

Original article

ICAM-1 and $\alpha 3\beta 1$ expression by bronchial epithelial cells and their *in vitro* modulation by inflammatory and anti-inflammatory mediators

Background: Adhesion molecules are involved in inflammatory and repair processes of the bronchial epithelium. ICAM-1 is mainly involved in inflammatory reactions, whereas integrins, such as $\alpha 3\beta 1$, are mainly involved in repair processes.

Methods: Using bronchial biopsies from 10 asthmatics and eight controls, we first evaluated by immunohistochemistry expression of $\alpha 3\beta 1$ and ICAM-1 in intact and damaged epithelium. Then, using the human pulmonary epithelial cell line WI-26 VA, we studied, by flow-cytometry, the modulation of ICAM-1 and $\alpha 3\beta 1$ expression, and, by ELISA, the release of fibronectin by proinflammatory cytokines, such as IL-5, and anti-inflammatory cytokines, such as IL-4, TGF- β , and EGF.

Results: $\alpha 3\beta 1$ expression was slightly higher in asthma than in controls, as well as in damaged epithelium than in undamaged epithelium. ICAM-1 expression was higher in asthma than in controls, and similarly distributed in intact or damaged epithelium. *In vitro*, $\alpha 3\beta 1$ was significantly increased by TGF- β , EGF, and IL-4, and significantly decreased by IL-5. Fibronectin release was significantly increased by TGF- β and IL-4, unchanged by EGF, and slightly but significantly decreased by IL-5. ICAM-1 expression was significantly decreased by TGF- β and IL-4, unchanged by EGF, and significantly increased by IL-5.

Conclusions: These differences in adhesion molecule expression and fibronectin release may be important in epithelial cell inflammation and repair.

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Asthma is a chronic inflammatory disease of the airways in which shedding of the bronchial epithelium is an important feature (1, 2). The desquamation of bronchial epithelial cells is followed by repair mechanisms to re-epithelialize the denuded luminal surface (3). Adhesion molecules, a heterogeneous group of molecules which mediate cell–cell and cell–matrix interactions, are probably involved in both inflammatory and repair processes within the epithelium layer. While the adhesion molecules of the immunoglobulin superfamily are mainly involved in inflammatory reactions, the adhesion molecules of the integrin family play an important role in repair processes (4).

Among the adhesion molecules of the immunoglobulin supergene family, the intercellular adhesion molecule-1 (ICAM-1) is involved in antigen presentation and in the mobilization of leukocytes to the inflammatory

sites (5). ICAM-1 expression by bronchial epithelial cells may promote leukocyte–epithelial cell interactions, which are crucial for the development of airway inflammation (6). ICAM-1 expression is increased on the surface of bronchial epithelial cells recovered from asthmatic subjects, and this increase is correlated with the clinical severity of asthma (7, 9).

Among the adhesion molecules of the integrin family, the $\alpha 3\beta 1$ integrin, the counterreceptor of several extracellular matrix (ECM) components (such as fibronectin, laminin, and collagen) (5), is mainly involved in repair processes. Because it mediates cell–matrix interactions, $\alpha 3\beta 1$ integrin is crucial for the simple attachment of cells on adhesive substrates and for more complex processes involved in cell migration and wound repair (5, 10). Since bronchial epithelial cells surrounding an injured area produce ECM components

(10), and the deposition of ECM components on the basement membrane is followed by the adhesion and spreading of bronchial epithelial cells, eventually leading to wound repair (11), the expression of $\alpha 3\beta 1$ integrin by bronchial epithelial cells may play an important role in the regulation of their interaction with ECM components, including fibronectin. In particular, $\alpha 3\beta 1$ is able to mediate fibronectin deposition and assembly, which are both important in the generation and maintenance of tissue architecture during repair processes (12).

$\alpha 3\beta 1$ and ICAM-1 expression has been previously evaluated in asthma and normal subjects (7, 9), but the topographic distribution of these markers in intact or damaged epithelium of asthmatic and normal subjects has not been examined.

We therefore carried out the present study to evaluate the expression of $\alpha 3\beta 1$ and ICAM-1 molecules in the epithelium of bronchial biopsies taken from normal and asthmatic subjects. We also examined the expression of both $\alpha 3\beta 1$ and ICAM-1 molecules in undamaged and damaged epithelium, in order to identify their topographic distribution in areas characterized by either a virtually absent (undamaged epithelium) or active (damaged epithelium) repair process. In addition, because of the different roles played by $\alpha 3\beta 1$ and ICAM-1 molecules in inflammation and repair, we put forward the hypothesis that the expression of these molecules might be regulated in a different fashion by mediators involved in the development of inflammatory or repair processes, respectively. To test this hypothesis, we cultured the human pulmonary epithelial cell line WI-26 (epithelial cells), which was previously used to study the role of epithelial cells in inflammatory mediators (13) and repair (14). Using these cells, we set up an *in vitro* model to assess ICAM-1 and $\alpha 3\beta 1$ modulation by IL-5, a proinflammatory cytokine involved in airway inflammation (15); by IL-4, an anti-inflammatory cytokine (16, 17); and by TGF- β and EGF, two growth factors able to play an important role in repair processes (10, 18). Moreover, we investigated the effects of the same mediators on the release of fibronectin.

Material and methods

Bronchial biopsies: subjects

Bronchial biopsies from central airways were obtained from 10 asthmatic subjects (19–52 years of age, 35 ± 9.7 years). Asthma was defined as previously described in detail (19). None of the subjects participating in this study were current smokers, and none had smoked during the previous 2 years. No subject had any bronchial or respiratory tract infection during the month preceding the study. Patients with asthma or chronic bronchitis were excluded from the study if they

had had a severe exacerbation or respiratory tract infection during the month preceding the study. Inhaled corticosteroids or oral corticosteroids had been withdrawn for at least 2 months before the commencement of the study, and use of nedocromil sodium or cromoglycate had been stopped for at least 2 weeks. No patient was taking a long-acting β_2 agonist or oral theophylline. Short-acting β_2 -agonists were stopped for 12 h. Forty percent of asthmatic patients were allergic to perennial allergens.

Eight healthy subjects (18–68 years of age; 46.3 ± 18.0 years) were enrolled in the study as a control group. Their pulmonary function was within normal range. They were nonallergic and none of them were smokers. No member of the control group had suffered from any respiratory tract infection during the month preceding the test.

The study was performed after informed consent was obtained from the participants, and approval of the ethics committee of the hospital was obtained.

Pathologic examination of bronchial mucosa

Bronchial biopsies. Biopsies were recovered by fiberoptic bronchoscopy performed as previously described in subsegmental bronchi (21). Specimens were embedded in Tissue Tek II OCT (Miles Scientific, Naperville, IL, USA), frozen within 30 min in methylbutane precooled in liquid nitrogen, and stored at -80°C . Cryostat sections 6 μm thick were cut, and tissue specimens were oriented for hematoxylin and eosin staining, and for immunohistochemistry staining, and fixed in acetone/methanol (4:1) for 2 min.

Epithelium shedding. The shedding was characterized as the percentage of length of basal cells uncovered by columnar cells (20, 21), as previously described (2).

Expression of ICAM-1 and $\alpha 3\beta 1$. Slides were incubated with BSA 0.5% followed by incubation with the mouse monoclonal antibody (mAb) anti-ICAM-1 and anti- $\alpha 3\beta 1$ (Dako, Glostrup, Denmark) at a dilution of 1:50 and 1:50 overnight, respectively. The reaction was revealed by the APAAP method (Dako APAAP Kit[®] (Dako, Glostrup, Denmark) according to the manufacturer's instructions. Phosphatase was visualized with new fuchsin as a substrate. Control slides were prepared using TBS and a nonrelated antibody instead of the specific primary monoclonal antibody. For each biopsy, the immunoreactivity for $\alpha 3\beta 1$ was expressed as the percentage of positively stained cells in areas characterized by epithelium shedding and damage or by intact epithelium. Cell counting was performed for the entire length of the bronchial biopsies. The percentage of positive cells was obtained with the following formula: number of positive cells/number of total cells counted $\times 100$. We

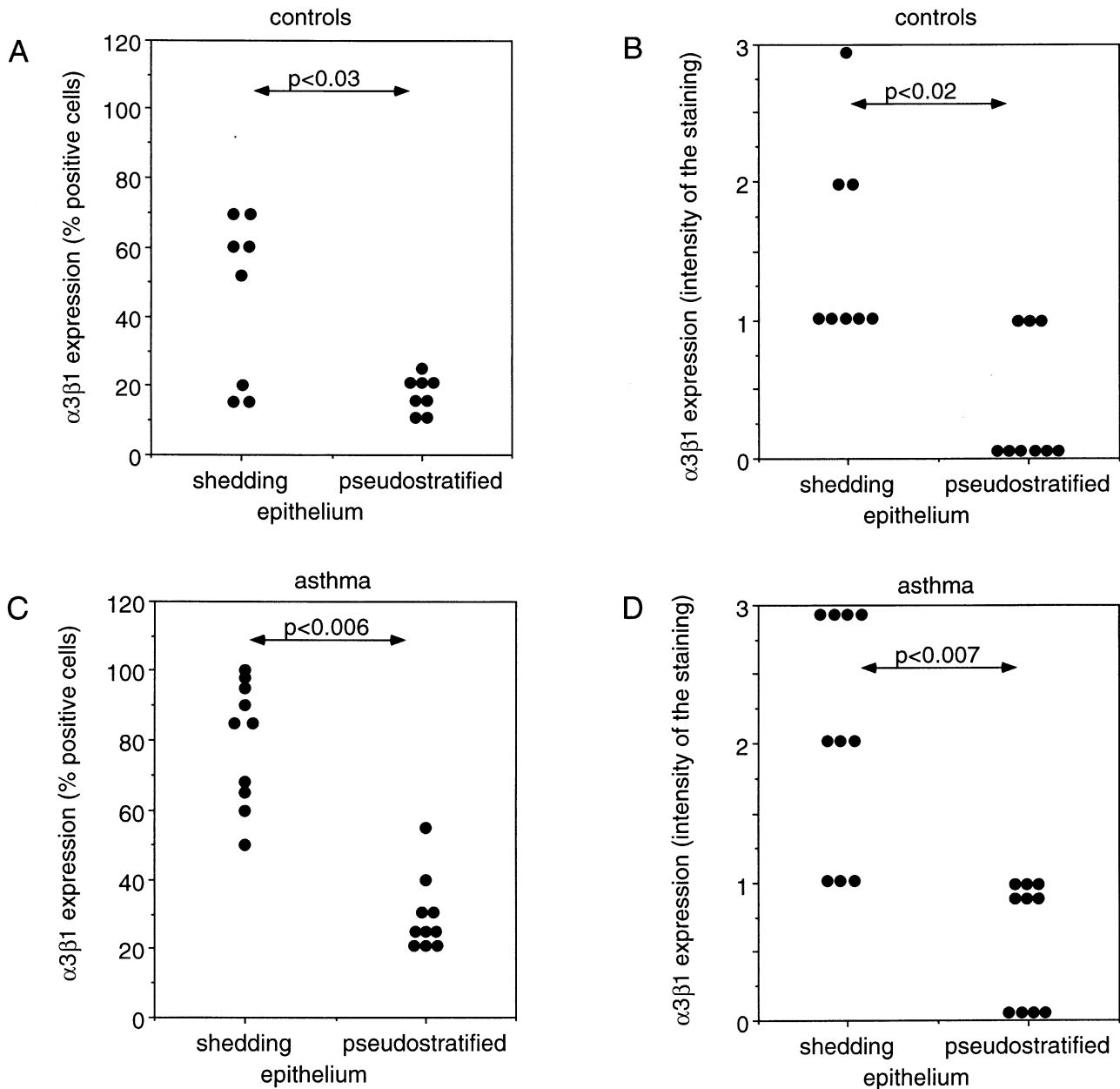


Figure 1. Expression of $\alpha 3\beta 1$ in areas characterized by epithelium shedding or by pseudostratified epithelium in bronchial biopsies taken from normal (A, B) and asthmatic subjects (C, D). Results are expressed as percent positive cells (A, C) or as intensity of staining (B, D).

also used a semiquantitative analysis to quantify the intensity of the staining. We used the following scores: 0 = negative; 1 = low immunoreactivity; 2 = medium immunoreactivity; 3 = high immunoreactivity. Areas of damaged epithelium were identified morphologically by the presence of compromised structure, lack of differentiated ciliated or secretory cells, and the height of the epithelium. $\alpha 3\beta 1$ expression was evaluated by two independent investigators (A.M.V., G.C.). The immunoreactivity for $\alpha 3\beta 1$ and ICAM-1 in areas characterized by epithelium shedding was evaluated only if a layer of basal epithelial cells still remained attached to the basement membrane.

In vitro study

Culture of WI-26 epithelial cells. WI-26 epithelial cells were purchased from the American Type Culture Collection (ATCC CCL 95.1 WI-26 VA4) (Rockville, MD, USA) and cultured as adherent monolayers in complete medium (CM) composed of RPMI 1640 plus 10% heat-inactivated (56°C, 30 min) fetal bovine serum plus 1% penicillin-streptomycin solution plus 1 mM L-glutamine (all from GIBCO, Paisley, UK).

Modulation of ICAM-1 and $\alpha 3\beta 1$ expression by WI-26 epithelial cells. Cultured WI-26 epithelial cells were

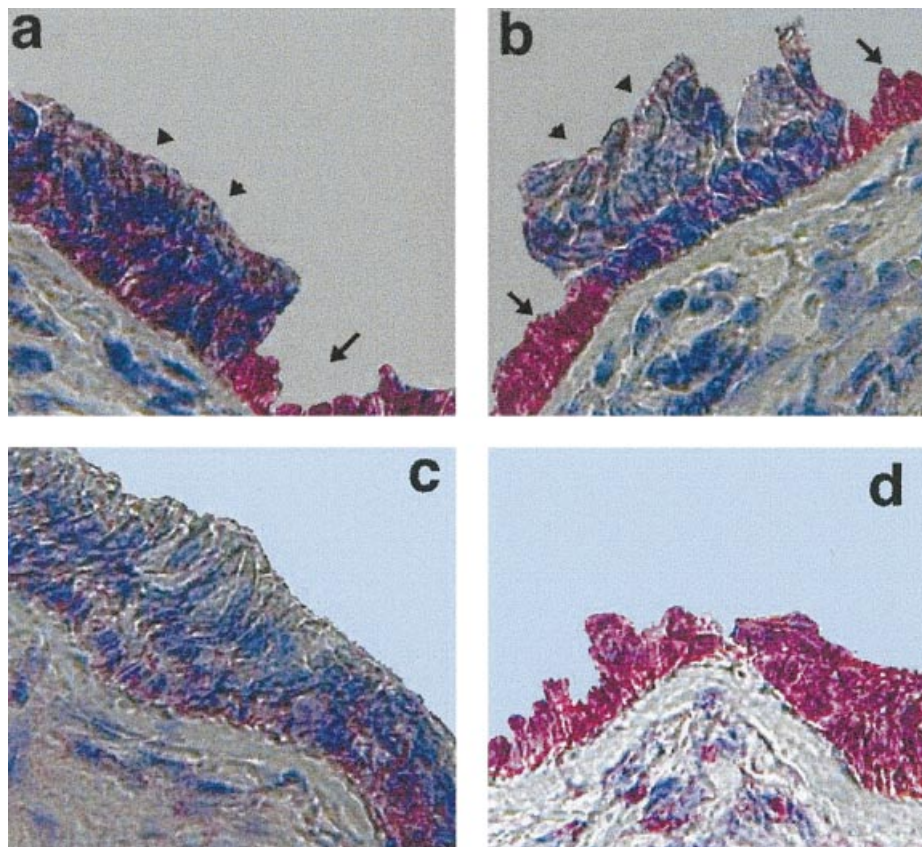


Figure 2. Expression of $\alpha 3\beta 1$ and ICAM-1 in bronchial biopsies obtained from asthmatic subjects (b, d) and control subjects (a, c). Panels a and b show immunoreactivity for $\alpha 3\beta 1$ in areas characterized by epithelium damage (arrows) and by pseudostratified epithelium (arrowheads). Panels c and d show increased ICAM-1 immunoreactivity in epithelium of asthmatic subjects as compared to normal subject.

harvested with a rubber policeman washed twice with phosphate buffer solution (PBS, GIBCO), counted with a hemocytometer, and resuspended in complete

Table 1. ICAM-1 and $\alpha 3\beta 1$ modulation by TGF- β , EGF, IL-4, and IL-5

TGF- β	ICAM-1	$\alpha 3\beta 1$	EGF	ICAM-1	$\alpha 3\beta 1$
Baseline	34.5 (33–39)	15 (12–15)	Baseline	35 (34–39)	15 (12–15)
0.002 nM	30 (28–34)*	10 (9–11)***	0.0006 nM	32 (32–38)*	15 (15–16)
0.02 nM	29 (20–38)	15 (14–20)	0.006 nM	30.5 (30–35)*	15.5 (15–20)
0.2 nM	26.5 (18–35)*	19 (16–21)	0.06 nM	30 (29–32)*	20 (20–21)*
2 nM	20 (17–20)**	30 (30–33)*	0.6 nM	29 (29–30)***	28 (27–28)*
20 nM	30 (30–35)	25 (20–28)*	6 nM	30 (29–30)*	22.5 (22–23)*
IL-4	ICAM-1	$\alpha 3\beta 1$	IL-5	ICAM-1	$\alpha 3\beta 1$
Baseline	41 (40–45)	15 (12–15)	Baseline	40.5 (40–42)	15 (12–15)
0.003 nM	39 (38–42)	20 (19–20)*	0.002 nM	41.5 (41–42)	13 (11–15)
0.03 nM	26.5 (24–28)*	30 (30–32)*	0.02 nM	42 (40–45)	14.5 (12–15)
0.3 nM	39 (37–40)	20 (20–20)*	0.2 nM	59.5 (56–60)*	5.5 (5–6)*
3 nM	41 (40–45)	19 (15–20)***	2 nM	51.5 (50–53)*	12 (12–15)

Results are expressed as median and 25–75th percentiles of mean intensity of fluorescence (MIF) of six experiments.

* $P < 0.03$ (calculated by comparison with baseline by Mann–Whitney U-test).

** $P < 0.006$ (calculated by comparison with baseline by Mann–Whitney U-test).

*** $P < 0.05$ (calculated by comparison with baseline by Mann–Whitney U-test).

medium at a concentration of 10^6 cells/ml. Aliquots (5 ml) of WI-26 epithelial cell suspension were delivered in 25 cm² tissue culture flasks and incubated (37°C, 5% CO₂) for 24 h in the absence or presence of TGF- β 1, or EGF, or IL-4, or IL-5. This time of incubation was selected on the basis of preliminary time-response curves, demonstrating optimal modulation of the expression of the investigated markers in the absence of significant cell death, as assessed by the trypan blue method. To select the concentrations of each of these stimuli, we performed dose-response curves using TGF- β 1 at concentrations ranging from 20 to 0.002 nM, EGF at concentrations ranging from 6 to 0.0006 nM, IL-4 at concentrations ranging from 3 to 0.003 nM, and IL-5 at concentrations ranging from 2 to 0.002 nM.

The expression of ICAM-1 and $\alpha 3\beta 1$ on the surface of WI-26 epithelial cell lines was determined by direct and indirect immunofluorescence, respectively, with a FACStar Plus (Becton Dickinson) analyzer equipped with an argon ion laser (Innova 70 Coherent) and Consort 32 computer support. After incubation with TGF- β , EGF, IL-4, and IL-5, the cells were harvested, washed with PBS, and resuspended in

ICAM-1 expression

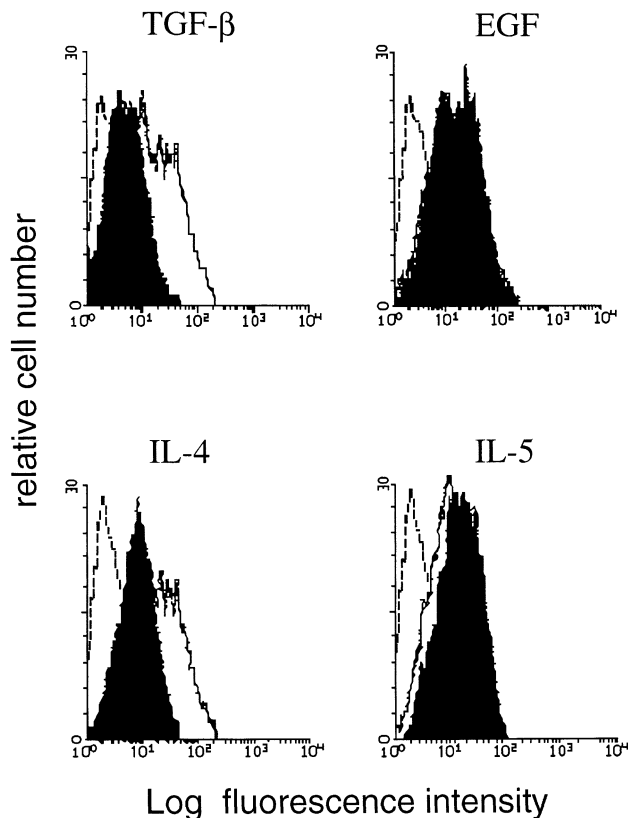


Figure 3. Representative cytofluorometric analysis of surface ICAM-1 expression by WI-26 cells after stimulation with TGF- β (2 nM), EGF (0.6 nM), IL-4 (0.03 nM), and IL-5 (0.2 nM). Stimulated cells (shaded portion) were analyzed on FACStar and compared to baseline (open portion). Control was performed by incubating cells with irrelevant isotype-matched mAb (broken-line portion). Results are expressed as relative cell number (y-axis) vs log fluorescence intensity (x-axis).

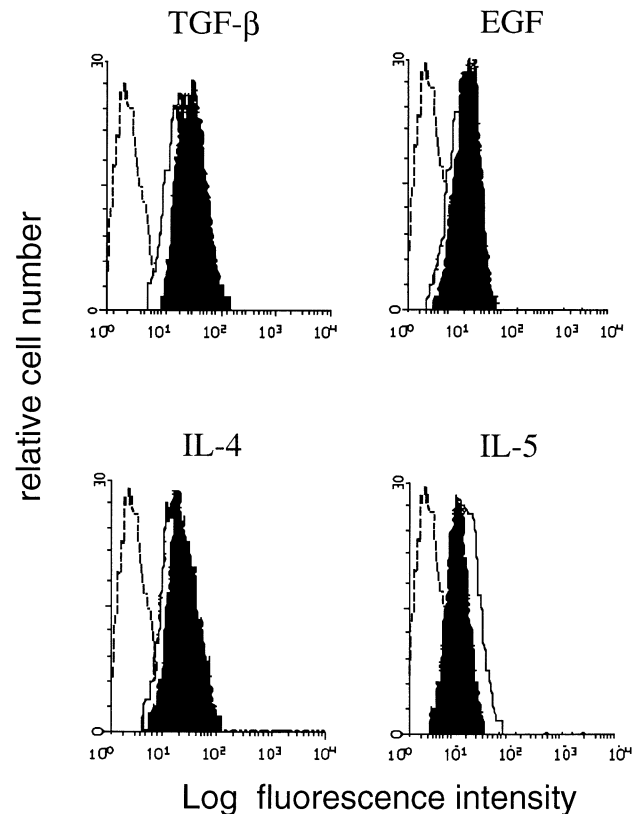
 $\alpha 3\beta 1$ expression

Figure 4. Representative cytofluorometric analysis of surface $\alpha 3\beta 1$ expression by WI-26 epithelial cells after stimulation with TGF- β (2 nM), EGF (0.6 nM), IL-4 (0.03 nM), and IL-5 (0.2 nM). Stimulated cells (shaded portion) were analyzed on a FACStar and compared to baseline (open portion). Control was performed by incubating cells with irrelevant isotype-matched mAb (broken-line portion). Results are expressed as relative cell number (y-axis) vs log fluorescence intensity (x-axis).

PBS at the concentration of $10 \times 10^6/\text{ml}$. With regard to ICAM-1 expression, 0.1-ml aliquots of WI-26 epithelial cells were incubated in the dark (30 min, 4°C) with phycoerythrin (PE)-conjugated mouse monoclonal antibody (mAb) antihuman ICAM-1 (clone LB2-PE, Becton Dickinson, Mountain View, CA, USA); with regard to $\alpha 3\beta 1$ expression, 0.1-ml aliquots of WI-26 epithelial cells were incubated in the dark (30 min, 4°C) with an anti- $\alpha 3\beta 1$ mAb (Dakopatts, Glostrup, Denmark), washed twice with PBS, and then incubated with FITC-conjugated rabbit antimouse immunoglobulins (Dakopatts, Glostrup, Denmark) (30 min, 4°C). PE-conjugated mouse antihuman IgG1 (X-928, Dakopatts, Glostrup, Denmark) served as negative control. FITC-conjugated mouse antihuman IgG1 (X-927, Dakopatts, Glostrup, Denmark) served as negative control. The cells stained with mouse antihuman ICAM-1 or anti-

$\alpha 3\beta 1$ were compared with cells incubated with the irrelevant mouse IgG1. Results were expressed as mean log fluorescence intensity. Cells were gated in order to exclude debris. We studied 5000 cells per sample, and a uniform response was obtained.

Measurement of fibronectin release by epithelial cells. Fibronectin was measured in the supernatant fluid by specific ELISA using antibodies and standards obtained from Calbiochem (La Jolla, CA, USA). The limit of detection was 50 pg/ml (22).

Statistical analysis

Statistical analysis was performed by nonparametric tests. The Wilcoxon W test was used for paired comparisons.

Fibronectin release

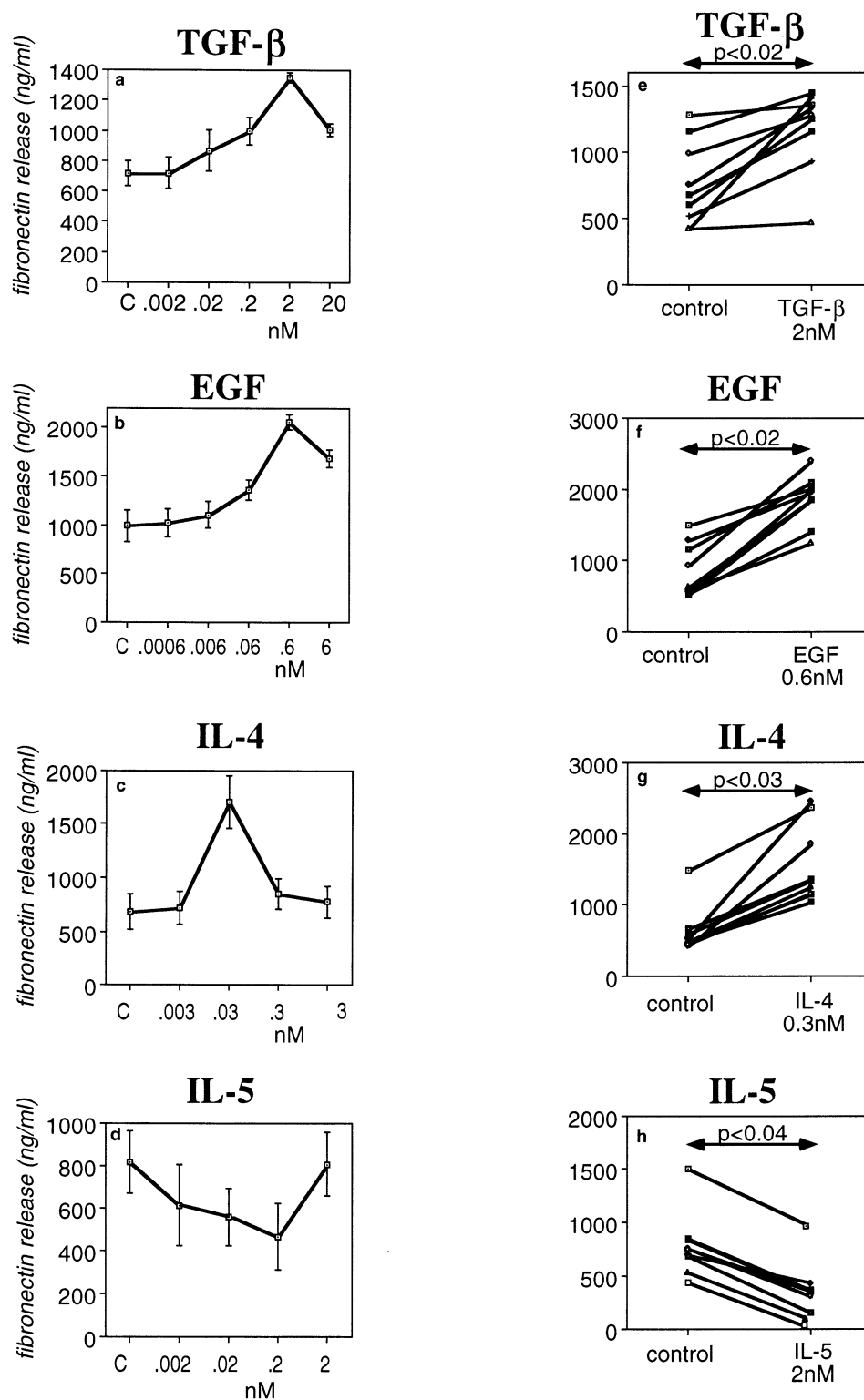


Figure 5. a–d) Fibronectin release by WI-26 epithelial cells after stimulation with TGF- β (from 0.002 to 20 nM), EGF (from 0.0006 to 6 nM), IL-4 (from 0.003 to 3 nM), and IL-5 (from 0.002 to 2 nM). Results are expressed as mean $\pm$ SD of six experiments. e–h) Fibronectin expression by WI-26 epithelial cells after stimulation with optimal dose of TGF- β (2 nM), EGF (0.6 nM), IL-4 (0.03 nM), and IL-5 (0.2 nM). Results are expressed as mean $\pm$ SD of nine experiments.

Results

Pathologic examination of bronchial mucosa

Epithelium shedding. Hematoxylin and eosin staining showed striking differences in the epithelium between the two groups of subjects. Shedding was absent or moderate in most normal subjects, whereas all patients with asthma exhibited various degrees of shedding, which was significantly higher (mean and 25–75th percentiles: 70 [65–75]) than in normal subjects (mean and 25–75th percentiles: 12.5 [10–22.5]) ($P < 0.004$, Mann–Whitney U-test).

Evaluation of $\alpha 3\beta 1$ and ICAM-1 expression. The expression of $\alpha 3\beta 1$ by bronchial epithelial cells was slightly but significantly higher in asthmatic than in control subjects ($P < 0.04$, Mann–Whitney U-test). In sections of biopsies taken from asthmatic subjects, the immunoreactivity for $\alpha 3\beta 1$, as well as the percentage of positively stained cells, was higher in areas of damaged epithelium than in areas of undamaged epithelium, within the same samples (Figs. 1 and 2). In bronchial biopsies of normal subjects, in areas with a damaged epithelium (which were found in only a few biopsies), $\alpha 3\beta 1$ immunoreactivity was also found to be increased (Figs. 1 and 2).

The expression of ICAM-1 by bronchial epithelial cells was significantly higher in asthmatic than in control subjects ($P < 0.001$, Mann–Whitney U-test) (Figs. 1 and 2). In asthma, ICAM-1 immunoreactivity was similar in areas of intact or damaged epithelium.

Evaluation of ICAM-1 expression by WI-26 cells. The expression of ICAM-1 by WI-26 bronchial epithelial cells and its modulation by cytokines was investigated at cellular level by cytofluorometric analysis. Six consecutive dose-response curves showed that while EGF did not significantly modify ICAM-1 expression, TGF- β , IL-4, and IL-5 were capable of modulating ICAM-1 expression by WI-26 epithelial cells in a dose-dependent fashion (Table 1; Fig. 3). In addition, dose-response curves showed that the concentrations of TGF- β , IL-4, EGF, and IL-5 capable of inducing the maximal effect on ICAM-1 expression were 2, 0.03, 0.6, and 0.2 nM, respectively (Table 1). These concentrations were then used to stimulate WI-26 epithelial cells. Among the anti-inflammatory cytokines used, TGF- β and IL-4 significantly decreased ICAM-1 expression, while EGF did not (TGF- β : $P < 0.005$; IL-4: $P < 0.03$, Wilcoxon-W test) (Fig. 3). On the other hand, IL-5 significantly increased ICAM-1 expression by WI-26 bronchial epithelial cells (IL-5: $P < 0.03$, Wilcoxon test) (Fig. 3).

Evaluation of $\alpha 3\beta 1$ expression by WI-26 cells. The

expression of $\alpha 3\beta 1$ by WI-26 bronchial epithelial cells and its modulation by cytokines were investigated at cellular level by cytofluorometric analysis. TGF- β , EGF, and IL-4 significantly increased $\alpha 3\beta 1$ expression (TGF- β and EGF: $P < 0.03$; IL-4: $P < 0.03$, Wilcoxon-W test) (Fig. 4). On the other hand, IL-5 induced a significant decrease of $\alpha 3\beta 1$ expression. ($P < 0.03$, Wilcoxon-W test) (Fig. 4). Six consecutive dose-response curves showed that the concentrations of TGF- β , IL-4, EGF, and IL-5 capable of inducing the maximal effect on $\alpha 3\beta 1$ expression by WI-26 epithelial cells were 2, 0.03, 0.6, and 0.2 nM, respectively, which were subsequently used to stimulate WI-26 epithelial cells (Table 1; Fig. 4).

Fibronectin release. Dose-response curves showed that the optimal concentrations of TGF- β , IL-4, EGF, and IL-5 were 2, 0.03, 0.6, and 0.2 nM, respectively (Fig. 5). TGF- β (2 nM), IL-4 (0.03 nM), and EGF (0.6 nM) were able to increase significantly the release of fibronectin (TGF- β : $P < 0.02$; IL-4: $P < 0.02$; EGF: $P < 0.03$, respectively; Wilcoxon test) (Fig. 5). On the other hand, IL-5 (0.2 nM) determined a slight but significant decrease of fibronectin release ($P < 0.04$, Wilcoxon-W test) (Fig. 5).

Discussion

The bronchial epithelium forms a dynamic barrier which actively participates in the inflammatory and repair processes involved in the pathogenesis of asthma. In asthma, epithelium is partly shed (23–25), and appears to be more fragile than in normal individuals. Recently, an increased interest has been shown in the mechanisms involved in the injury-repair cycle of the bronchial epithelium, as a response to the exposure to inflammatory stimuli. It has been suggested that epithelial injury activates a repair response of basal cells targeted at the re-epithelialization (25) of the wound. In order to be well oriented, such a repair process requires the release of ECM components (including fibronectin and collagens) and of chemotactic factors that establish a chemotactic gradient influencing the migration of bronchial epithelial cells (4, 10). Another fundamental biologic step in repair processes is the ability of the cells to adhere to the ECM and then to migrate toward the wound. These latter events require an overexpression of integrins, a heterogeneous group of molecules capable of modulating the interactions between cell and extracellular matrix components (4). Among integrins, an important role is played by the $\alpha 3\beta 1$ integrin, which functions as a receptor mediating not only the adhesion of cells to the ECM but also the deposition and matrix assembly of fibronectin, contributing to cell adhesion, migration, and growth, as well as to the maintenance of the

architecture of newly generated tissues (12). In the healing of dermal wounds, both the expression of $\alpha 3\beta 1$ integrin and the deposition of ECM are dramatically increased (26). In addition, $\alpha 3\beta 1$ integrin expression on the basal surfaces of keratinocytes may facilitate the formation of ECM during wound healing (27).

The results of the present study show that $\alpha 3\beta 1$ expression is increased at the sites of epithelial damage, and lend further support to the hypothesis that this integrin may play an important role in postinflammatory repair processes within the bronchial epithelium. This hypothesis is also supported by the marked difference between $\alpha 3\beta 1$ and ICAM-1 expression. ICAM-1 is indeed a molecule involved in epithelial-leukocyte interaction and in the development of airway inflammation (5, 6). Its expression on epithelial cells is increased in asthma (8) and correlated with the clinical severity of the disease (7). Therefore, the overexpression of ICAM-1 by bronchial epithelial cells may be crucial in the maintenance (9) and activation (28) of eosinophils within the epithelium layer, potentially leading to the injury and damage of bronchial epithelial cells. In this study, we demonstrated that ICAM-1 is overexpressed in the bronchial epithelium of asthmatics, in both damaged and intact epithelium.

Because of the presence of an increased expression of $\alpha 3\beta 1$ in areas of damaged epithelium and the striking difference between $\alpha 3\beta 1$ and ICAM-1 expression, we put forward the hypothesis that the expression of these molecules might be regulated in a different fashion by mediators involved in inflammatory or repair processes. To test this hypothesis, we used an *in vitro* model represented by cultured human pulmonary epithelial cells. These cells were exposed to IL-5, a powerful proinflammatory cytokine able to recruit and activate eosinophils (29) and to regulate their trafficking across the airway wall, and to IL-4, TGF- β , and EGF, which possess some anti-inflammatory properties (16, 30) and

are able to promote wound healing (10, 18). EGF and TGF- β are indeed able to act on post-inflammatory repair processes. EGF participates in airway repair processes targeted at the resurfacing of a wound with new epithelium (31) by stimulating the proliferation and migration of epithelial cells, and by increasing the synthesis of proteins, such as fibronectin (32). TGF- β is able to upregulate $\alpha V\beta 1$ expression in guinea-pig epithelial cells in culture (33), thus modifying the adhesion and migration characteristics of epithelial cells. In addition, TGF- β induces an increase in fibronectin production by bovine bronchial epithelial cells (10) and increases their attachment to matrix proteins (34). Interestingly, the expression of both these growth factors was previously found to be increased in the bronchial epithelium of asthmatic subjects (35), suggesting a role for both TGF- β and EGF in the regulation of the injury-repair cycle of the bronchial epithelium in asthma.

The *in vitro* results of the present study suggest that cytokines able to promote bronchial inflammation (such as IL-5) exert an opposite effect on the expression of ICAM-1 and $\alpha 3\beta 1$, and on the release of fibronectin by WI-26 epithelial cells, as compared to cytokines able to reduce bronchial inflammation and to promote tissue repair (such as TGF- β , EGF, and IL-4). IL-5 is able to increase ICAM-1 expression and to reduce $\alpha 3\beta 1$ expression and fibronectin release by WI-26 epithelial cells. On the other hand, IL-4 and TGF- β are able to decrease ICAM-1 expression and to increase $\alpha 3\beta 1$ expression and fibronectin release by the same cells. This latter effect was also exerted by EGF.

In conclusion, these results highlight the ability of the epithelium to adapt its biological behavior to mediators exerting either a proinflammatory or an anti-inflammatory effect, and show and confirm the ability of these cells to act as a dynamic barrier to pulmonary microenvironmental stimuli.

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