Modulation of Cadherin and Catenins Expression by Tumor Necrosis Factor-α and Dexamethasone in Human Bronchial Epithelial Cells

Nathalie Carayol, Alison Campbell, Isabelle Vachier, Brigitte Mainprice, Jean Bousquet, Philippe Godard, and Pascal Chanez

Clinique des Maladies Respiratoires, Hôpital Arnaud de Villeneuve, Montpellier, France

Asthma is an inflammatory disease, and the epithelial mesenchymal unit appears to be of importance in regulating the disease mechanisms. Cell–cell adhesion plays an important role in tissue morphogenesis and homeostasis and is commonly mediated by cadherins, a family of Ca^{2+}-dependent transmembrane adhesion receptors. The cadherin family is involved in control of the cellular architecture. Proinflammatory cytokines such as tumor necrosis factor (TNF)-α are involved in asthma and may interfere with epithelial integrity. In the present study, we investigated the role of TNF-α and dexamethasone on the expression of E-cadherin, β-catenin, and γ-catenin. We used two bronchial epithelial cell models: primary small airway epithelial cell cultures and primary culture obtained from human bronchial tubes. After 48 h of TNF-α stimulation with or without dexamethasone expression of E-cadherin, β-catenin and γ-catenin were analyzed using Western blot analysis and immunofluorescence. This study showed a decrease in the expression of adhesion molecules in both epithelial cell cultures after stimulation. Dexamethasone and anti–TNF-α inhibited this effect. In unstimulated cells, E-cadherin and β- and γ-catenin expression was membranous, expressed only on the lateral cell wall with minimal cytoplasmic expression. Immunoreactivity was cytoplasmic in stimulated cells. We demonstrated, using Western blot analysis and immunofluorescence, that proinflammatory cytokines could be responsible for structural damage to the epithelium and that this process was potentially reversed by steroids.

Asthma is an inflammatory disease of the airways, and the epithelial mesenchymal unit appears to be of importance in its mechanisms (1). These cells provide a primary defense and can also have an active role in the immune response. In asthma, the airway epithelium is highly fragile, with a corresponding thickening of the basement membrane, which is possibly part of the defense mechanism. Bronchial epithelial cells of patients with asthma are less viable and more "fragile" than those from control subjects, meaning that the damage of the epithelium as the loss of large numbers of pseudostratified columnar epithelial cells may be a predominant feature (2–4). In addition, bronchial epithelial cells of asthmatic patients appear to be more activated both in terms of cell surface markers and release of inflammatory mediators (5, 6).

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Address correspondence to: Dr. Pascal Chanez, Clinique des Maladies Respiratoires, Hôpital Arnaud de Villeneuve, 371 Av. du Doyen Gaston Giraud, 34295 Montpellier Cedex 5, France. E-mail: chanez@montp.inserm.fr

Abbreviations: bovine serum albumin, BSA; ethylenediaminetetraacetic acid, EDTA; human bronchial epithelial cells, HBEC; human umbilical vein cells, HUVEC; interferon, IFN; interleukin, IL; nuclear factor, NF; phosphate-buffered saline, PBS; small airway epithelial cell, SAEC; tumor necrosis factor, TNF.

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Cell–cell adhesion plays an important role in tissue morphogenesis and homeostasis (7) and is commonly mediated by cadherins, a family of Ca^{2+}-dependent transmembrane adhesion receptors. The cadherin family, which is involved in control of the cellular architecture, is composed of glycoproteins including E- (epithelial), N- (nerve), and P- (placenta) cadherin. They have homophilic interactions with similar receptors on neighboring cells, whereas their cytoplasmic domain interacts with the cytoskeleton (8). These interactions are mediated via β-catenin or its closely related homolog γ-catenin (plakoglobin), which interacts with microfilaments through α-actinin (9) and α-catenin (10). β-catenin and γ-catenin interact directly with the cytoplasmic domain of cadherins in a mutually exclusive way (11). In addition, β-catenin has been identified as a direct component of the wnt/wingless signaling cascade (12, 13). In the absence of wnt signaling, β-catenin is constitutively phosphorylated and targeted for ubiquitination and subsequent proteosomal degradation (14). Inhibition of this phosphorylation leads to β-catenin stabilization and accumulation in the cytoplasm and the nucleus where it forms a complex with transcription factors (15, 16).

Tumor necrosis factor (TNF)-α mediates cell–cell dissociation associated with disordered expression of cadherin and β-catenin (19) in endometrial epithelial cells. Moreover, TNF-α induces dissociation of vascular endothelial cadherin from the intercellular junction in human umbilical vein cells (HUVEC) (20). Finally, long-term exposure to elevated levels of TNF-α and interferon (IFN)-γ could induce destruction of salivary gland parenchyma in Sjögren syndrome via an increase in cell death processes (21). Differences in the expression of E-cadherin and catenins have been described in biopsies from patients with lung cancer (17, 18), suggesting that modifications in E-cadherin binding to catenins may be involved in weakening the bronchial epithelium in asthma.

In asthma, TNF-α, interleukin (IL)-1β, and IFN-γ are prominent potentially released proinflammatory cytokines (22, 23), and glucocorticoids are the first-line therapy. The most striking effect of glucocorticoids is that they inhibit the expression of multiple inflammatory genes (cytokines, enzymes, receptors, and adhesions molecules). Dexamethasone has been reported to upregulate cadherin expression in human fibrosarcoma cells**.** Dexamethasone treatment of HT-1080 cell aggregates more than doubles their cohesivity, and Western blot analysis shows a corresponding increase in cadherin expression (24). Moreover, glucocorticoids play a fundamental role in the function and maintenance of cell–cell contact in mammary epithelia by inducing tight junction formation (25).

In the present study, we assessed the importance of intercellular adhesion molecule expression and regulation in bronchial epithelial cell cohesion processes as well as their associations with morphologic changes. For this purpose, we first investigated the normal localization of E-cadherin and β- and γ-catenin on human bronchial section. We then evaluated the effect of TNF-α stimulation, with or without the addition of dexamethasone, on E-cadherin, β- and γ-catenin, and cytokeratin expression by Western blot analysis and their cellular localization by immunofluorescence *in vitro*. We used two airway epithelial cell models: primary small airway epithelial cell (SAEC) culture and cells obtained at surgery from human bronchial tubes. To evaluate the inflammatory status of our epithelial cell models, the results were correlated with the release of proinflammatory cytokines such as IL-8 and RANTES and the expression of transcription factor nuclear factor (NF)-κB.

Materials and Methods

Study Design

To assess the importance of E-cadherin and β- and γ-catenin expression and regulation in bronchial epithelial cell cohesion processes, we performed a four-step analysis. We used human bronchial section and two airway epithelial cell models: primary SAEC culture and primary cultures of cells obtained from human bronchial tubes.

First, we assessed the *in situ* localization of E-cadherin and β- and γ-catenin by immunohistochemistry. Second, we studied, using Western blot analysis, whether TNF-α and dexamethasone would be able to quantitatively modulate E-cadherin and β- and γ-catenin expression. Third, we investigated the effect of inflammatory and antiinflammatory stimulation on epithelial cell function by measuring IL-8 and RANTES release and NF-κB regulation.

β- and γ-catenin are known to be involved in the Wnt signaling pathway; therefore, we used immunofluorescence to investigate differences in localization of these adhesion molecules and to investigate possible cellular morphological changes.

Human Bronchial Epithelial Cell Cultures

Primary SAEC cultures were obtained from Bio-Whittaker (Walkersville, MD). Cells were monolayed in tissue culture flasks (Costar, Cambridge, MA) and incubated at 37°C in a 5% CO_2 humidified atmosphere. SAEC were maintained in Small Airway cells Growth Medium (SAGM) and serum-free small airway epithelium basal medium (Clonetics, San Diego, CA) supplemented with growth factors. Cells were fed every 2 d and subcultivated when confluent by detachment with trypsin (0.25 mg/ml) and ethylenediaminetetraacetic acid (EDTA) (0.1 mg/ml) for 3 min at 37°C without scraping. Then trypsin neutralization solution (Clonetics) was added to the cell suspension, and cells were centrifuged at 400 × *g* for 7 min and resuspended in culture medium. After the first passage, cells were cultured in 12-well plates until confluence.

Human bronchial epithelial cells (HBEC) were obtained from human bronchial tube surgery biopsies. The bronchial samples were taken from a normal area of bronchi removed at surgery from exsmoker patients suffering of lung cancer with a normal lung function. After excision, the bronchial tubes were washed and incubated overnight at 4°C with 0.38 mg/ml hyaluronidase, 0.75 mg/ml collagenase, 1 mg/ml protease, and 0.3 mg/ml DNase in RPMI 1640 (Gibco, Grand Island, NY) and then filtered through a 70-μm mesh nylon cell strainer. Pieces of epithelium retained on the strainer were recovered with SAGM culture medium and transferred into 12-well plates (Nunc, Naperville, IL).

Immunohistochemistry

Bronchial samples obtained from surgery were immediately fixed at −20°C in acetone containing protease inhibitors (iodoacetamine and phenylmethylsulfonyl). Specimens were processed and embedded in the water-soluble resin glycolmethacrylate (JB4; Polysciences, Los Angeles, CA). Endogenous peroxidase activity was blocked by incubating sections (2 μm) with hydrogen peroxide. Nonspecifc immunoreaction was blocked by bovine serum albumin (BSA) for 20 min. Sections were incubated at room temperature with the primary monoclonal antibodies: anti–β-catenin, γ-catenin, and E-cadherin (250 ng/ml) (Transduction Laboratories, Lexington, KY) for 1 h; biotinylated $F(ab')_2$ fragments of rabbit anti-mouse immunoglobulins (Ig) were used as secondary antibody (Dako, Glostrup, Denmark) for 30 min. In control experiments, the primary antibody was replaced by an irrelevant antibody, mouse IgG1 (Dako). Revelation was performed using ABcomplexe-HRP (Dako), and tissue-bounded immune complexes were rendered visible by hydrogen peroxide with 3,3′-diaminobenzidine (Sigma, St. Louis, MO). Sections were counterstained with Mayer's hematoxylin.

Stimulation of SAEC and HBEC Cultures

First, a dose-response curve was performed on SAEC to determine by Western blot analysis the maximal concentration of TNF-α used in subsequent experiments (from 0.1–100 ng/ml) for 48 h (R&D Systems, Abington, UK). The maximal effect was observed for the TNF-α concentration of 50 ng/ml. Then SAEC and HBEC were stimulated with culture medium supplemented with TNF-α (50 ng/ml) for 48 h. To test stimulation specificity, the antibody against TNF-α at 50 ng/ml (R&D Systems, Minneapolis, MN) was added to the stimulated medium. Subsequently, dexamethasone (10^{-7} M) was added at the same time to the stimulated medium.

Western Blot Analysis

After specific stimulation, whole cells were washed with cold phosphate-buffered saline (PBS) and lysed in 10 mM Tris-HCl (pH 7.4), 50 mM NaCl, 5 mM EDTA, 1% Nonidet P-40, and 10 μg/ml phenylmethylsulfonyl fluoride. Cell extracts were transferred in microcentrifuge tubes, mixed, and left on ice for 10 min. After one freeze/thaw cycle, they were centrifuged at 12,000 × *g* for 5 min at 4°C. The supernatant sample was taken for protein estimation and the remainder adjusted with 4× Laemmli dissociation buffer. Total protein (10 μg) was subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis on 4–12% gradient gels (Novex, San Diego, CA) and then blotted onto nitrocellulose membranes. Blots were blocked with PBS containing 3% BSA, 0.1% Tween 20, and probe with anti–E-cadherin (1:2,500), β-catenin (1:500), γ-catenin (1:2,000) monoclonal primary antibodies, and anti-p65 (subunit of NF-κB) polyclonal antibody (Santa Cruz, CA). After serial washes with PBS containing 0.1% Tween 20, membranes were incubated with peroxidase-conjugated goat anti-mouse antibody (Sigma) as secondary antibody at 1:3,000 dilution. The monoclonal antibody against the housekeeping gene β-actin 1:3,000 (Sigma) was used as internal control. Revelation was performed using an enhanced chemiluminescence system (NEN, Boston, MA) followed by autoradiography. The density of bands was determined by autoradiography. Autoradiographic films were analyzed by densitometric scanning using a camera (monochrome CCD camera [RS-170; COHU, San Diego, CA]), coupled to a specific software (NIH Image analysis program). All the results were expressed as the ratio of the density of the specific band corrected with the density of the band obtained for β-actin.

Immunofluorescence

After specific stimulation, HBEC were obtained by cytospinning, and, alternatively, SAEC were grown to confluence on specific im-

munostaining chambers (lab-tek). Cells were fixed in 4% paraformaldehyde and permeabilized for 4 min at 0°C using 0.5% (vol/vol) Triton X-100 in PBS. After blocking in PBS containing donkey serum, cells were incubated for 1 h at room temperature with the anti E-cadherin, β-catenin and γ-catenin monoclonal antibodies, and pan-cytokeratin (Dako) diluted to 1:100 in the same buffer. Labeling was performed using a Cy 3-conjugated AffinePure donkey anti-mouse IgG (Jackson ImmunoResearch, West Grove, PA). After extensive washes, cells were mounted and observed with a Optiphot-2 fluorescence microscope (Nikon, Tokyo, Japan).

Measurement of RANTES and IL-8 Release by BEC

IL-8 and RANTES were measured in cell-free supernatants of specific stimulated cells using commercial enzyme-linked immunosorbent assay (ELISA) kits (Quantikine; R&D Systems). ELISA was performed according to the manufacturer's instructions.

Statistical Methods

Quantitative results are expressed as mean ± SEM of three experiments performed in duplicate. All results are compared with a 100% basal value. The effects of cytokine and dexamethasone are analyzed using a paired Student's *t* test and considered significant at $P < 0.05$.

Results

E-cadherin, β-Catenin, and γ-Catenin Expression in Bronchial Sections

For comparisons, the three antibodies were applied on serial sections of the same sample. Immunoreactivity for anti–E-cadherin, β-catenin, and γ-catenin was observed in the same region of the section, at epithelial level. No cytoplasmic staining was observed, and the immunoreactivity was shown on the lateral walls of goblets and ciliated cells. At light microscopic observation level, the immunostaining appeared at cell surfaces or interfaces. No labeling was observed on basal cells. Sporadic, probably intermediate cells showed immunostaining (Figure 1).

Quantitative Regulation of E-Cadherin, β-Catenin, and γ-Catenin Expression in Epithelial Cells

Western blot analysis showed that SAEC and HBEC exhibited constitutive expression of E-cadherin and β- and γ-catenin (Figure 2). Three different experiments were performed in duplicate.

TNF-α stimulation of SAEC or HBEC for 48 h significantly decreased E-cadherin and β-catenin, and to a lesser extent γ-catenin. The maximum decrease in E-cadherin and β-catenin was observed at 50 ng/ml TNF-α concentration. When anti–TNF-α (50 ng/ml) was added to the stimulated medium, the levels of E-cadherin and β- and γ-catenin returned to the level of unstimulated cells. The results are expressed as a percentage of constitutive expression. Dexamethasone (10^{-7}) partly reversed this effect of TNF-α.

Quantitative Regulation of Cytokine Expression in Epithelial Cells

In the supernatant of specific stimulated cells (SAEC and HBEC), we measured IL-8 and RANTES levels using ELISA kits. As expected, TNF-α increased the release of both IL-8 and RANTES, whereas dexamethasone reversed this decrease, and anti–TNF-α returned to basal values (Figure 3A).

Quantitative Regulation of NF-κB

Figure 3B shows the regulation of NF-κB expression using TNF-α (50 ng/ml) with and without dexamethasone (10^{-7} M)

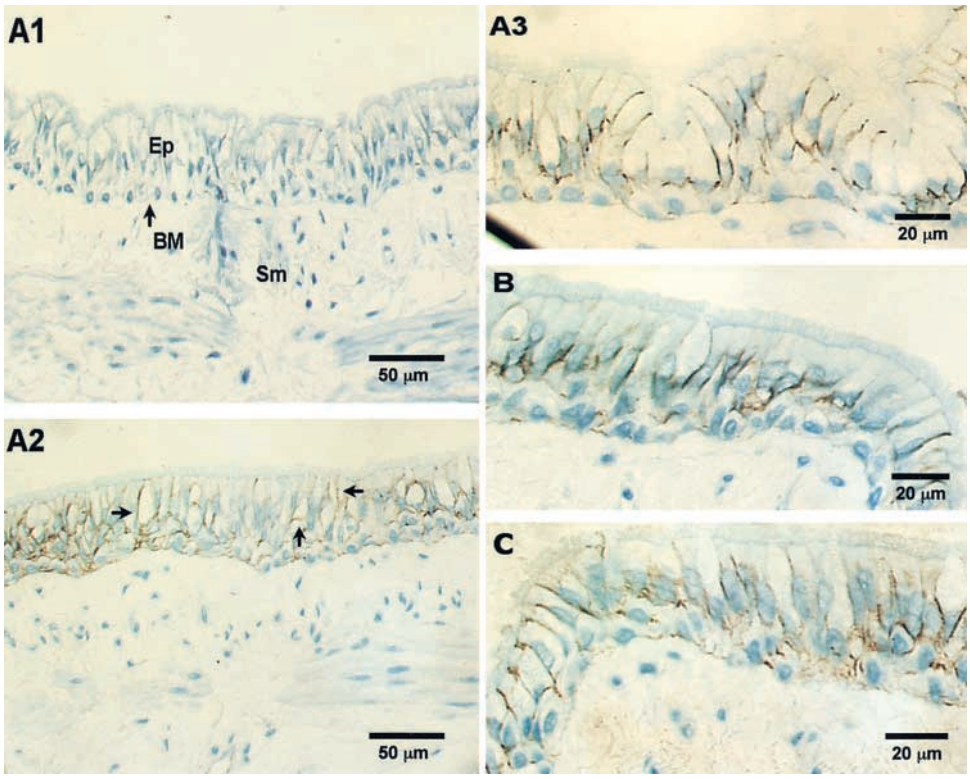

Figure 1. Immunolocalization of E-cadherin (*A*), β-catenin (*B*), and γ-catenin (*C*) in epithelial cells of bronchial mucosa using monoclonal antibodies. Serial sections of Glycolmethacrylate (GMA)-embedded samples were used for immunostaining. All micrographs were in the same part of the specimen. (*A1*) Negative control using mouse IgG1. Ep, epithelium; BM and *arrows*, basement membrane; Sm, submucosa. (*A2* and *A3*) Epithelial cells stained by a monoclonal anti–E-cadherin (*arrows*). (*B*) Epithelial cells stained by a monoclonal anti–β-catenin. (*C*) Epithelial cells stained by a monoclonal anti–γ-catenin. All the labeling was observed on lateral walls of goblets and ciliated cells. No staining was observed in basement membrane or submucosa.

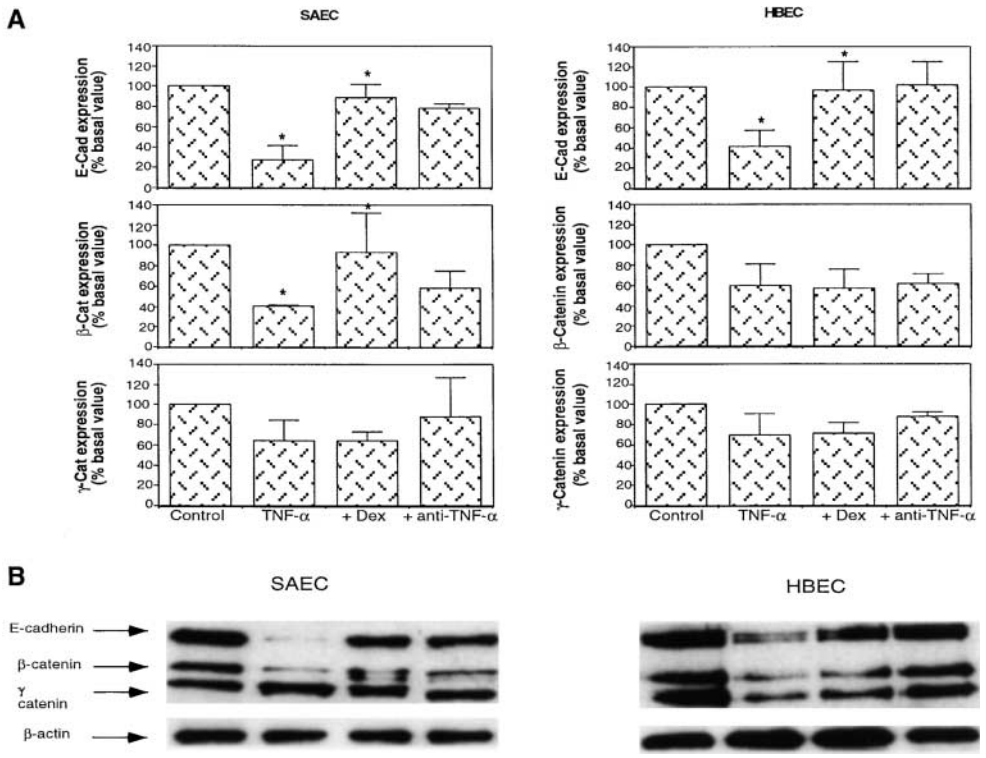

Figure 2. Effect of TNF-α (50 ng/ml) stimulation on E-cadherin, β-catenin, and γ-catenin expression as determined by Western blot analysis on SAEC and HBEC (*A*) and representative Western blot analysis of E-cadherin, β-catenin, and γ-catenin expression in SAEC and HBEC (*B*). SAEC and HBEC were stimulated for 48 h with TNF-α with or without dexamethasone and anti–TNF-α antibody. Total proteins were isolated, and Western blot analysis was performed with anti–E-cadherin (120 kD), β-catenin (92 kD), γ-catenin (82 kD) antibodies, and a housekeeping gene, β-actin (45 kD), as described in MATERIALS AND METHODS. *Lane 1*: SAEC or HBEC control expression. *Lane 2*: SAEC or HBEC 48 h after TNF-α stimulation. *Lane 3*: SAEC or HBEC after TNF-α and dexamethasone stimulation. *Lane 4*: SAEC or HBEC after TNF-α and anti–TNF-α antibody stimulation. The β-actin signal was used as loading control. Autoradiographic films were analyzed by densitometric scanning using a monochrome CCD camera coupled to the NIH image analysis program. The reported values are means of three independent experiments, expressed as percentage of unstimulated control cells (± standard error of the mean (SEM)). + Dex, TNF-α + 10^{-7} M dexamethasone; + anti-TNF-α, TNF-α + anti–TNF-α antibody (50 ng/ml).

and anti-TNF-α (50 ng/ml) for three experiments performed in duplicate on SAEC.

TNF-α significantly increased NF-κB expression. Dexamethasone and anti–TNF-α reversed the effects of TNF-α.

Qualitative Regulation of E-Cadherin, β-Catenin, and γ-Catenin

E-cadherin and γ-catenin expression was assessed by immunofluorescence after 48 h of stimulation by TNF-α (50 ng/ml) ± dexamethasone (10^{-7} M). In unstimulated cells, E-cadherin expression was membranous, characterizing the lateral cell wall with minimal cytoplasmic expression (Figures 4A-a and 4B-1). In stimulated cells, immunoreactivity remained membranous for E-cadherin, but cytoplasmic expression was increased (Figures 4A-d and 4B-4). When dexamethasone was added, E-cadherin immunoreactivity became membranous again, and cytoplasmic expression was reduced (Figures 4A-g and 4B-7). The distribution of γ-catenin differed somewhat from the pattern obtained for E-cadherin. Although there was some staining around the cell periphery, γ-catenin was detected mainly as cytoplasmic granules, which were predominantly perinuclear, possibly in the endoplasmic reticulum (Figures 4A-b and 4B-2). In stimulated cells and with dexamethasone, γ-catenin expression was concentrated in granules juxtaposed to the nuclei (Figures 4A-e, 4A-h, 4B-5, and 4B-8).

Qualitative Regulation of Cytokeratin Expression

In the absence of stimulation, most cells demonstrated a normal cytokeratin filamentous network and a polygonal shape (Figures 4A-c and 4B-3). After TNF-α stimulation, we observed an abnormal aggregated cytokeratin distribution. Most cells had a rounder shape, and some of them appeared as drawn-out cells with long extensions (Figures 4A-f and 4B-6). When dexamethasone was added, most cells returned to a polygonal shape (Figures 4A-i and 4B-9).

Nuclear Translocation and Time Kinetics with Dexamethasone

β-catenin expression was assessed by immunofluorescence after 6, 12, and 24 h of stimulation by TNF-α (50 ng/ml) with or without dexamethasone (10^{-7}) in SAEC and HBEC. At 24 h, immunoreactivity remained membranous, and cytoplasmic expression was increased in stimulated cells (Figures 5-b and 5-f). When dexamethasone was added, immunoreactivity became membranous again (Figures 5-d and 5-h). In contrast, at 6 h incubation, the immunoreactivity of stimulated cells increased in the cytoplasm, and when dexamethasone was added, the immunoreactivity was mainly expressed in the nucleus of most cells (Figures 5-c and 5-g).

Discussion

In the present study, immunohistochemical analysis of human bronchi sections showed constitutive membranous expression of E-cadherin, β-catenin, and γ-catenin in goblets and ciliated cells of human bronchial mucosa. This expression was not found on basal cells. This constitutive level was confirmed using Western blot analysis of SAEC and HBEC. We used a purified primary human epithelial cell culture (SAEC) to study the effect of TNF-α on puri-

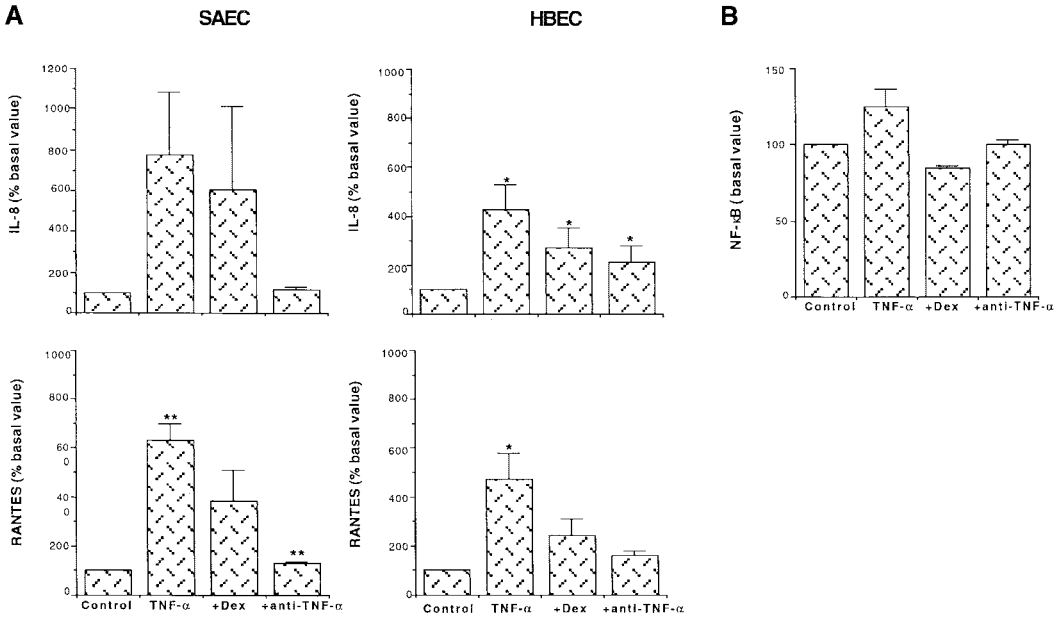

Figure 3. Effect of TNF-α on IL-8 and RANTES release in SAEC and HBEC (*A*) and NF-κB (*B*). After 48 h of stimulation with TNF-α (50 ng/ml) with or without dexamethasone (10^{-7} M) and anti–TNF-α antibody (50 ng/ml), IL-8 and RANTES were measured in cell-free supernatants of specific stimulated cells using commercial ELISA kits and NF-κB by Western blot analysis. The reported values are means of three independent experiments, expressed as percentage of unstimulated control cells (± SEM). + Dex, TNF-α + 10^{-7} M dexamethasone; + anti–TNF-α, TNF-α + anti–TNF-α antibody (50 ng/ml).

fied epithelial cells, and we confirmed our results obtained on HBEC, to test our hypothesis on "pieces" of human bronchial epithelium, a closer *in vivo* model. In these cells, Western blot analysis showed that TNF-α significantly decreased E-cadherin and β-catenin expression. This decrease was specific because an antibody against TNF-α was able to reverse it. Moreover, the addition of dexamethasone also reversed the effect of TNF-α. The decreased expression of cadherin and catenins in airway epithelial cells induced by TNF-α may be related to the inflammatory state. Using an ELISA kit, we observed that IL-8 and RANTES were increased by TNF-α. The addition of an anti–TNF-α antibody or dexamethasone inhibited this effect. Moreover, we demonstrated using Western blot anal-

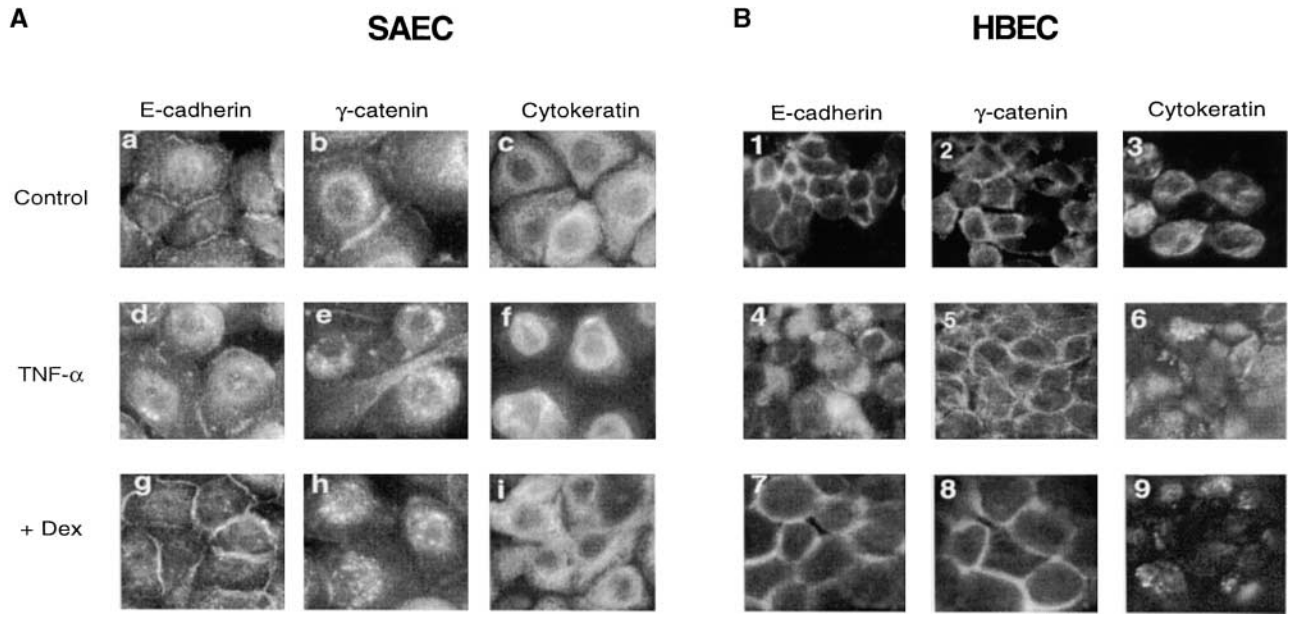

Figure 4. Subcellular distribution of E-cadherin, γ-catenin, and cytokeratin in SAEC (*A*) and HBEC (*B*). After 48 h of stimulation with TNF-α with or without dexamethasone and anti–TNF-α antibody. Cells were fixed with paraformaldehyde. After permeabilization immunostaining was performed with anti–E-cadherin (*A-a*, *A-d*, *A-g* and *B-1*, *B-4*, *B-7*), γ-catenin (*A-b*, *A-e*, *A-h* and *B-2*, *B-5*, *B-8*), and anti–pan-cytokeratin (*A-c*, *A-f*, *A-i* and *B-3*, *B-6*, *B-9*) antibodies. Immunostaining was observed with an Optiphot-2 fluorescence microscope (Nikon, Tokyo, Japan) equipped with a 1024 laser scanning confocal imaging system (Bio-Rad, Hercules, CA). + Dex, TNF-α + 10^{-7} M dexamethasone; + anti–TNF-α, TNF-α + anti–TNF-α antibody (50 ng/ml).

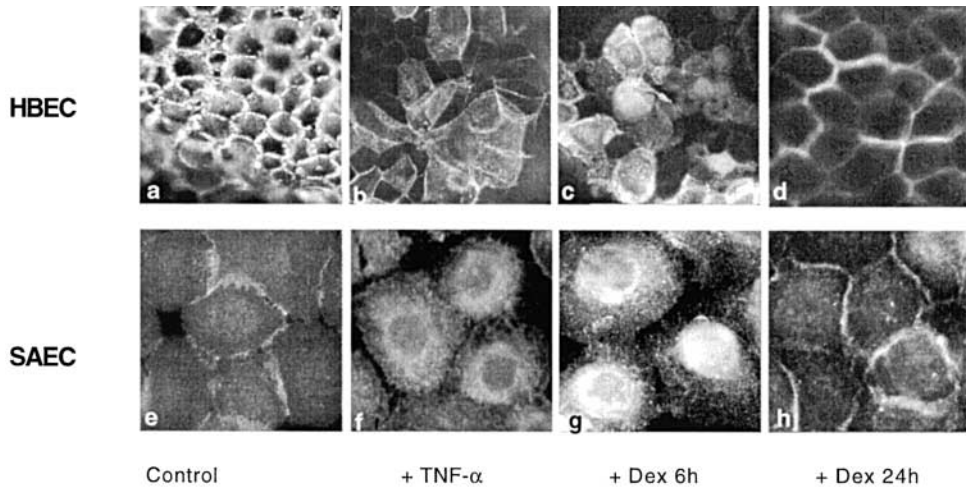

Figure 5. Subcellular distribution of β-catenin in SAEC and HBEC. After 24 h of TNF-α stimulation (*b* and *f*) and 6 h (*c* and *g*) or 24 h (*d* and *h*) of TNF-α with or without dexamethasone stimulation, cells were fixed with paraformaldehyde. After permeabilization, immunostaining was performed with β-catenin. Immunostaining was observed with an Optiphot-2 fluorescence microscope (Nikon). + Dex, TNF-α + 10^{-7} M dexamethasone; + anti–TNF-α, TNF-α + anti–TNF-α antibody (50 ng/ml).

ysis that NF-κB was involved in this regulation process. Finally, we performed immunofluorescence analysis to better assess the importance of this expression with respect to the epithelial cohesion structure. We found that the membranous localization of β-catenin and E-cadherin was associated with a cohesive epithelial structure with a normal morphologic structure shown by cytokeratin immunostaining. The addition of TNF-α, which delocalized the adhesion molecules, also involved a morphologic change in the cells, which were less cohesive with a rounder shape. This phenomenon was reversed by the addition of dexamethasone. The time kinetics of this delocalization demonstrated a transient nuclear localization of β-catenin 6 h after the addition of dexamethasone.

Bronchial epithelial cells of patients with asthma are less viable and more "fragile" than those from control subjects (2). This fragility may be explained first by the results of the *in situ* study that showed the absence of E-cadherin, β-catenin, and γ-catenin on basal cells, creating a suprabasal plan allowing shedding of epithelial cells to occur as proposed by Montefort and colleagues (26). Second, the *in vitro* study showed that there is a TNF-α–induced decrease in cadherin and catenin expression, which could lead to a decrease in epithelial cohesion. In the same way, Perry and coworkers reported a reduction in E-cadherin expression and a modest effect on β-catenin after TNF-α, IL-1β, and IFN-γ stimulation in celiac disease (27). Jakob and coworkers demonstrated that TNF-α and IL-1β act on Langherans cells like dendritic cells to regulate E-cadherin function by reducing mRNA levels and then attenuating E-cadherin–mediated adhesion (28). Even if our model using TNF-α presents limitations of cells in cultures, Montefort and colleagues, using bronchial biopsies, have already suggested that, in asthma, mechanisms involving reduced expression of intraepithelial intercellular adhesion molecules may account for epithelial disruption (29).

In addition, bronchial epithelial cells of patients with asthma appear to be more activated both in terms of cell surface markers and release of inflammatory mediators (5, 6). In our study, we focused on IL-8 and RANTES release (30, 31) and NF-κB expression, which are described to be involved in asthma (32) and activated by a wide variety of different stimuli, including inflammatory cytokines such as TNF-α. These observations indicate that the bronchial epithelium could be a major source of IL-8 and RANTES and that their production could be amplified substantially by TNF-α. The bronchial epithelium is able to modulate inflammatory events, and these observations suggest that it could contribute to promoting and sustaining the inflammatory process and then the fragility of the epithelium associated with decreased cell cohesion. We demonstrated a quantitative decrease in adhesion molecule that may be related to the loss of epithelium cohesion in patients with asthma.

We then performed immunofluorescence analysis to better assess the importance of this expression on the epithelial cohesion structure. We observed that after 48 h of TNF-α stimulation, most cells had a rounder shape, and some of them appeared as drawn-out cells with long extensions. Moreover, we detected an abnormal aggregated distribution of filamentous cytokeratin. These observations are consistent with the loss of adhesion previously suggested by the decreased expression of cadherin and catenins. Moreover, we noted increased cytoplasmic expression of E-cadherin and β-catenin, even though membrane immunostaining of these two molecules remained present. This suggests a redistribution of the molecule induced by an inflammatory situation.

Adrenal glucocorticoids, cortisol and corticosterone, have important physiologic actions on growth, development, the immune system, and inflammatory processes. Dexamethasone and other powerful synthetic glucocorticoids are in widespread clinical use to treat other disease states (33). Glucocorticoids are the most potent and effective agents for controlling chronic inflammatory diseases. Glucocorticoids exert their effects by interaction of their specific glucocorticoid receptor with transcription factors such as activator protein 1 and NF-κB (34, 35).

In our study, we confirmed that dexamethasone effectively inhibited the effect induced by TNF-α on inflammatory cytokine release (IL-8 and RANTES). Dexamethasone also restored the level of adhesion molecules, which confirms its antiinflammatory effects. Moreover, E-cadherin and β-catenin immunoreactivity became membra-

nous again, suggesting that the antiinflammatory effect of dexamethasone could lead to epithelial recohesion (24, 25). This was confirmed by our observation that most cells took on a polygonal shape again with a cohesive structure after the addition of dexamethasone.

Moreover, the time-course distribution of β-catenin indicated strong β-catenin nuclear staining at 6 h, which disappeared after 24 and 48 h. Karin and colleagues showed that brief glucocorticoid inhibition of NF-κB led to short-term β-catenin stabilization in the cytoplasm (36). Then β-catenin can translocate to the nucleus and form a complex with transcription factors of the (Lef)/Tcf family (37, 38). Based on several observations in our study, it could be hypothesized that dexamethasone, which decreases NF-κB by potentially inhibiting IkB-α degradation, may lead to β-catenin translocation and then interfere in the balance of adhesion complexes formed by E-cadherin and catenins (39).

Epithelial cells perform multiple functions related to the modulation of local inflammation, wound healing, differentiation, and establishment of polarity requiring interaction of their cell surface molecules (40).

Our observations highlight possible mechanisms involved in inducing airway epithelial cell damage or repair. Inflammatory mediators involved in the asthmatic pathologic process, such as TNF-α, likely interfere with epithelial wound repair mechanisms (41), and antiinflammatory agents, such as dexamethasone, are involved in the epithelial healing mechanism.

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References

1. Holgate, S. T., D. E. Davies, P. M. Lackie, S. J. Wilson, S. M. Puddicombe, and J. L. Lordan. 2000. Epithelial-mesenchymal interactions in the pathogenesis of asthma. *J. Allergy Clin. Immunol.* 105:193–204.
2. Campbell, A., A. Vignola, P. Chanez, I. Couret, F. B. Michel, J. Bousquet, and P. H. Godard. 1992. Functional assessment of viability of epithelial cells: comparison of viability and mediator release in healthy subjects and asthmatics. *Chest* 101:25S–27S.
3. Jeffery, P. K. 1992. Pathology of asthma. *Br. Med. Bull.* 48:23–39.
4. Rennard, S. I., D. J. Romberger, R. A. Robbins, and J. R. Spurzem. 1995. Is asthma an epithelial disease? *Chest* 107:127S–131S.
5. Holgate, S. T., P. M. Lackie, D. E. Davies, W. R. Roche, and A. F. Walls. 1999. The bronchial epithelium as a key regulator of airway inflammation and remodelling in asthma. *Clin. Exp. Allergy* 2:90–95.
6. Montefort, S., W. R. Roche, and S. T. Holgate. 1993. Bronchial epithelial shedding in asthmatics and non-asthmatics. *Respir. Med.* 87:9–11.
7. Gumbiner, B. M. 1996. Cell adhesion: the molecular basis of tissue architecture and morphogenesis. *Cell* 84:345–357.
8. Takeichi, M. 1995. Morphogenetic roles of classic cadherins. *Curr. Opin. Cell Biol.* 7:619–627.
9. Knudsen, K. A., A. P. Soler, K. R. Johnson, and M. J. Wheelock. 1995. Interaction of alpha-actinin with the cadherin/catenin cell-cell adhesion complex via alpha-catenin. *J. Cell Biol.* 130:67–77.
10. Kemler, R. 1993. From cadherins to catenins: cytoplasmic protein interactions and regulation of cell adhesion. *Trends Genet.* 9:317–321.
11. Aberle, H., H. Schwartz, and R. Kemler. 1996. Cadherin-catenin complex: protein interactions and their implications for cadherin function. *J. Cell. Biochem.* 61:514–523.
12. Barth, A. I., I. S. Nathke, and W. J. Nelson. 1997. Cadherins, catenins and APC protein: interplay between cytoskeletal complexes and signaling pathways. *Curr. Opin. Cell Biol.* 9:683–690.
13. Willert, K., and R. Nusse. 1998. Beta-catenin: a key mediator of Wnt signaling. *J. Biol. Chem.* 273:95–102.
14. Aberle, H., A. Bauer, J. Stappert, A. Kispert, and R. Kemler. 1997. beta-catenin is a target for the ubiquitin-proteasome pathway. *EMBO J.* 16:3797–3804.
15. Novak, A., and S. Dedhar. 1999. Signaling through beta-catenin and Lef/Tcf. *Cell. Mol. Life Sci.* 56:523–537.
16. Sharpe, C., N. Lawrence, and A. Martinez Arias. 2001. Wnt signalling: a theme with nuclear variations. *Bioessays* 23:311–318.
17. Kanai, Y., T. Oda, Y. Shimoyama, A. Ochiai, T. Oyama, K. Yoshiura, S. Akimoto, T. Yamada, and S. Hirohashi. 1994. Alterations of the cadherin-catenin cell adhesion system in cancers. *Princess Takamatsu Symp.* 24:51–62.
18. Smythe, W. R., J. P. Williams, M. J. Wheelock, K. R. Johnson, L. R. Kaiser, and S. M. Albelda. 1999. Cadherin and catenin expression in normal human bronchial epithelium and non-small cell lung cancer. *Lung Cancer* 24:157–168.
19. Tabibzadeh, S., Q. F. Kong, S. Kapur, P. G. Satyaswaroop, and K. Aktories. 1995. Tumour necrosis factor-alpha-mediated dyscohesion of epithelial cells is associated with disordered expression of cadherin/beta-catenin and disassembly of actin filaments. *Hum. Reprod.* 10:994–1004.
20. Wojciak-Stothard, B., A. Entwistle, R. Garg, and A. J. Ridley. 1998. Regulation of TNF-alpha-induced reorganization of the actin cytoskeleton and cell-cell junctions by Rho, Rac, and Cdc42 in human endothelial cells. *J. Cell. Physiol.* 176:150–165.
21. Wu, A., Z. Chen, M. Tsokos, B. O'Connell, and I. Ambudkar. 1996. Interferon-g induced cell death in a cultured human salivary gland cell line. *J. Cell Physiol.* 167:297–304.
22. Hallsworth, M. P., C. P. Soh, S. J. Lane, J. P. Arm, and T. H. Lee. 1994. Selective enhancement of GM-CSF, TNF-alpha, IL-1 beta and IL-8 production by monocytes and macrophages of asthmatic subjects. *Eur. Respir. J.* 7:1096–1102.
23. Borish, L., J. J. Mascali, J. Dishuck, W. R. Beam, R. J. Martin, and L. J. Rosenwasser. 1992. Detection of alveolar macrophage-derived IL-1 beta in asthma: inhibition with corticosteroids. *J. Immunol.* 149:3078–3082.
24. Foty, R. A., S. A. Corbett, J. E. Schwarzbauer, and M. S. Steinberg. 1998. Dexamethasone up-regulates cadherin expression and cohesion of HT-1080 human fibrosarcoma cells. *Cancer Res.* 58:3586–3589.
25. Zettl, K. S., M. D. Sjaastad, P. M. Riskin, G. Parry, T. E. Machen, and G. L. Firestone. 1992. Glucocorticoid-induced formation of tight junctions in mouse mammary epithelial cells in vitro. *Proc. Natl. Acad. Sci. USA* 89:9069–9073.
26. Montefort, S., J. A. Roberts, R. Beasley, S. T. Holgate, and W. R. Roche. 1992. The site of disruption of the bronchial epithelium in asthmatic and non-asthmatic subjects. *Thorax* 47:499–503.
27. Perry, I., C. Tselepis, J. Hoyland, T. H. Iqbal, D. S. A. Sanders, B. T. Cooper, and J. A. Z. Jankowski. 1999. Reduced cadherin/catenin complex expression in celiac disease can be reproduced in vitro by cytokine stimulation. *Lab. Invest.* 79:1489–1499.
28. Jakob, T., and M. C. Udey. 1998. Regulation of E-cadherin-mediated adhesion in Langerhans cell-like dendritic cells by inflammatory mediators that mobilize Langerhans cells in vivo. *J. Immunol.* 160:4067–4073.
29. Montefort, S., R. Djukanovic, S. T. Holgate, and W. R. Roche. 1993. Ciliated cell damage in the bronchial epithelium of asthmatics and non-asthmatics. *Clin. Exp. Allergy* 23:185–189.
30. Kwon, O. J., B. T. Au, P. D. Collins, J. N. Baraniuk, I. M. Adcock, K. F. Chung, and P. J. Barnes. 1994. Inhibition of interleukin-8 expression by dexamethasone in human cultured airway epithelial cells. *Immunology* 81:389–394.
31. Stellato, C., L. A. Beck, G. A. Gorgone, D. Proud, T. J. Schall, S. J. Ono, L. M. Lichtenstein, and R. P. Schleimer. 1995. Expression of the chemokine RANTES by a human bronchial epithelial cell line: modulation by cytokines and glucocorticoids. *J. Immunol.* 155:410–418.
32. Barnes, P., and M. Karin. 1997. Nuclear factor-kappaB- a pivotal transcription factor in chronic inflammatory diseases. *N. Engl. J. Med.* 336c:1066–1071.
33. Cole, T. J., J. A. Blendy, A. P. Monaghan, W. Schmid, A. Aguzzi, and G. Schutz. 1995. Molecular genetic analysis of glucocorticoid signaling during mouse development. *Steroids* 60:93–96.
34. Wissink, S., E. C. van Heerde, B. vand der Burg, and P. T. van der Saag. 1998. A dual mechanism mediates repression of NF-kappaB activity by glucocorticoids. *Mol. Endocrinol.* 12:355–363.
35. Pfeilschifter, J., and H. Muhl. 1999. Immunopharmacology: anti-inflammatory therapy targeting transcription factors. *Eur. J. Pharmacol.* 375:237–245.
36. Karin, M., and Y. BenNeriah. 2000. Phosphorylation meets ubiquitination: the control of NF-kappa B activity. *Annu. Rev. Immunol.* 18:621–623.
37. Polakis, P. 2000. Wnt signaling and cancer. *Genes Dev.* 14:1837–1851.
38. Rubinfeld, B., I. Albert, E. Porfiri, S. Munemitsu, and P. Polakis. 1997. Loss of beta-catenin regulation by the APC tumor suppressor protein correlates with loss of structure due to common somatic mutations of the gene. *Cancer Res.* 57:4624–4630.
39. Fagotto, F., N. Funayama, U. Gluck, and B. Gumbiner. 1996. Bindings to cadherins antagonizes the signaling activity of b-catenin during axis formation in xenopus. *J. Cell Biol.* 132:1105–1114.
40. Rennard, S. I. 1996. Repair mechanisms in asthma. *J. Allergy Clin. Immunol.* 98:S278–S286.
41. Laitinen, A., E. M. Karjalainen, A. Altraja, and L. A. Laitinen. 2000. Histopathologic features of early and progressive asthma. *J. Allergy Clin. Immunol.* 105:S509–S513.