Histamine alters E-cadherin cell adhesion to increase human airway epithelial permeability

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Histamine alters E-cadherin cell adhesion to increase human airway epithelial permeability. J Appl Physiol 95: 394–401, 2003; 10.1152/japplphysiol.01134.2002.—During the immediate response to an inhaled allergen, there is an increase in the paracellular permeability of the airway epithelium. Histamine is an important agonist released during the immediate response to inhaled allergen. We hypothesized that histamine would increase human airway epithelial paracellular permeability and that it would do this by interrupting E-cadherin-based cell adhesion. Histamine, applied to the basolateral surface, increased the paracellular permeability of cultured human airway epithelium, and this effect of histamine was blocked by the histamine receptor antagonist promethazine. ECV304 cells express a histamine receptor, N-cadherin, and elements of the tight junction, including claudins, but they do not express E-cadherin. Histamine increased the paracellular permeability of ECV304 cells transfected with a vector and expressing E-cadherin but not ECV304 cells expressing lac-Z in the same vector. L cells do not express the histamine receptor, cadherins, or claudins. Histamine decreased adhesion of L cells expressing the human histamine receptor and E-cadherin to an E-cadherin-Fc fusion protein. Histamine did not alter the adhesion to the E-cadherin fusion protein of L cells expressing either the histamine receptor or E-cadherin alone. When applied to the apical surface, adenovirus poorly infects airway epithelial cells because its receptor, CAR, is restricted to the basolateral surface of the cells. When histamine was applied to the basolateral surface of airway epithelial cells, infection of the cells by adenovirus increased by approximately one log. This effect of histamine was also blocked by promethazine. Histamine increases airway paracellular permeability and increases susceptibility of airway epithelial cells to infection by adenovirus by interrupting E-cadherin adhesion.

THE AIRWAY EPITHELIUM is a barrier to the environment, a regulator of the content of airway surface liquid, and a source of cytokines and other products that regulate airway physiology and protect the airways and more distal lung from injury and infection. In sensitized humans and laboratory animals, an acute antigen challenge causes an increase in the presence of plasma proteins in fluid recovered from the airways and an increase in the movement of macromolecules from the airways into the plasma (3, 9, 17). The interpretation of these observations has been that acute antigen challenge increases the permeability of both bronchial microvascular and epithelial barriers.

Recently, Goto et al. (11) more precisely documented increased airway epithelial permeability to macromolecules after antigen challenge in sensitized guinea pigs. In their study, less E-cadherin was present on the basolateral membrane of the airway epithelial cells during the late phase of the response to the inhaled allergen. They detected fragments of E-cadherin in fluid recovered from the airways during the late phase and hypothesized that the loss of E-cadherin from the cell surfaces reflected proteolytic cleavage. In contrast to the changes in the amount of E-cadherin present on the airway epithelial cell surfaces during the late phase of the response to antigen challenge, Goto et al. detected no change in the amount of E-cadherin on the airway epithelial cells during the immediate response. However, despite the persistence of E-cadherin on the cells’ surface, there was an increase in airway epithelial permeability to macromolecules during the immediate response that was similar to the increase in permeability seen during the late response. They offered no explanation for this increase in epithelial permeability during the immediate response to the allergen.

Several agents relevant to the sensitized state alter the permeability characteristics of epithelia, including proteinases in house dust mite feces, oxidants and peroxidases from inflammatory cells, cationic proteins, histamine, and methacholine (3, 4, 19, 23). Histamine, released from sensitized mast cells in the microenvironment of the airway epithelium, participates in the immediate response to inhaled allergen and increases airway permeability in laboratory animals (3). We previously observed that histamine interrupts VE-cadherin-based adhesion of endothelium (22). Based on these observations, we hypothesized that histamine

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would increase airway epithelial permeability and alter E-cadherin-based epithelial cell-cell adhesion.

To test this hypothesis, we conducted a series of experiments on different types of cells. First, we confirmed that histamine, acting through the H1 receptor, decreased the transepithelial resistance of differentiated human airway epithelium. To determine whether histamine affected the transepithelial resistance through a mechanism dependent on E-cadherin, we examined the effects of histamine on the transepithelial resistance of monolayers of ECV304 cells. ECV304 cells express the H1 receptor and form tight junctions but do not normally express E-cadherin. We examined the change in the transepithelial resistance of monolayers of mock-transfected ECV304 cells and ECV304 cells transfected with E-cadherin and found that histamine only decreased the transepithelial resistance of ECV304 cells expressing E-cadherin.

In the ECV304 cells, the effects of histamine might have represented an effect of histamine on an interaction of E-cadherin with the tight junction, or they might reflect an effect of histamine directly on E-cadherin-based adhesion. To directly examine E-cadherin-based adhesion, we transfected L cells with the histamine receptor and E-cadherin, neither of which is normally expressed by L cells. We then examined adhesion of the L cells transfected with the H1 receptor and E-cadherin to an E-cadherin-Fc fusion protein, before and after the cells were exposed to histamine. Histamine decreased the adhesion of L cells expressing both the H1 receptor and E-cadherin, but histamine did not affect the adhesion of L cells expressing either E-cadherin or the H1 receptor alone.

The effect of histamine on the airway transepithelial resistance was brief. We examined the susceptibility of airway epithelium to infection with adenovirus from the apical compartment to determine whether the effect of histamine on the epithelial barrier was significant enough to affect epithelial function. Because the receptor for adenovirus, CAR, is restricted to the basolateral membrane of airway epithelium, adenoviral infection of airway epithelium from the apical surface is very inefficient. If histamine caused a significant disruption of airway epithelial resistance, it might enhance the efficiency of adenoviral infection of the epithelium from the apical surface. The results of our investigations indicate that histamine decreases the transepithelial resistance of airway epithelium by decreasing E-cadherin-based adhesion and that this has significant functional effects.

**METHODS**

**Materials.** Fibronectin was from Collaborative Research (Bedford, MA). Tissue culture media and serum were from the Tissue Culture Core, University of Iowa. ECV304 cells and L cells were from American Type Culture Collection (Manassas, VA). The pLKneo plasmid, the E-cadherin cDNA, and the E-cadherin-human Fc fusion protein cDNA were a generous gift from W. James Nelson. Antibody to E-cadherin (rr1, mouse monoclonal) was from the Hybridomas Studies Bank (University of Iowa). Anti-ZO-1 antibody was from Transduction Laboratories (Lexington, KY). Secondary antibody was sheep anti-mouse IgG, conjugated with horseradish peroxidase from Amersham. Fura 2 was from Molecular Probes (Eugene, OR). This study was approved by University of Iowa Institution Review Board (no. 9507432).

**Cell culture.** Airway epithelial cells were isolated from bronchial tissue obtained from six normal people and four people with cystic fibrosis (CF). Cells were seeded onto collagen-coated, semipermeable membranes (0.6 cm² Millipore; HA; Millipore, Bedford, MA) and grown at the air-liquid interface as previously described (14). Culture medium, a 1:1 mixture of DMEM and Ham’s F-12 medium, was supplemented with 2% Ultroser G (BioSepra, Villeneuve, France) and initially with 100 μM penicillin, 100 μg/ml streptomycin, 50 μg/ml gentamicin, 15 μg/ml colchicine, 125 μg/ml ceftazidime, and 2 μg/ml fusazolone. Basolateral culture medium was changed every 2–4 days. All epithelia were studied at least 14 days after seeding when they had differentiated. Samples of the epithelia were evaluated with scanning electron microscopy for the development of a ciliated apical surface.

ECV304 cells were grown in M199 supplemented with 10% FBS, basal medium Eagle’s vitamins and amino acids, glucose (5 mM), glutamine (2 mM), penicillin (100 μg/ml), and streptomycin (100 μg/ml). L cells were grown in DMEM with 10% FBS, penicillin (100 μg/ml), and streptomycin (100 μg/ml).

ECV304 or L cells transfected with E-cadherin in the vector pLKneo were grown as above with the addition of G418 (1 mg/ml) for selection. L cells expressing E-cadherin in pLKneo and the human histamine receptor (H1) in pcDNA 3.1 were grown as above with the addition of G418 (1.4 mg/ml) and zeocin (1 mg/ml) for selection. When zeocin was present, penicillin and streptomycin were eliminated.

Human embryonal kidney (HEK) 293 cells containing a cDNA for the E-cadherin-human Fc fusion protein were grown in DMEM with 10% FBS and hygromycin (200 μg/ml) as described by Chen and Nelson (7).

**Measurement of transepithelial electrical properties.** For measurement of transepithelial electrical properties, epithelia were mounted in Ussing chambers and studied as previously described (14). Epithelia were bathed in apical and basolateral solutions containing (in mM) 135 NaCl, 2.4 K₃H₂PO₄, 0.6 KH₂PO₄, 1.2 CaCl₂, 1.2 MgCl₂, 10 dextrose, and 5 HEPES, at pH 7.2, 37°C, and gassed with 100% O₂. Transepithelial resistance was measured before and after application of histamine by applying a 2-mV spike every 5 s and measuring the inverse of the electrical conductance. To independently assess the effect of histamine on the paracellular component of transepithelial resistance, we first blocked the epithelial sodium channel of non-CF airway epithelia with 10 μM amiloride and non-CFTR chloride channels with 10 μM DIDS. This completely blocked the transient increase in current seen in non-CF epithelia after the addition of histamine.

**Plasmid preparation and transfection.** A cDNA for the human histamine receptor was developed with primers based on the sequence published by Fukui et al. (10).

RNA was made from ECV304 cells by using RNA Stat (Tel-Test). ECV304 cDNA was made with the oligo(dt) primers in the Advantage RT for PCR kit (Clontech Laboratories). A PCR reaction was set up to amplify the human histamine (H1) receptor using the cDNA from the above RT reaction and the oligonucleotides 5'-ATAACTGGCGGCTGCTTGGG-3' and 5'-ATCCCCAGAGCCTCCCTTAG-3' corresponding to the NH₂-terminal and COOH-terminal regions of the gene. The pFX platinum PCR kit (Invitrogen)
was used. The product was run on a 1% agarose gel and then purified with β-agarose I (New England Biolabs). The purified PCR product was then ligated into pCR-Blunt (Invitrogen). The directionality of human histamine receptor within pCR-Blunt was determined by restriction analysis. The human histamine receptor sequence was removed from the pCR-Blunt vector and ligated into pcDNA 3.1 (zeo) at the HindIII and XbaI sites. Sequence and directionality were verified by sequencing with the T7 promoter primer and the bioassayable growth hormone reverse primer.

L cells were transfected with this plasmid by using Lipofectamine Plus per the manufacturer’s directions (Invitrogen). Transfected cells were grown in DMEM with 10% FBS and selected with zeocin (1 mg/ml). Clones were isolated on the basis of an increase in cell calcium in response to histamine (see below).

E-cadherin, in the vector pLKneo, was used in ECV304 cells as previously described (22).

L cells expressing the histamine receptor in the vector pcDNA 3.1 zeo were transfected with the E-cadherin-pLKneo plasmid by using Lipofectamine Plus. Doubly transfected cells were available after 1 week at 1 mg/ml (C418 1.4 mg/ml). Clones were isolated on the basis of surface expression [fluorescence-activated cell sorting (FACS)] of E-cadherin.

HEK 293 cells transfected with a cDNA for the E-cadherin-human Fc fusion protein were grown as described by Chen and Nelson (7).

Analysis of protein expression. Protein was solubilized from E-cadherin-transfected cells with SDS sample buffer, and equal masses of cell proteins were separated on 8% PAGE gels, transferred to PVDF membranes in 25 mM Tris, and 150 mM NaCl, and equal masses of cell proteins were separated on 8% PAGE gels, transferred to PVDF membranes in 25 mM Tris, and 150 mM NaCl, and then incubated with primary antibody for 1 h at room temperature with rr1 (conditioned medium diluted 1:10 in PBS-EGTA). After two rinses with PBS containing 0.5 mM EGTA, HBSCGA (in mM: 135 NaCl, 1.2 CaCl2, 1.2 MgCl2, 10 HEPES, 10 glucose, and 0.05% bovine serum albumin, pH 7.4 with KOH) was added to the wells and allowed to bind overnight at 5°C (PBS-EGTA alone is added to control wells). In experiments with rr1, the wells were rinsed three times with PBS-EGTA and incubated 2 h at room temperature with rr1 (conditioned media diluted 1:10 in PBS-EGTA). After two rinses with PBS containing 0.5 mM EGTA, HBSCGA containing calcine-AM (5 ng/ml) and incubating for 30 min at 37°C. The cells were then rinsed with PBS, harvested (10-min incubation in 137 mM NaCl, 4.2 mM NaHCO3, 5.4 mM KCl, 5.6 mM glucose, and 0.5 mM EDTA (lifting solution)). The cells were suspended in PBS, centrifuged at 150 g for 5 min, resuspended, and rotated in PBS containing primary antibody for 1 h at 27°C, washed twice with PBS, and resuspended in PBS containing sheep anti-mouse IgG conjugated to FITC. Cells were rotated every 1 h at 27°C, resuspended in PBS with propidium iodide, and then analyzed for surface expression of E-cadherin by FACS.

To detect surface expression of E-cadherin-transfected and nontransfected cells, cells were grown as indicated above. Cells expressing E-cadherin in the dexamethasone responsive vector pLKneo were cultured with the addition of dexamethasone (1 μM) for 18 h, cultured without dexamethasone for 24 h, washed in PBS, and released from the plates in 137 mM NaCl, 4.2 mM NaHCO3, 5.4 mM KCl, 5.6 mM glucose, and 0.5 mM EDTA (lifting solution). The cells were suspended in PBS, centrifuged at 150 g for 5 min, resuspended, and rotated in PBS containing primary antibody for 1 h at 27°C, washed twice with PBS, and resuspended in PBS containing sheep anti-mouse IgG conjugated to FITC. Cells were rotated every 1 h at 27°C, resuspended in PBS with propidium iodide, and then analyzed for surface expression of E-cadherin by FACS.

A reliable antibody for the human histamine receptor is not available. As a surrogate indicator of surface expression of the histamine receptor, transfected L cells were loaded with fura 2 and examined for an increase in cell calcium in response to histamine, as previously described (22).

β-Galactosidase activity was measured with a commercial β-galactosidase method (Galacto-Light, Tropix, Bedford, MA) as previously described (20).

Preparation of E-cadherin-Fc fusion protein. HEK 293 cells transfected with the cDNA for E-cadherin-Fc in pcDM8 were grown in MEM with immunoglobulin-depleted FBS for 48 h. The conditioned medium was collected, and the fusion protein was isolated by affinity chromatography on a protein A-Sepharose column. The fusion protein was eluted from the column with citric acid (pH 3.0) and identified as a single band running at a molecular mass of 112 kDa on a Coomassie-stained PAGE gel and by Western blotting with rr1 (anti-E-cadherin).

Cell impedance measurements. Cell-cell adhesion of ECV304 cells was assayed by measuring the impedance of a cell-covered electrode and comparing it with the impedance of a cell-free electrode as described (22). The total impedance was partitioned into its three components: the cell-cell resistance, the cell-matrix resistance, and the membrane capacitance as described. To do this, we measured the impedance of the cell-covered electrode at three different frequencies (1, 4, and 16 kHz) every second. For each of these time points, we used the downhill simplex method to determine the set of parameters (cell-cell resistance, cell matrix resistance, and membrane capacitance) that best fit the observed data (22). The software for data acquisition and analysis was written with LabVIEW (National Instruments, Austin, TX).

Cells were plated on the electrode at 1.5 × 106 cells per cm2. Twenty-four hours after they were plated, ECV304 cells and cells transfected with the pLKneo constructs were exposed to dexamethasone for 18 h followed by an additional 24 h in the absence of dexamethasone before being studied.

Adhesion of transfected L cells to cadherin-Fc. We adapted the adhesion assay used by Lilien, Balsamo, and colleagues (2) to measure cadherin-dependent adhesion to cadherin-Fc coated microtiter plate surfaces (2). Reacti- Bind Protein G-coated strip plates were rinsed three times with 0.05% Tween 20 in PBS. One hundred microliters of the cadherin-Fc fusion protein (30 μg/ml) in PBS with 0.5 mM EGTA (PBS-EGTA) were added to the wells and allowed to bind overnight at 5°C (PBS-EGTA alone is added to control wells). In experiments with rr1, the wells were rinsed three times with PBS-EGTA and incubated 2 h at room temperature with rr1 (conditioned media diluted 1:10 in PBS-EGTA). After two rinses with PBS containing 0.5 mM EGTA, HBSCGA (in mM: 135 NaCl, 1.2 CaCl2, 1.2 MgCl2, 10 HEPES, 10 glucose, and 0.05% bovine serum albumin, pH 7.4 with KOH) was added to the wells and incubated at room temperature for 1 h. After an additional rinse with HBSCGA, the wells were ready for the addition of cells.

Cells transfected with E-cadherin in the dexamethasone responsive vector pLKneo were seeded at 70% confluence in 60-mm tissue culture dishes. After 4 h, the medium was replaced with medium containing 1 μM dexamethasone. Two days later, the cells were labeled by replacing the medium with HBSSGA containing calcine-AM (5 ng/ml) and incubating for 30 min at 37°C. The cells were then rinsed with PBS, harvested (10-min incubation in 137 mM NaCl, 4.2 mM NaHCO3, 5.4 mM KCl, 5.6 mM glucose, and 0.5 mM EDTA, pH 7.2), pelleted by mild centrifugation, and resuspended in HBSCGA at a final concentration of 100,000 cells/ml.

Two hundred microliters of the cell suspension were added to each well and allowed to bind for 45 min at 37°C. Each eight-well strip was then individually treated with histamine for the indicated time, removed from the cassette, and rinsed three times with HBSCGA to remove nonadherent cells. The fluorescence remaining in each well was then measured (EG&G Wallac Victor2, Gaithersburg, MD) and used as an estimate of the relative number of adherent cells after background subtraction.

CAR-GFP localization in human airway epithelia. Well differentiated primary human airway epithelia were grown and infected with Ad-CAR-GFP as previously described (14, 21). Two days after infection, cells were permeabilized with
0.1% Triton X-100 and incubated with primary ZO-1 MAb (610967, Becton Dickinson Transduction Laboratories, Lexington, KY) followed by secondary goat anti-mouse Alexa 568 (A11019, Molecular Probes). Filters were cut out, mounted on glass slides, and coverslipped with Vectashield mounting media (Vector Laboratories, Burlingame, CA). Images were acquired with a Bio-Rad MRC-1024 laser scanning confocal microscope (Hercules, CA) mounted on a Nikon E600 microscope (Melville, NY) using a ×60 oil immersion lens.

Fig. 1. A: human airway epithelial cells were cultured at an air-liquid interface (14) and mounted in Ussing chambers to examine the effects of histamine on trans-epithelial electrical resistance ($R_t$) as described in the text. At the time indicated by the bar, different concentrations of histamine were added to the monolayers and changes in $R_t$ were recorded. Concentrations of histamine of $10^{-5}$ M and greater initiated a prompt and transient decrease in $R_t$. B: human airway epithelial cells were examined as in A except that some of the monolayers were pretreated with the H1 receptor antagonist, promethazine ($10^{-3}$ M). Promethazine itself caused a slow gradual increase in $R_t$ (data not shown) and completely blocked the effects of histamine ($10^{-4}$ M) on $R_t$. C: airway epithelial cells from subjects with cystic fibrosis (CF) and cultured at an air-liquid interface were exposed to histamine ($10^{-4}$ M). There was no current activated in the CF cells, indicating that the current activated in cells from normal subjects is dependent on the presence of CFTR. Similar to cells from normal subjects, histamine decreased the $R_t$ (increased amplitude of the current spike) across monolayers of CF cells. This is a representative sample of the response of CF epithelia.

Fig. 2. ECV304 cells (ECV) expressing lac-Z (A) or E-cadherin (C) in the pLKneo vector and Madin-Darby canine kidney (MDCK) cells, used as a positive control (B), were labeled with primary antibody to E-cadherin and fluorescein-tagged secondary antibody and analyzed by fluorescence-activated cell sorting (FACS). ECV304 cells expressing lac-Z in pLKneo did not demonstrate surface expression of E-cadherin. ECV304 cells expressing E-cadherin in pLKneo had surface expression of E-cadherin comparable to that of MDCK cells.
Infection of epithelial cells with adenovirus. Recombinant adenovirus expressing β-galactosidase, AdβGal, was prepared as described previously by the University of Iowa Gene Transfer Vector Core (21) at titers of \(10^{10}\) infectious units/ml. Airway epithelia were allowed to reach confluence and develop a transepithelial electrical resistance; all epithelia had transepithelial resistance values of \(>500\) Ω·cm². Fourteen days after seeding was completed, 50 multiplicity of infection of the recombinant viruses (in 10 μl of PBS) were added to the apical surface for 30 min. After 30 min, the viral suspension was removed, and the epithelia were rinsed twice with PBS. After infection, the epithelia were incubated at 37°C for an additional 48 h.

Transepithelial resistance was measured with an ohmmeter (EVOM, World Precision Instrument, Sarasota, FL) before and after infection, and transepithelial resistance was not altered by application of virus. To study the effect of histamine on the transepithelial barrier that prevents adenovirus access to the basolateral side where its receptor is localized, we treated the basolateral surface of the epithelia with 10⁻⁴ M histamine, 10⁻⁵ M promethazine, and the combination of promethazine and histamine.

Statistical analysis. Changes in transepithelial resistance, cell-cell impedance, cell adhesion, and cells infected with adenovirus were compared by ANOVA, and individual group comparisons were done by using a Tukey’s honestly significant difference test for post hoc comparisons of means. Differences were considered significant at the \(P < 0.05\) level.

RESULTS

Effects of histamine on differentiated human airway epithelium. When histamine was applied to the basolateral surface of differentiated human airway epithelium, it caused an immediate 20 ± 5.5% fall in transepithelial resistance that persisted for 3–5 min \((n = 38\) from six different donors) (Fig. 1A). In contrast, histamine applied to the apical surface of the epithelium had no effect in epithelia from four different donors who were among the total of six (data not shown). The H1 receptor antagonist, promethazine, prevented the effects of histamine and by itself caused a gradual 14.9 ± 1.87% increase in resistance over 10 min (Fig. 1B). The small transient increase in short-circuit current initiated by histamine could not explain the change in transepithelial resistance. Histamine did not initiate the increase in short-circuit current in CF airway epithelia, suggesting that it resulted from activation of CFTR. However, histamine did cause a 21.2 ±
2.1% transient decrease in transepithelial resistance of CF epithelia (n = 12 from four different donors) (Fig. 1C). This decrease in resistance was the same as that seen in non-CF epithelia.

Effects of histamine on monolayer resistance of cells transfected with E-cadherin. ECV304 cells express P- and N-cadherin but not E-cadherin. They form tight junctions and respond to histamine with an increase in cell calcium (22). ECV304 cells transfected with E-cadherin in the pLKneo vector expressed E-cadherin on the cell surface similar to surface expression of E-cadherin in Madin-Darby canine kidney cells (Fig. 2).

Histamine decreased the cell-cell resistance of a monolayer of ECV304 cells transfected with E-cadherin by 50 ± 5%, but histamine did not alter the resistance of a monolayer of ECV304 cells transfected with lac-Z in pLKneo (Fig. 3). Hence, the effects of histamine on the resistance created by the cell-cell junctions was dependent on the presence of E-cadherin.

Effects of histamine on adhesion of L cells transfected with E-cadherin and the histamine receptor. In ECV304 cells, E-cadherin and elements of the tight junction contribute to the electrical resistance of the monolayer. To determine whether histamine decreased the transepithelial resistance by altering the interaction of E-cadherin with elements of the tight junction or whether histamine directly affected E-cadherin-based adhesion, we examined the effects of histamine on the adhesion of L cells transfected with E-cadherin and the human histamine receptor to an E-cadherin-human-Fc fusion protein.

Fourfold more L cells expressing E-cadherin than L cells expressing just the histamine receptor adhered to the fusion protein, E-cadherin-Fc, bound to a protein A-coated microtiter plate (Fig. 4). Adhesion of the E-cadherin-expressing cells to the fusion protein was entirely blocked by preexposing the fusion protein to antibody to E-cadherin (Fig. 4).

Histamine increased cell calcium in L cells expressing the human histamine receptor with or without coexpression of E-cadherin but not L cells expressing only lac-Z in the pLKneo vector (Fig. 5).

Histamine decreased adhesion to E-cadherin-Fc of L cells expressing both E-cadherin and the histamine receptor but not L cells expressing either the histamine receptor or E-cadherin alone (Fig. 6). Hence, histamine directly interrupted E-cadherin homophilic adhesion.

Effects of histamine on infection of human airway epithelial cells with adenovirus. We investigated the possibility that the histamine-mediated transient disruption in transepithelial resistance would increase the ability of apically applied adenovirus to infect human airway epithelia through the basolateral side in a fiber/CAR-dependent mechanism. As previously described, CAR localizes below the tight junctions (Zho-1) and cannot be accessed by adenovirus applied from the apical side (21) (Fig. 7A). Human airway epithelia

Fig. 5. Histamine increased cell calcium in L cells expressing the human histamine receptor (L-H1) or the human histamine receptor and E-cadherin (L-H1-Ecad) but not in L cells alone.

Fig. 6. Histamine decreased adhesion to the E-cadherin-Fc fusion protein of L cells expressing both E-cadherin and the human histamine receptor (L-H1-Ecad) but not L cells expressing either E-cadherin (L-Ecad) or the histamine receptor (L-H1) alone.
expressed adenovirus-linked β-galactosidase activity at almost a log greater when the epithelia were treated with histamine from the basolateral side after application of adenovirus to the apical surface of the epithelia. Moreover, prior treatment of the epithelia with promethazine blocked the ability of histamine to increase apical infection with adenovirus (Fig. 7B).

**DISCUSSION**

The barrier created by the airway epithelium segregates the apical and basolateral compartments. This limits exposure of subapical tissues and receptors to proteins, chemicals, bacteria, and viruses. Hence, the epithelial barrier is an important determinant of how the airways respond to the environment.

Several earlier studies had documented that the integrity of the airway epithelial barrier was compromised during the response of the airways to an inhaled allergen, and histamine was identified as one of the agonists that contributed to these changes in epithelial permeability (3, 9, 11, 17). We examined whether histamine would alter the permeability of a differentiated airway epithelium in vitro. When airway epithelial cells are grown at the air-liquid interface, they differentiate into ciliated cells with a phenotype similar to in vivo airway epithelium (14). We found that histamine caused an almost immediate decrease in the transepithelial resistance of these differentiated airway epithelial cells, and the resistance spontaneously recovered to basal levels within 5–10 min. The decrease in resistance was blocked by the H1 receptor antagonist, promethazine. The dose-response curve was almost identical to what we previously detected in endothelial cells (6). Other agonists such as serotonin and leukotrienes are also released in the airway during the immediate response to inhaled allergen, but we do not know whether they would have effects similar to those of histamine.

E-cadherin is one of the classic cadherins and is essential for development and maintenance of epithelia (1, 12). E-cadherin mediates calcium-dependent, homophilic adhesion between adjacent cells as part of a complex of proteins including α-, β-, and p120-catenin that integrate the complex with the cortical actin cytoskeleton (13). E-cadherin-based adhesion is dependent on the levels of expression of E-cadherin (12). Adhesion may also be regulated by phosphorylation of elements of the complex and by IQGAP (8, 15).

We hypothesized that histamine altered the trans epithelial resistance of the airway epithelium by affecting E-cadherin-based adhesion. ECV304 cells express both endothelial and epithelial proteins but do not express E-cadherin (5, 18, 22). The resistance of a monolayer of ECV304 cells did not change when the cells were exposed to histamine, but the resistance of ECV304 cells expressing E-cadherin fell in response to histamine. This is consistent with histamine affecting E-cadherin-based adhesion. However, ECV304 cells also express claudin 2 by RT-PCR, and they develop tight junctions (Shasby laboratory, data not shown). Because E-cadherin interacts with tight junctions, the change in impedance could reflect a change in E-cadherin adhesion to E-cadherin on adjacent cells, or it could reflect a change in the interaction of E-cadherin with elements of the tight junction. To isolate the function of homophilic E-cadherin adhesion, we examined the adhesion of L cells expressing E-cadherin and the human histamine receptor to an E-cadherin-human Fe fusion protein adherent to the surface of protein A-coated microtiter plates. Histamine decreased the adhesion of L cells expressing E-cadherin and the histamine receptor but not the adhesion of L cells expressing either alone. Hence, histamine directly alters E-cadherin-based adhesion.

Histamine decreased the transepithelial resistance by ~20%, and the resistance recovered within 5–10 min. These changes are modest, and their potential significance is uncertain. To examine one potential implication, we asked whether this brief change in the epithelial barrier would affect the efficiency with which adenovirus infected airway epithelial cells. The adenoviral receptor, CAR, is restricted to the basolateral surface of airway epithelial cells, and infection of the cells by apical application of virus is very low (16, 20). However, when the cells were exposed to histamine, the infection of the airway epithelial cells by adenoviro...
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Histamine applied to the apical surface increased by almost one log. This is consistent with the changes in trans-epithelial resistance and demonstrates that histamine causes a functionally significant decrease in the tight junction and the effective barrier of the airway epithelium.

In summary, histamine alters E-cadherin-based adhesion of airway epithelial cells. This is a reversible process with no apparent cell toxicity. It exposes basolateral cell receptors to agents normally restricted to the apical surface and may be an important process that alters the response of the asthmatic airway to the environment.

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