

Caveolin-1 Controls Airway Epithelial Barrier Function

Implications for Asthma

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The molecular basis for airway epithelial fragility in asthma has remained unclear. We investigated whether the loss of caveolin-1, the major component of caveolae and a known stabilizer of adherens junctions, contributes to epithelial barrier dysfunction in asthma. We studied the expression of caveolin-1 and adhesion molecules E-cadherin and β -catenin in airway sections, and we cultured bronchial epithelial cells from patients with asthma and from healthy control subjects. To determine the functional role of caveolin-1, we investigated the effects of caveolin-1 up-regulation and down-regulation on E-cadherin expression, barrier function, and proallergic activity in the human bronchial epithelial cell lines 16HBE and BEAS-2B. The membrane expression of caveolin-1 was significantly lower in airway epithelia from patients with asthma than from subjects without asthma, and this lower expression was maintained *in vitro* upon air-liquid interface and submerged culturing. Importantly, reduced caveolin-1 expression was accompanied by a loss of junctional E-cadherin and β -catenin expression, disrupted epithelial barrier function, and increased levels of the proallergic cytokine thymic stromal lymphopoietin (TSLP). Furthermore, E-cadherin redistribution upon exposure to epidermal growth factor or house dust mite was paralleled by the internalization of caveolin-1 in 16HBE cells. These effects appear to be causally related, because the short, interfering RNA down-regulation of caveolin-1 resulted in the delocalization of E-cadherin and barrier dysfunction in 16HBE cells. Moreover, caveolin-1 overexpression improved barrier function and reduced TSLP expression in BEAS-2B cells. Together, our data demonstrate a crucial role for caveolin-1 in epithelial cell-cell adhesion, with important consequences for epithelial barrier function and the promotion of Th2 responses in asthma.

Keywords: asthma; caveolin-1; E-cadherin; epithelial barrier; thymic stromal lymphopoietin

Allergic asthma is characterized by allergen-induced airway inflammation, hyperresponsiveness, and remodeling. The airway epithelium forms the first structural barrier against environmental insults, including deposited aeroallergens, and plays an important role in the initiation of allergic airway inflammation and remodeling.

(Received in original form March 15, 2013 and in final form May 15, 2013)

This study was supported by the Dutch Royal Academy of Arts and Sciences and by grants from the Canadian Lung Association and the Klosterfrau Foundation (T.-L.H.). T.-L.H. is a recipient of salary awards from the Canadian Institutes of Health Research/Canadian Lung Association/GlaxoSmithKline, Integrated and Mentored Pulmonary and Cardiovascular Training strategic training, the Michael Smith Foundation for Health Research, and the Parker B. Francis Foundation.

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This article has an online supplement, which is accessible from this issue's table of contents at www.atsjournals.org

Am J Respir Cell Mol Biol Vol 49, Iss. 4, pp 662–671, Oct 2013

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Originally Published in Press as DOI: 10.1165/rcmb.2013-0124OC on June 6, 2013

Internet address: www.atsjournals.org

CLINICAL RELEVANCE

We show that caveolin-1 expression is crucial for the maintenance of airway epithelial barrier function, that caveolin-1 expression is reduced in airway epithelial junctions from patients with asthma, and that the loss of caveolin-1 expression is accompanied not only by altered structure, but also by an increased Th2-promoting activity of airway epithelium. Together, our findings may open avenues to improved therapeutic strategies directed toward the maintenance and/or restoration of the epithelial barrier and its function in asthma.

Intercellular adhesions form the structural basis of epithelial integrity and include tight junctions, adherens junctions (AJs), and desmosomes. E-cadherin mechanically connects adjacent cells by forming AJs, initiating the formation of other cell-cell contacts (1–3). Many aeroallergens, including house dust mite (HDM), are known to cause epithelial damage (4, 5), and we previously reported that HDM induces epithelial barrier dysfunction and disrupts E-cadherin-mediated cell-cell adhesion (6, 7). Airway epithelial integrity is often compromised in asthma, as demonstrated by increased permeability to allergens, the detachment of ciliated cells, decreased E-cadherin expression, disrupted tight junctions (8–10), and the increased expression of the repair marker epidermal growth factor (EGF) receptor (EGFR) (11–14). In addition, *in vitro* air-liquid interface (ALI)-differentiated bronchial epithelial cells from patients with asthma display disrupted tight junctions (15) and reduced E-cadherin expression, compared with healthy control subjects (10, 16), indicating that the epithelium in patients with asthma is intrinsically unable to reconstitute intercellular adhesions. A role for the aberrant differentiation of asthmatic airway epithelium is supported by increased numbers of cells expressing the basal-cell markers cytokeratin-5 and p63 (17). Of importance, these intrinsic phenotypic changes are accompanied by increased proinflammatory cytokine secretion upon exposure to environmental insults (17). We previously observed that the down-regulation of E-cadherin results in an increased expression of proallergic cytokines (2), indicating that a loss of epithelial integrity promotes airway inflammation. Biopsy studies in children demonstrated a damaged epithelial phenotype before a definitive diagnosis of asthma, further suggesting that epithelial changes may be causal and not merely secondary to the development of asthma (18). Therefore, the need is urgent for novel therapeutic strategies directed toward the maintenance and/or restoration of epithelial barrier function.

We aimed to improve insights regarding the molecular basis for the loss of E-cadherin-mediated cell-cell contacts in asthma. Caveolae, which are microdomains rich in the scaffolding protein caveolin-1, are thought to stabilize AJs (19–22). Recent data indicated that caveolin-1 expression is reduced in the airways of patients with asthma and in the lungs of *Aspergillus*

TABLE 1. SUBJECT CHARACTERISTICS: LUNG TISSUE

Sex	Age (yr)	Ethnicity	Cause of Death*	Medical History	Known Medications	Subbasement Membrane Fibrosis
Female	42	White	Head trauma	None	None	None
Male	22	White	Head trauma	None	None	None
Male	23	White	Head trauma	None	None	None
Male	18	White	Head trauma	None	None	None
Male	24	White	Head trauma	None	None	None
Female	47	White	Head trauma	None	None	None
Male	36	White	Anoxia/asthma	Asthma diagnosis at age 10 yr	Albuterol, prednisone	Yes
Male	11	White	Anoxia/asthma	Asthma diagnosis at age 2 yr	Albuterol	Yes
Female	21	White	Tylenol overdose	Asthma diagnosis, cervical cancer	Albuterol, salmeterol/fluticasone	Yes
Female	15	White	Anoxia/asthma	Environmental allergies and asthma diagnosis	Albuterol, salmeterol/fluticasone, prednisone	Yes
Male	26	White	Anoxia and brain injury	Asthma diagnosed in childhood	Albuterol	Yes
Male	23	White	Asthma	Asthma diagnosis at age 3 yr	Albuterol	Yes

* Donor deaths are primarily attributable to head trauma in patients without asthma. Five of six patients with asthma are thought to have died during exacerbations of their asthma.

fumigatus-challenged mice (23). Therefore, we hypothesized that caveolin-1 internalization contributes to the disruption of E-cadherin-mediated cell-cell contacts and dysregulated barrier immunity in the airway epithelium of patients with asthma. We studied whether caveolin-1 expression is reduced in asthmatic-derived airway epithelium, and whether alterations in caveolin-1 membrane expression are accompanied by changes in E-cadherin expression and barrier function in airway epithelium.

MATERIALS AND METHODS

Subjects and Cell Culture

Deidentified asthmatic ($n = 6$) and nonasthmatic ($n = 6$) donor lungs not suitable for transplantation and donated for medical research were obtained through the International Institute for the Advancement of Medicine (Edison, NJ). Primary bronchial epithelial cells (PBECs) were isolated from these donors by protease digestion, as previously described (24). In addition, bronchial brushings were obtained from individuals with mild asthma ($n = 5$) and from healthy individuals ($n = 6$). Subject characteristics are listed in Tables 1 and 2. PBECs were cultured in hormonally supplemented bronchial epithelium growth medium containing bovine pituitary extract, triiodothyronine, epinephrine, hydrocortisone, and retinoic acid (BEGM; Lonza, Walkersville, MD) (25–27), and ALI cultures were grown as previously described (24). Transepithelial resistance (TER) was measured using a voltohmmeter (EVOM; World Precision Instruments, Sarasota, FL). This study was approved by the Research Ethics Board of the University of British Columbia, and by the Medical Ethics Committee of the University Medical Center of Groningen. All subjects provided written, informed consent.

16HBE cells were kindly provided by Dr. D. C. Gruenert (University of California at San Francisco, San Francisco, CA), and were cultured in Eagle's Minimum Essential medium/10% FCS, as described elsewhere (2). BEAS-2B cells were grown in RPMI medium/10% FCS, as described elsewhere (3).

Immunofluorescent Staining

Cells grown on LabTeks (Nunc; Thermo Fisher Scientific, Waltham, MA) were washed with PBS/CaCl₂, fixed in ice-cold acetone (90%) for 30 minutes, and blocked in PBS/5% BSA for 60 minutes. ALI cultures and sections of airway from the same patients were deparaffinized as previously described (24). Cells and sections were stained with primary antibodies (1:200) against E-cadherin, caveolin-1 (Santa Cruz Biotechnology, Santa Cruz, CA), or β -catenin (BD Biosciences, Mississauga, ON, Canada), secondary goat-anti-mouse IgG Alexa Fluor 488 or goat-anti-rabbit IgG Alexa Fluor 594 (Invitrogen, Grand Island, NY), and 4'6-diamidino-2-phenylindole (1 μ g/ml). Images were acquired and analyzed as described elsewhere (24).

Electrical Cell-Substrate Impedance Sensing

Cells were grown on electrode arrays, and adhesion measurements were based on changes in resistance and capacitance to current flow

applied at 400 Hz and 40 kHz, using electrical cell-substrate impedance sensing (ECIS; Applied Biophysics, Troy, NY), as described elsewhere (3, 28).

Immunoblotting

Western blotting and immunodetection were performed using rabbit anti-E-cadherin and mouse anti-caveolin antibodies, with anti- β -actin as loading control (Santa Cruz Biotechnology) (29).

Quantitative PCR

RNA was isolated, cDNA was synthesized with the iScript cDNA Synthesis Kit (Bio-Rad, Hercules, CA), and gene expression was analyzed using TaqMan (Applied Biosystems, Foster City, CA)-validated probes for caveolin-1, thymic stromal lymphopoietin (TSLP), and the housekeeping genes β_2 -microglobulin ($\beta_2\mu$ G) and peptidylprolyl isomerase A, according to the manufacturer's guidelines.

Short, Interfering RNA and Plasmid Transfection

Cells were seeded at 5×10^4 cells/well and transfected with either E-cadherin, caveolin-1, or scrambled short, interfering RNA (siRNA; 20 μ M), full-length caveolin-1 cDNA overexpression plasmid (catalogue number SC119082; Origene, Rockville, MD), or empty pCMV-XL5 vector (final concentration, 250 ng plasmid), using Lipofectamine 2000 (Invitrogen) and cultured for 3 days, as described elsewhere (2, 6).

TABLE 2. SUBJECT CHARACTERISTICS: BRONCHIAL BRUSHINGS

Subjects*	Asthma [†] ($n = 5$)	Control ($n = 6$)
Age (yr)	42 (33–50)	33 (21–49)
Sex (male, %)	3 (60)	3 (50)
Pack-yr	0.7 (0–4)	0 (0–0)
FEV ₁ (% predicted)	93 (76–114)	107 (101–122)
FEV ₁ /FVC (%)	72 (58–78)	79 (75–94)

Definition of abbreviations: AMP, adenosine monophosphate; FEV₁ (% predicted), forced expiratory volume during the first second as percentage of predicted value; FVC, forced vital capacity; PC₂₀, the lowest concentration of AMP which caused $\geq 20\%$ fall in FEV₁.

Values are presented as median (range) or as number (%).

* Subjects were all nonsmokers (≤ 10 pack-yr, no smoking in the last yr), free of other lung diseases, and they did not use inhaled corticosteroids during the 4 weeks preceding the study.

[†] Patients with asthma were included on the basis of presence of allergy (assessed by skin test or Phadiatop; Thermo Scientific), FEV₁ > 80% predicted, and bronchial hyperresponsiveness (assessed by PC₂₀ AMP < 16 mg/ml, PC₂₀ methacholine < 8 mg/ml, or PC₂₀ histamine < 8 mg/ml).

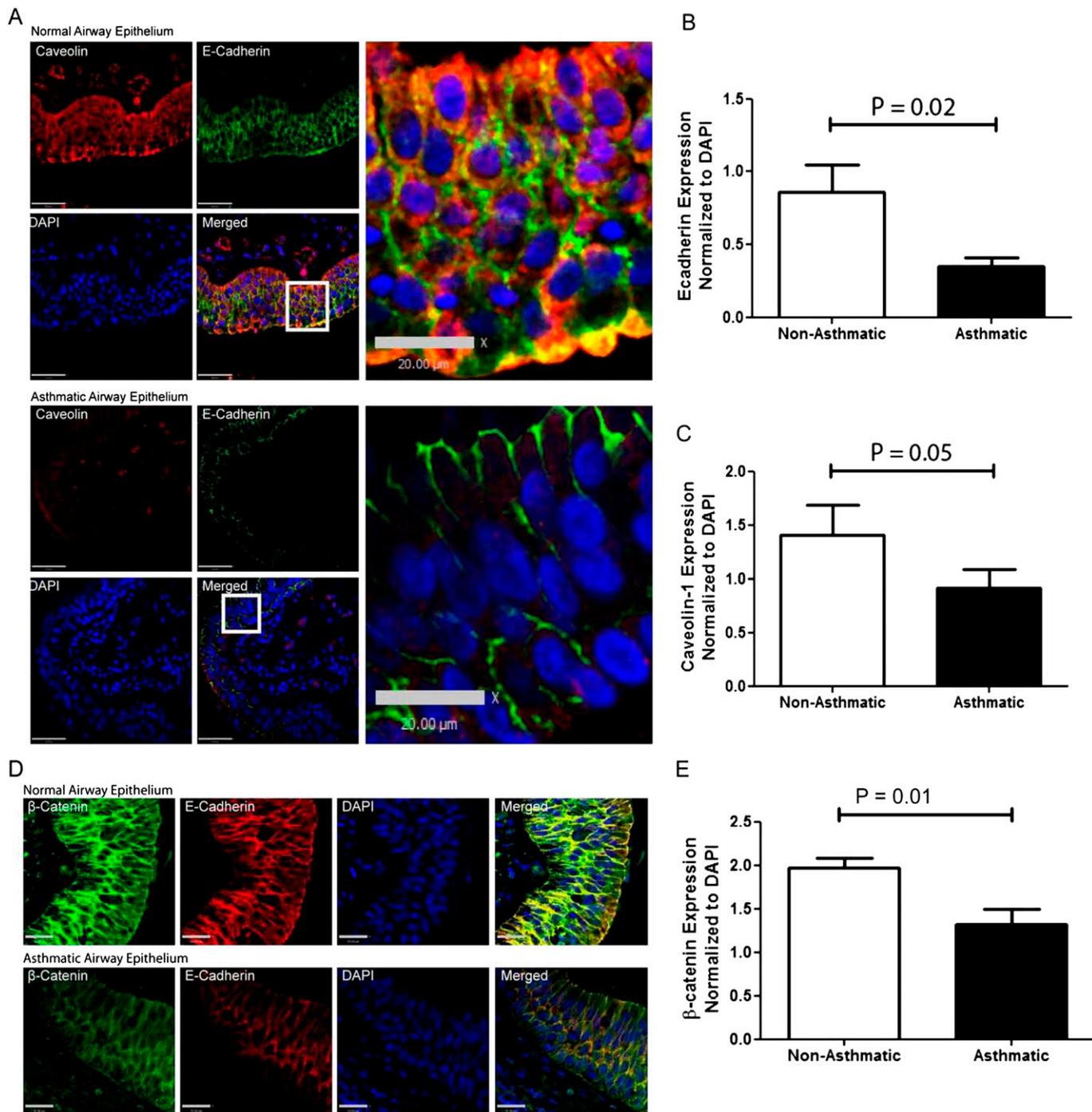


Figure 1. Reduced epithelial E-cadherin, caveolin-1, and β -catenin expression in asthmatic airways. Airway sections from subjects with asthma ($n = 6$) and from subjects without asthma ($n = 6$) were stained for (A) 4'-diamidino-2-phenylindole (DAPI; blue), E-cadherin (green), and caveolin-1 (red), and for (D) β -catenin (green) and E-cadherin (red). (B, C, and E) E-cadherin, caveolin-1, and β -catenin levels were quantified and normalized to DAPI to correct for cell numbers, and are presented as percent positive staining/unit length \pm SEM. Representative images are shown. Boxed areas in A are what is zoomed-in in the right panel. Scale bars = 100 μ m.

ELISA

TSLP, IL-33, IL-6, and IL-8 were measured in cell-free supernatants according to the manufacturer's guidelines (R&D Systems, Abingdon, UK).

Statistical Analysis

Differences between groups were analyzed according to the Mann-Whitney test or two-way ANOVA (time curves), and differences between paired data were analyzed according to the Student *t* test.

RESULTS

Aberrant Expression of E-Cadherin, β -Catenin, and Caveolin-1 in Asthmatic Epithelium

We first studied whether the reduced expression of E-cadherin in airway epithelia from patients with asthma is accompanied by a loss of membrane caveolin-1. Similar to our previous findings (17), lung sections from patients with severe asthma displayed a reduced bronchial epithelial expression of E-cadherin,

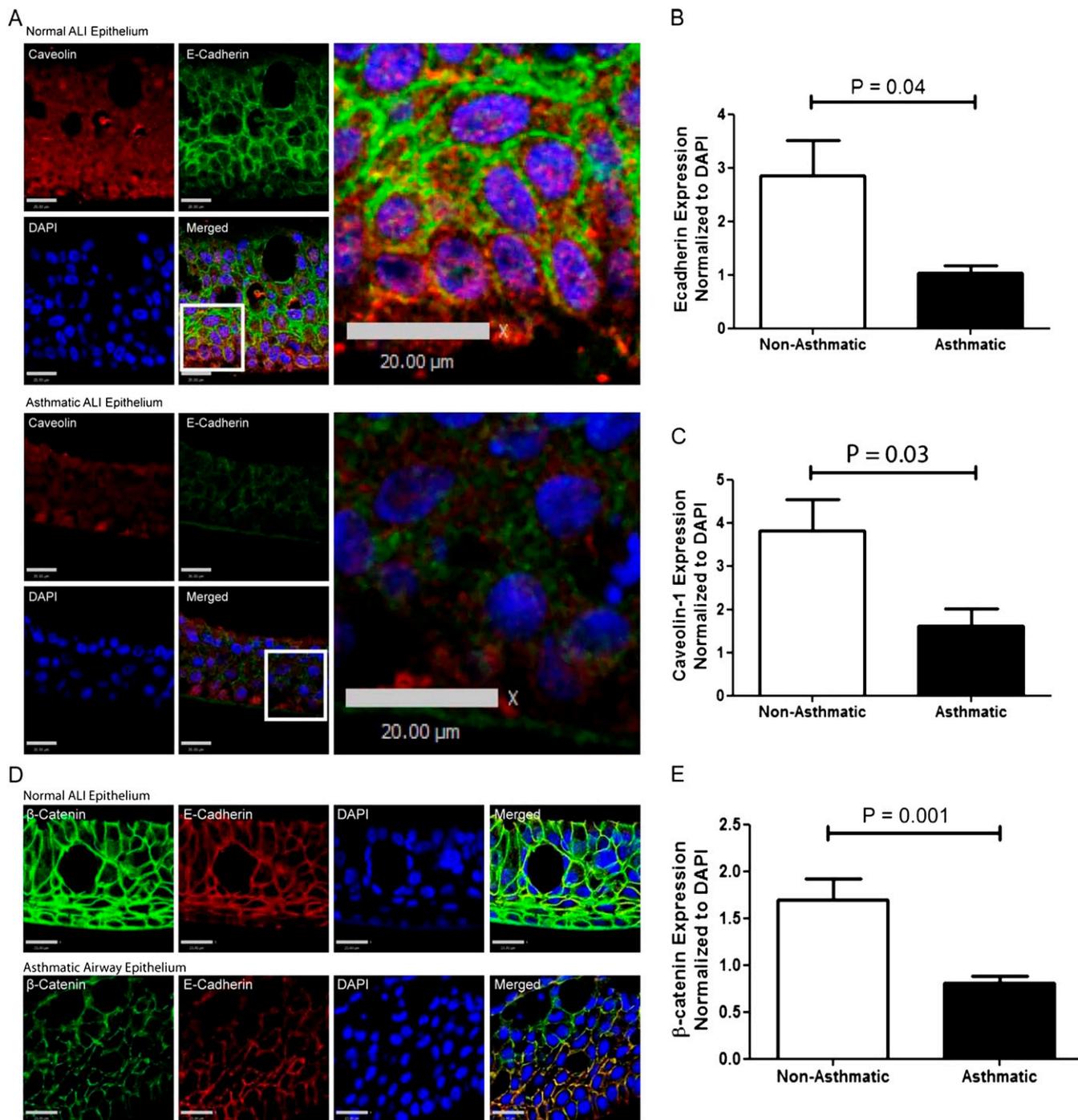


Figure 2. Reduced epithelial E-cadherin, caveolin-1, and β -catenin expression in asthmatic air-liquid interface (ALI) cultures. Airway epithelial-cell ALI cultures from subjects with asthma ($n = 6$) and from subjects without asthma ($n = 6$) were stained for (A) E-cadherin (green) and caveolin-1 (red), and for (D) β -catenin (green) and E-cadherin (red). (B, C, and E) E-cadherin, caveolin-1, and β -catenin levels were quantified and normalized to DAPI to correct for cell numbers, and are presented as percent positive staining/unit length \pm SEM. Boxed areas in A are what is zoomed-in in the right panel. Representative images are shown. Scale bars = 100 μ m.

as shown by immunofluorescence images (Figure 1A) and analysis (Figure 1B). Strikingly, caveolin-1 expression was significantly lower in the epithelia of patients with asthma compared with control subjects without asthma, with a loss of membrane expression, especially at sites where E-cadherin staining was absent (Figures 1A and 1C). In support of a role for caveolin-1 in the stabilization of AJs via the membrane recruitment of β -catenin, we observed that the reduced membrane expression of E-cadherin was accompanied by reduced junctional β -catenin in epithelia derived from patients

with asthma, compared with patients without asthma (Figures 1D and 1E).

Of note, these epithelial changes in asthmatic airway tissue appeared to be intrinsic, because cultured PBECs from the same patients with asthma displayed similar defects in E-cadherin (Figures 2A and 2B) and caveolin-1 expression (Figures 2A and 2C) upon differentiation under ALI conditions. In addition, the observed decrease in β -catenin expression in asthmatic compared with nonasthmatic airway tissue was maintained in asthma-derived ALI cultures (Figures 2D and 2E).

Epithelial Barrier Dysfunction and Aberrant Expression of Caveolin-1 in Asthmatic Epithelium

Next, we studied whether the defects in E-cadherin and caveolin-1 expression of asthmatic epithelia resulted in reduced barrier function. In line with our previous findings (17), we did not observe a significant difference in TER between asthma-derived and control ALI cultures (Figure 3A). The use of ECIS enables a more sensitive and accurate monitoring of epithelial resistance, with the ability to distinguish between cell-cell and cell-matrix contacts (3, 28), but does not allow for measurements in ALI cultures on transwell membranes. Therefore, we used submerged cultures of PBECs obtained by bronchial brushings of patients with asthma and healthy control subjects. PBECs demonstrated a growth curve typical for primary cells (3). A further increase in epithelial resistance was observed upon the removal of growth factors/hormones in PBECs from healthy control subjects, but not from patients with asthma, demonstrating significantly lower resistance levels (Figure 3B). This difference was observed

for low-frequency resistance, but not high-frequency capacitance (data not shown), indicating the reinforcement of cell-cell contacts (3), potentially as a consequence of further cell differentiation/polarization upon the deprivation of factors that keep cells in a proliferative state. This was accompanied by lower levels of caveolin-1 in PBECs from patients with asthma than from control subjects (Figure 3C), accentuating that reduced epithelial caveolin-1 expression is also a feature of mild-to-moderate asthma. In contrast to protein expression, no significant differences in caveolin-1 mRNA expression were observed (Figure 3D), suggesting post-transcriptional/post-translational alterations.

Internalization of Caveolin-1 and Redistribution of E-Cadherin upon EGF Stimulation in 16HBE Cells

To underscore further the role of caveolin-1 in the loss of E-cadherin-mediated cell-cell adhesion and barrier dysfunction in asthma, we performed mechanistic studies in human bronchial epithelial cell lines. We first studied whether a loss of membrane

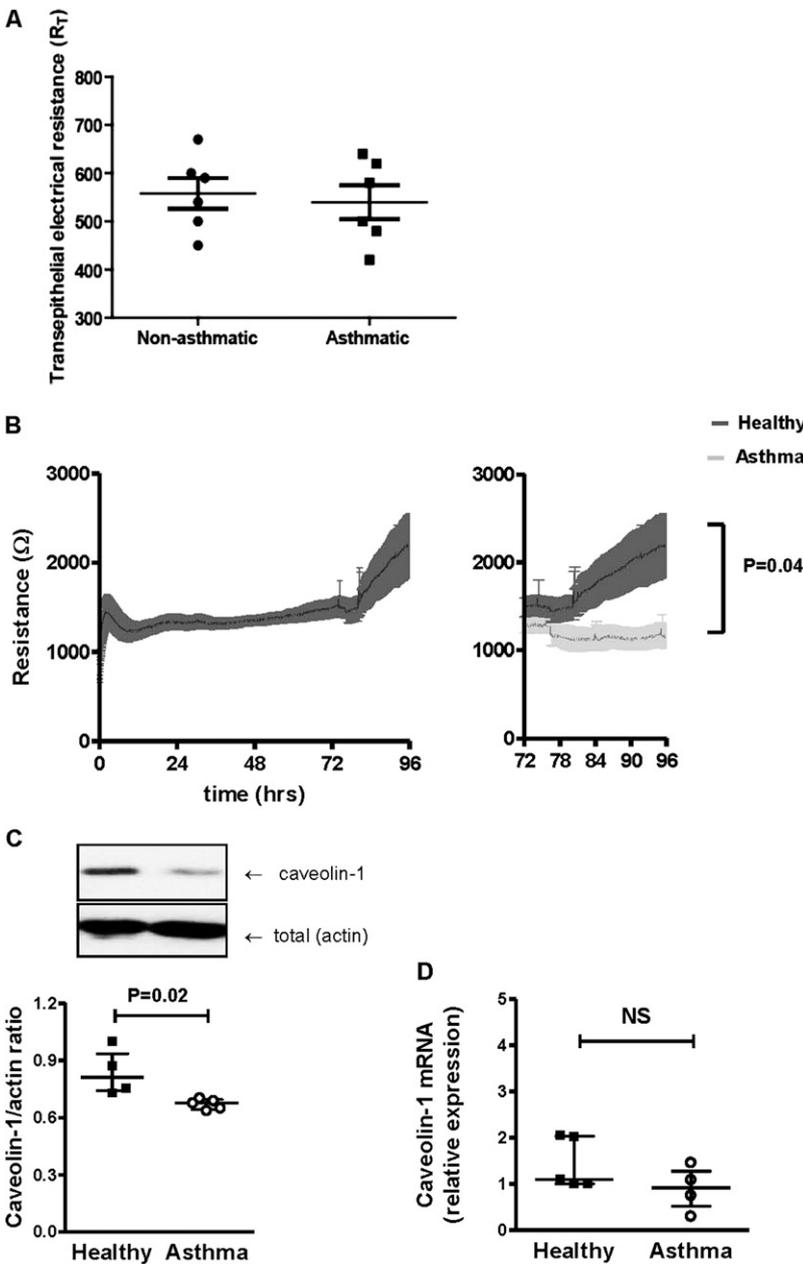


Figure 3. Reduced barrier function and caveolin-1 expression in submerged cultured asthma primary bronchial epithelial cells (PBECs). (A) Transepithelial resistance was measured in airway epithelial-cell ALI cultures from subjects with asthma ($n = 6$) and from subjects without asthma ($n = 6$). R_T , transepithelial resistance. (B) Cells of six healthy and five asthmatic donors were seeded in duplicate, grown on electrical cell-substrate impedance sensing (ECIS) arrays for 3 days, and placed in hormone-deprived medium for 24 hours. Resistance (mean \pm SEM) was measured by ECIS. Cells were seeded in duplicates, grown in 24-well plates for 3 days, and placed in hormone-deprived medium for 24 hours. Caveolin-1 was detected by (C) Western blotting, related to actin, or (D) quantitative PCR, related to house-keeping genes and expressed as fold change, compared with one of the healthy cultures ($2^{-\Delta\Delta Ct}$). Medians \pm interquartile ranges (IQRs) are shown. * $P < 0.05$. NS, no significance.

caveolin-1 was paralleled by the redistribution of E-cadherin stimulation in 16HBE cells, which display high endogenous levels of caveolin-1 and E-cadherin and tight barrier function (3). Because HDM-induced E-cadherin redistribution is mediated by EGFR, we exposed 16HBE cells to EGF (for 60 min), which indeed resulted in the internalization of caveolin-1 (Figure 4), as well as the disrupted expression of E-cadherin, especially at sites where caveolin-1 staining was profoundly cytoplasmic (Figure 4). The involvement of an EGFR-induced loss of membrane-localized caveolin-1 in HDM-induced barrier dysfunction was further supported by the observation that HDM and EGF induced a similar effect on caveolin-1 expression (Figure 4).

Down-Regulation of Caveolin-1 Results in Disrupted E-Cadherin Expression in 16HBE Cells

To assess whether the junctional loss of E-cadherin and barrier dysfunction are indeed consequences of reduced caveolin-1 membrane expression, we studied the effects of siRNA down-regulation of caveolin-1 in 16HBE cells. The down-regulation of E-cadherin by siRNA treatment did not affect the expression of caveolin-1, indicating that reduced E-cadherin expression is not causal in the loss of caveolin-1 expression (Figure 5A). In turn, the siRNA down-regulation of caveolin-1, which was optimal between 2 and 4 days upon transfection (Figure 5A), resulted in a redistribution of E-cadherin, with a similar pattern as observed upon EGF

stimulation (Figure 5B). Importantly, the down-regulation of caveolin-1 also impaired the ability of 16HBE cells to form cell-cell contacts, as reflected by reduced epithelial resistance (Figure 5C). In contrast, the siRNA down-regulation of caveolin-1 did not affect epithelial high-frequency capacitance (Figure 5C), the most sensitive parameter for changes in cell-matrix attachments. Moreover, we tested the effects of caveolin-1 overexpression on epithelial barrier function in the human bronchial epithelial cell line BEAS-2B, because these cells demonstrate a low endogenous expression of cell-cell adhesion molecules and a low capacity to form cell-cell contacts (3). The overexpression of caveolin-1 modestly but significantly increased low-frequency resistance at 24 hours (but not after prolonged periods) after transfection (Figures 5E and 5F). Again, no effects were observed on high-frequency capacitance (data not shown), indicating caveolin-1 affects cell-cell rather than cell-matrix adhesion.

Caveolin-1 Expression Is Related to Proallergic Epithelial Activity

To determine whether the loss of E-cadherin and caveolin-1 expression is accompanied by a proinflammatory response of the airway epithelium, ALI cultures from the asthmatic and nonasthmatic patients of Figure 2 were analyzed for TSLP, IL-33, IL-6, and IL-8 secretion. Asthma-derived ALI cultures released higher levels of TSLP and IL-33 at baseline compared with

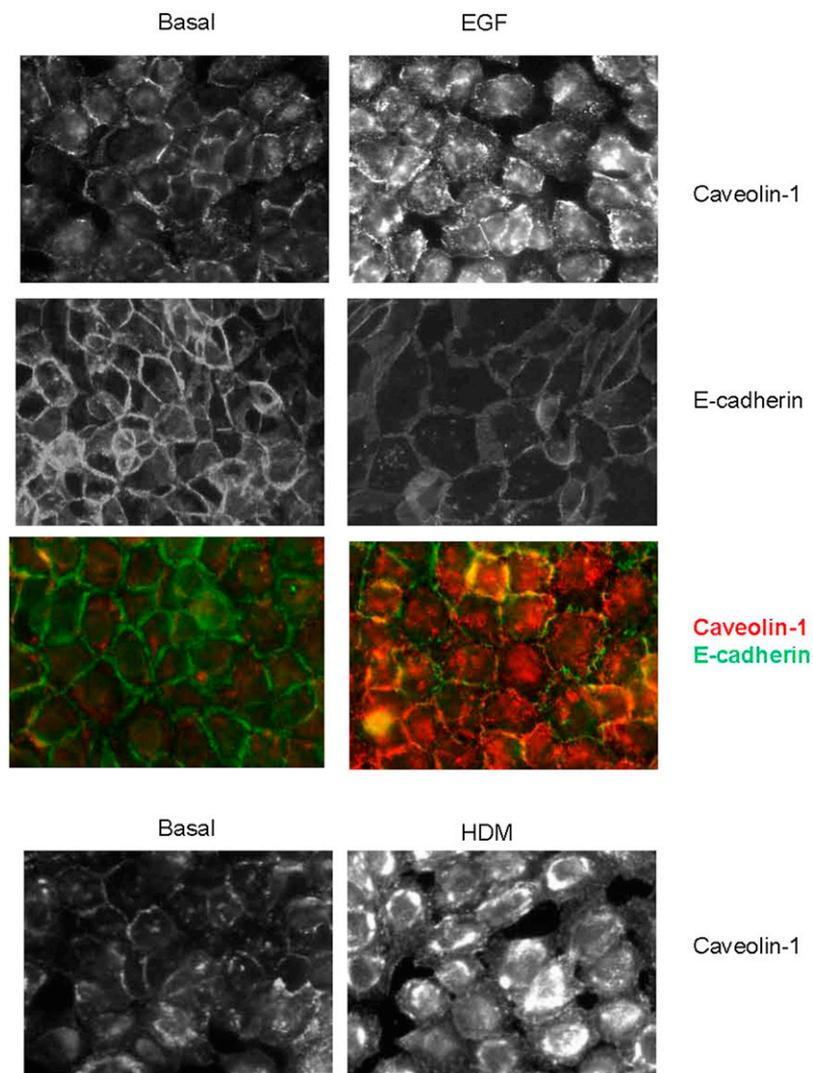


Figure 4. House dust mite (HDM)-induced and epidermal growth factor (EGF)-induced delocalization of E-cadherin is paralleled by caveolin-1 internalization in 16HBE cells. 16HBE cells were grown to confluence on coverslips, serum-deprived overnight, and subsequently exposed for 2 hours to EGF (10 ng/ml) or HDM (50 μ g/ml), as indicated. Caveolin-1 (red) and E-cadherin (green) were detected by immunofluorescent staining.

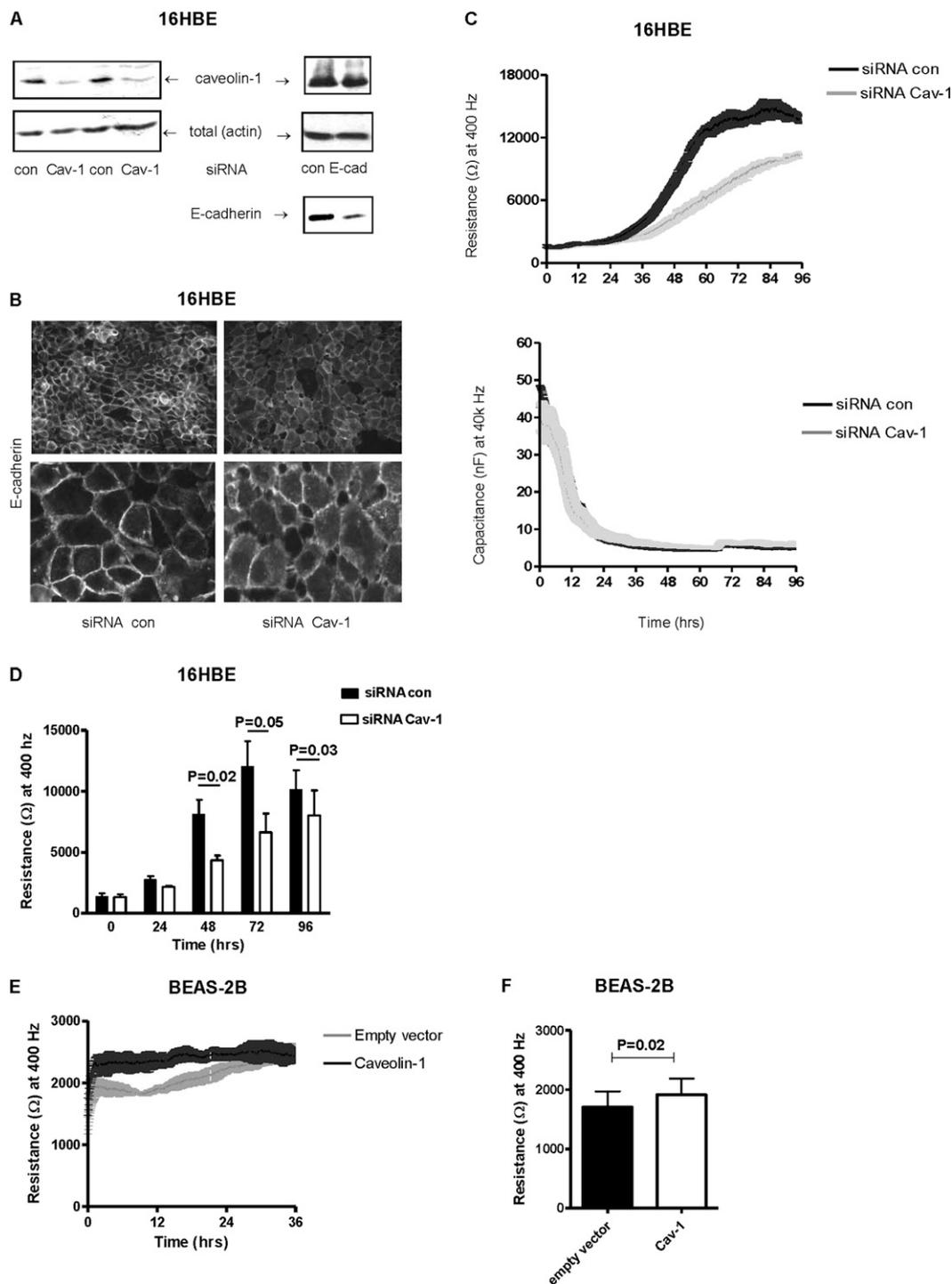


Figure 5. Caveolin-1 expression regulates resistance and E-cadherin distribution in human bronchial epithelia. Cells were seeded in duplicate. 16HBE cells were transfected with control (con), caveolin-1 (Cav-1), or E-cadherin (E-cad) short, interfering RNA (siRNA), BEAS-2B cells with empty vector, or caveolin-1 construct for 3 days. Caveolin-1 and/or E-cadherin were detected by (A) Western blotting or (B) immunofluorescent staining. (C–E) Resistance values (means \pm SEMs, $n = 3$ –4). Representative images of 3–4 independent experiments are shown.

cultures from subjects without asthma, with maintained epithelial integrity (Figures 6A and 6B). As observed previously (17), pleiotropic inflammatory cytokines such as IL-6 and IL-8 were not significantly different between asthmatic and nonasthmatic cultures (Figures 6C and 6D), suggesting specificity for Th2-promoting cytokines. To confirm that caveolin-1 expression regulates TSLP production, we tested the effects of caveolin-1 siRNA knockdown and overexpression on TSLP mRNA expression in BEAS-2B cells, with their low endogenous expression of cell-cell adhesion molecules. We observed that an approximately 70% knockdown of caveolin-1 expression resulted in a modest, nonsignificant increase in TSLP expression, whereas caveolin-1

overexpression induced a significant reduction in TSLP expression (Figures 6E and 6F), but not IL-6 and IL-8 expression (data not shown). This is in line with a regulatory role for caveolin-1/E-cadherin in TSLP expression.

DISCUSSION

We show for the first time that reduced caveolin-1 expression is related to the loss of E-cadherin-mediated cell-cell adhesion in airway epithelia from subjects without asthma. This appears to be a causal relationship, because the siRNA down-regulation of caveolin-1 results in decreased electrical resistance in 16HBE

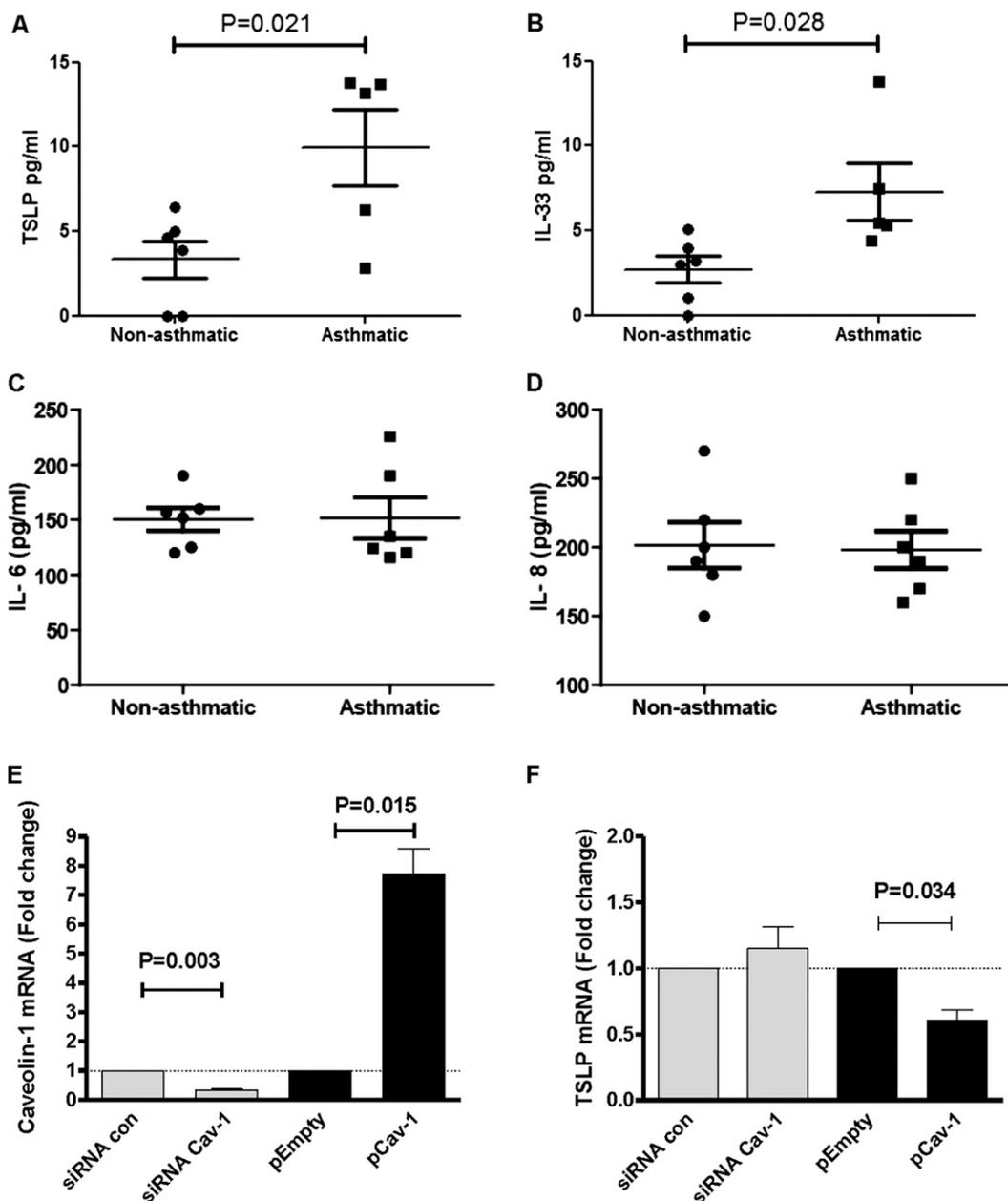


Figure 6. Thymic stromal lymphopoietin (TSLP) and IL-33 levels, but not IL-6 and IL-8 levels, are increased in asthmatic epithelia, and TSLP is down-regulated by caveolin-1 overexpression in BEAS-2B cells. (A) TSLP, (B) IL-33, (C) IL-6, and (D) IL-8 levels (medians \pm IQRs) were determined in basolateral supernatants from nonasthmatic and asthmatic epithelial-cell ALI cultures. (E) Caveolin-1 and (F) TSLP mRNA expression was determined in BEAS-2B cells transfected with empty vector (pEmpty), caveolin-1 construct (pCav-1), control (con) siRNA, and caveolin-1 (Cav-1) siRNA, detected by quantitative PCR and related to housekeeping genes, and expressed as fold change (mean \pm SEM, $n = 3$).

cells and reduced junctional E-cadherin expression, which we have previously shown to cause barrier dysfunction in 16HBE cells (2). This indicates that caveolin-1 may be a key contributor to epithelial barrier dysfunction in asthma. This may have important consequences, because a loss of airway epithelial barrier function may not only facilitate the access of allergens to submucosal allergen-presenting cells, but also enhance the Th2-promoting responses of the airway epithelium (2, 17). Accordingly, we demonstrate that asthma-derived ALI cultures, displaying delocalized E-cadherin and caveolin-1 expression, produce elevated levels of Th2-promoting cytokines TSLP and IL-33, but not inflammatory modulators IL-6 and IL-8. The role of caveolin-1 in Th2-mediated airway inflammation is further supported by the finding that caveolin-1 overexpression reduces TSLP expression in BEAS-2B cells, which is in line with our previous results demonstrating that E-cadherin regulates TSLP expression in bronchial epithelium (2).

We have previously shown that E-cadherin expression is reduced in asthmatic epithelia (17). Here, we show for the first time

that reduced E-cadherin expression is accompanied by a reduction in caveolin-1 expression in the airway epithelia of patients with asthma, suggesting that the loss of caveolin-1 may be involved in the disrupted expression of E-cadherin at the cell membrane. In line with our findings, Bains and colleagues recently reported lower caveolin-1 expression in airway-wall biopsies from patients with asthma than from control subjects (23), although Bains and colleagues did not link caveolin-1 expression to E-cadherin-mediated cell-cell contacts and airway epithelial integrity. Our findings further enhance our understanding about the functionality of caveolin-1. We observed that the reduced expression of caveolin-1 in PBECs from subjects with asthma is accompanied by lower electrical resistance than in healthy control subjects, as measured by ECIS. However, we did not detect differences in TER between ALI cultures derived from subjects with asthma and those derived from healthy control subjects, as demonstrated by Xiao and colleagues (15), which may be attributable to the limited sensitivity to changes in cell-cell contacts using the conventional TER method.

In addition to the regulation of barrier function and immune responses (1), the loss of E-cadherin may promote airway remodeling through enhanced epithelial plasticity (14). A role for caveolin-1 in the altered epithelial structure in asthma is supported by the increase in allergen-induced airway remodeling in a mouse model of asthma upon the down-regulation of caveolin-1 (34). We recently identified epithelial plasticity as a potential process underlying allergen-induced airway remodeling in asthma (6, 24, 35). Changes in epithelial plasticity and the induction of the epithelial-to-mesenchymal transition (EMT) in human tumor cells overexpressing EGFR involves the EGF-induced loss of caveolin-1 (31) by its internalization and recruitment to endosomes (30–33), whereas caveolin-1 expression suppresses the EMT in pancreatic cancer cells (36). Our data suggest that this suppressive role of caveolin-1 in epithelial plasticity is mediated by promoting the localization of E-cadherin and β -catenin in AJs, limiting free cytosolic β -catenin levels, the translocation of β -catenin to the nucleus, and its transcriptional activation (37). Our data further indicate that HDM-induced signaling through the EGFR (3, 6, 7) also leads to the EGFR-dependent internalization of caveolin-1, with a subsequent redistribution of E-cadherin *in vitro*. Furthermore, our data support the role of caveolin-1 in the membrane recruitment of the E-cadherin/ β -catenin complex and the stabilization of cell–cell adhesion, rather than an active role for caveolin-1 in E-cadherin endocytosis (31). E-cadherin and caveolin-1 did not colocalize in the cytoplasm, although cytoplasmic caveolin-1 was accompanied by the disrupted membrane expression of E-cadherin. The disrupted membrane expression of E-cadherin upon the siRNA down-regulation of caveolin-1 in 16HBE cells further confirms the role of caveolin-1 in the stabilization of epithelial cell–cell contacts. We anticipate that upon its internalization, the degradation of caveolin-1 by the ubiquitin–proteasomal pathway may be responsible for the reduction in its total levels in epithelial cells from patients with asthma, compared with patients without asthma. We did not observe differences in caveolin-1 mRNA expression between PBECs from asthmatic and control donors, suggesting that the changes in caveolin-1 expression in asthma are at the protein level.

Increased EGFR expression has been reported in asthma (13), and a cytosine adenine-repeat polymorphism in the EGFR gene has been associated with both the presence and severity of asthma (38). Thus, genetic variability could, at least in part, be responsible for the endocytosis of caveolin-1 in asthma epithelia, that is, as a consequence of enhanced EGFR activity in response to repeated exposures to environmental allergens such as HDM. Similarly, enhanced EGFR activity in asthma-derived epithelia can occur *in vitro* upon exposure to the EGF present in the growth medium. We propose that long-term EGF exposure may contribute to the reduced expression of caveolin-1 observed in the epithelia of patients with asthma and the concomitant loss of E-cadherin/ β -catenin complexes from AJs. Our present and previous data suggest that blocking EGFR receptor activity to prevent caveolin-1 down-regulation and the subsequent disruption of E-cadherin-mediated cell–cell adhesion may constitute a novel strategy for reinforcing epithelial barrier function. We previously observed that EGFR activation by its ligand EGF reduces airway epithelial barrier function, an effect most likely mediated by the disruption of cell–cell adhesion, but not cell–matrix adhesion (39). Furthermore, EGF impaired the recovery of cell–cell contacts upon wounding by electroporation, whereas EGF promoted epithelial migration during the initial phase of the wounding response (7). Accordingly, a beneficial role for EGF has been proposed in epithelial repair. Xiao and colleagues recently demonstrated

protective effects of long-term EGF treatment on bronchial epithelial integrity in epithelial cells cultured at ALIs derived from asthmatic but not healthy individuals (15). In view of these data, specific therapeutic strategies to prevent caveolin-1 internalization and promote the (membrane) expression of caveolin-1 to improve the airway epithelial barrier in asthma may be more promising than strategies to block EGFR activity.

In conclusion, our data show that caveolin-1 membrane expression acts to stabilize E-cadherin and β -catenin at AJs to maintain epithelial barrier function. Furthermore, our data provide supportive evidence that the loss of membrane caveolin-1 in airway epithelia underlies the loss of E-cadherin-mediated cell–cell contacts, barrier dysfunction, and Th2-mediated airway inflammation in asthma. Targeting caveolin-1 may therefore constitute a new therapeutic strategy to improve mucosal barrier function and ameliorate disease severity in asthma.

Author disclosures are available with the text of this article at www.atsjournals.org.

Acknowledgments: The authors thank N. M. Kliphuis for performing the resistance measurements in primary bronchial epithelial cells.

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