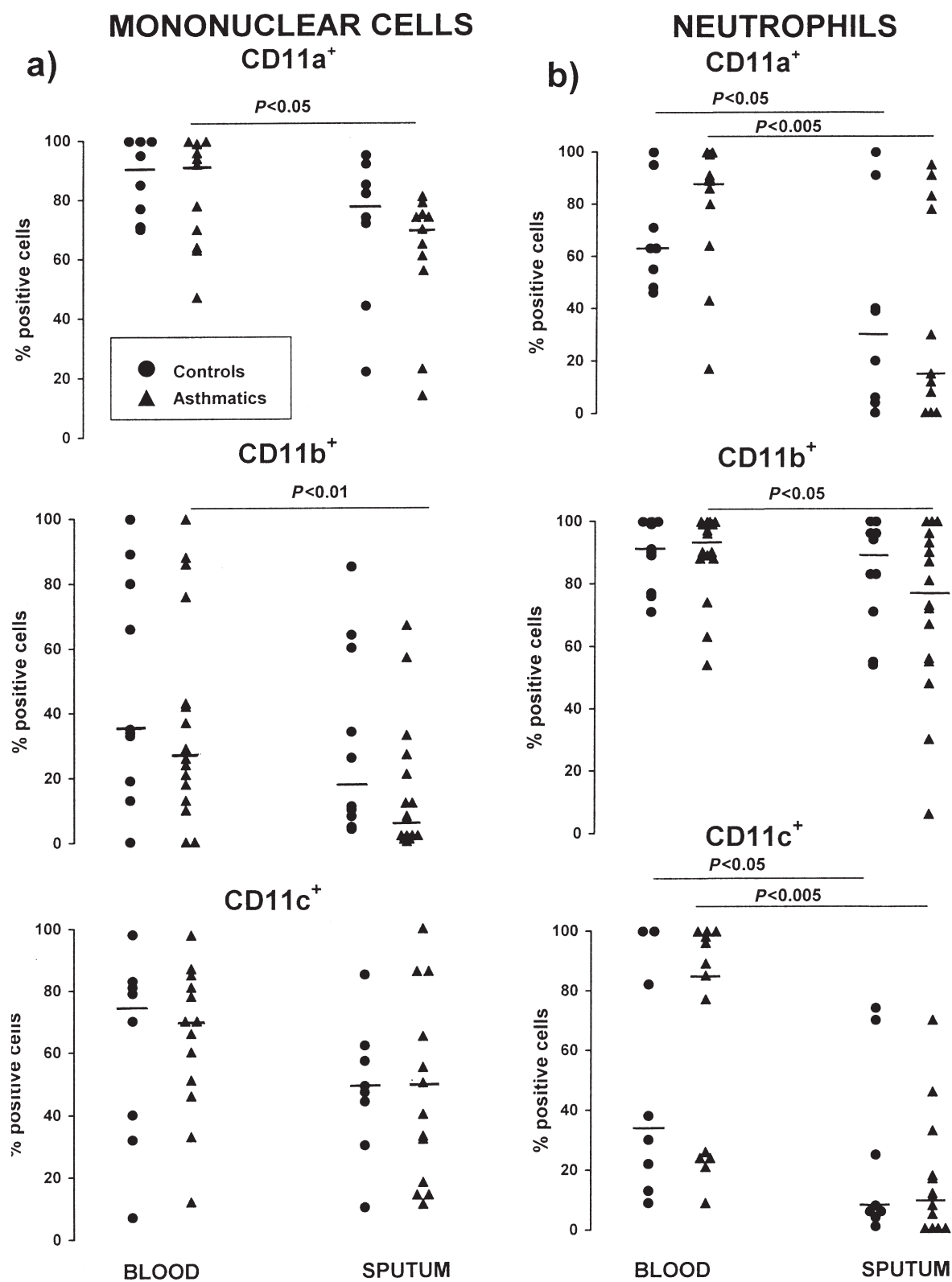


Figure 1. a) Expression of CD11a, CD11b, and CD11c on sputum mononuclear cells compared with peripheral blood mononuclear cells in normal controls and asthmatic subjects. b) Expression of CD11a, CD11b, and CD11c on sputum neutrophils compared with peripheral blood neutrophils in normal controls and asthmatic subjects. Median values are represented by horizontal bars.

corresponding percentages in blood. No differences were observed in control subjects, whereas CD11a and CD11b in asthmatic subjects were consistently less expressed on sputum cells. Fig. 1b compares the percentages of CD11a-, CD11b-, and CD11c-positive neutrophils in blood with the corresponding percentages in sputum. CD11a- and CD11c-positive neutrophils were reduced in sputum compared with blood in both control and in asthmatic subjects. In addition, CD11b expression on sputum neutrophils in the asthmatics was significantly lower than in their blood counterparts. No differences in integrin expression were detected on blood and induced-sputum leukocytes between asthmatic and control subjects. The expression of integrin α -chain on sputum eosinophils was evaluated in the nine asthmatics who showed a percentage of eosinophils greater than 5%. The percentages of eosinophils that exhibited immunoreactivity for the integrin α -chains (CD11a: $1.9 \pm 1.2\%$; CD11b: $14.5 \pm 4.8\%$; and CD11c: $6.3 \pm 1.5\%$) were lower than those of neutrophils. Immunostaining of blood eosinophils was negative.

The results for integrin expression were unchanged, excluding asthmatics taking inhaled steroids. No differences were detected between nonsmokers and subjects with smoking history for any of the measurements.

Discussion

This study reports some novel findings on *in vivo* expression of β_2 -integrins on leukocytes in the airway lumen. The integrin α -chains CD11a, CD11b, and CD11c were detected on sputum mononuclear cells and neutrophils in both normal subjects and asthmatic subjects. We demonstrated that the expression of CD11a and CD11b on sputum mononuclear cells is reduced compared with their blood counterparts in asthmatic subjects, but not in normal controls. In both groups of subjects, neutrophils in the airway lumen tended to decrease their expression of integrin α -chains compared with circulating neutrophils.

The lack of differences in integrin expression between asthmatic subjects and controls is in line with previous investigations of adhesion molecules in stable asthma. No differences in integrin α -chains were reported between asthmatic subjects with almost normal lung function and

control groups in studies on BAL macrophages (8, 9). Enhanced expression of macrophage CD11b was detected in a more severely asthmatic subgroup (9). No differences in the distribution of adhesion molecules were observed in the bronchial mucosa in stable atopic asthma compared with nonasthmatic controls (10–12), except for a trend for increased expression of ICAM-1 and LFA-1 in bronchial epithelium (10, 11). CD11b and L-selectin expression detected by flow cytometry on eosinophils and neutrophils from blood or induced sputum did not differ between asthmatics and controls (13).

Explanations of the relative decrease of the CD11a and CD11b molecules in sputum mononuclear cells may include a loss of cell-surface adhesion proteins during migration of mononuclear cells to the lumen of the airway in asthma or a selective retention by the airway mucosa of the leukocyte subsets expressing more adhesion molecules. This finding is in line with the findings of other investigators who demonstrated that circulating monocytes after migration into tissues give rise to cells of the mononuclear phagocyte series exhibiting low expression of CD11/CD18 (8). Apparently, the downregulation of integrins on macrophages is related to maturation and is compatible with the phenotype of resident tissue macrophages (14). This suggests that a higher proportion of mononuclear cells have reached their final maturation stage in asthmatic than normal airways. Another interpretation of our findings is that the inflammatory response in asthma is associated with a higher proportion of leukocytes internalizing integrin molecules, resulting in an apparent loss of immunoreactivity when assessed by immunocytology, as observed in mouse macrophages after culture (15).

We conclude that the sputum of asthmatics is characterized by increased percentages of eosinophils and decreased expression of integrins on mononuclear cells. The results suggest that downregulation of integrins occurs in asthmatic airways after cell recruitment from circulation.

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