PAR2 activation interrupts E-cadherin adhesion and compromises the airway epithelial barrier: protective effect of β-agonists

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Winter, Michael C., Sandra S. Shasby, Dana R. Ries, and D. Michael Shasby. PAR2 activation interrupts E-cadherin adhesion and compromises the airway epithelial barrier: protective effect of β-agonists. Am J Physiol Lung Cell Mol Physiol 291: L628–L635, 2006. First published May 19, 2006; doi:10.1152/ajplung.00046.2006.—The airway epithelium is an important barrier between the environment and subepithelial tissues. The epithelium is also divided into functionally restricted apical and basolateral domains, and this restriction is dependent on the elements of the barrier. The protease-activated receptor-2 (PAR2) receptor is expressed in airway epithelium, and its activation initiates multiple effects including enhanced airway inflammation and reactivity. We hypothesized that activation of PAR2 would interrupt E-cadherin adhesion and compromise the airway epithelial barrier. The PAR2-activating peptide (PAR2-AP, SLIGRL) caused an immediate ∼50% decrease in the transepithelial resistance of primary human airway epithelium that persisted for 6–10 min. The decrease in resistance was accompanied by an increase in mannitol flux across the epithelium and occurred in cystic fibrosis transmembrane conductance receptor (CFTR) epithelium pretreated with amiloride to block Na and Cl conductances, confirming that the decrease in resistance represented an increase in paracellular conductance. In parallel experiments, activation of PAR2 interrupted the adhesion of E-cadherin-expressing L cells and of primary airway epithelial cells to an immobilized E-cadherin extracellular domain, confirming the hypothesis that activation of PAR2 interrupts E-cadherin adhesion. Selective interruption of E-cadherin adhesion with antibody to E-cadherin decreased the transepithelial resistance of primary airway epithelium by >80%. Pretreatment of airway epithelium or the E-cadherin-expressing L cells with the long-acting β-agonist salmeterol prevented PAR2 activation from interrupting E-cadherin adhesion and compromising the airway epithelial barrier. Activation of PAR2 interrupts E-cadherin adhesion and compromises the airway epithelial barrier.

protease-activated receptor; histamine; barrier function

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. The epithelium protects the airways and more distal lung from injury and infection. Airway epithelium expresses protease-activated receptor-2 (PAR2), a receptor that itself has been linked to regulation of ion secretion, activation of signaling leading to cytokine production, metalloproteinase activation, conduction of signaling initiated by dust mite antigens, and airway hyperreactivity (1, 5, 15, 20, 25).

In sensitized humans and laboratory animals, an acute antigen challenge caused 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 (2, 7, 17). These observations indicate that the acute antigen challenge increased the permeability of both bronchial microvascular and airway epithelial barriers. Infusion of PAR2-activating peptide (PAR2-AP) into mouse airways initiated an inflammatory response that increased lung endothelial and epithelial permeability. The increased permeability was partially dependent on the release of neuropeptides (22). Infusion of PAR2-AP into the intestine increased paracellular permeability of intestinal epithelium, which was partly dependent on interferon-γ and activation of myosin light chain kinase (4). In the endothelium, activation of PAR1 by thrombin is a well-established pathway leading to interruption of VE-cadherin adhesion and disruption of the endothelial barrier (11). We had previously reported that histamine altered systemic endothelial and airway epithelial barriers by interrupting cadherin-based adhesion (26, 27, 29). We hypothesized that, in addition to the recognized effects of PAR2 activation on airway epithelium, activation of PAR2 would initiate signaling in airway epithelial cells that would directly interrupt E-cadherin adhesion and thereby the integrity of the airway epithelial barrier.

Stimulation of β-receptors, with consequent increases in cellular CAMP, has many effects on airway epithelial barriers, including activation of ion and water channels, enhancement of ciliary activity, and enhancement of migration and wound healing (19). CAMP acting through Rap-1 pathways enhances and stabilizes both adherens junction- and integrin-based cell adhesion (8, 10, 11, 16, 18). In endothelium, increases in cellular CAMP limit the effects of adherens junction-disrupting agonists that activate histamine and PAR receptors (13, 26, 27). CAMP limits the effects of these barrier-disrupting agonists by affecting the cell cytoskeleton and by affecting sites of cell adhesion (8, 10, 11, 13, 16, 18, 27). On the basis of these precedents, we further hypothesized that β-agonists would stabilize E-cadherin adhesion in the setting of activation of the histamine or PAR2 receptor.

MATERIALS AND METHODS

Materials. Tissue culture media and serum were from the Tissue Culture Core, University of Iowa. L cells were from American Type Culture Collection (Rockville, MD). Antibody to VE-cadherin (mouse IgG, monoclonal, clone 55-7H1) was from PharMingen, antibody to E-cadherin (11, mouse monoclonal) was from the Hybridoma Studies Bank (University of Iowa), and anti-Epac1 was from Upstate Cell Signaling Solutions (Lake Placid, NY). Calcein AM was from Molecular Probes (Junction City, OR). Reacti-Bind protein G-coated strip plates were from Pierce (Rockford, IL). Peptides were from Sigma-Genosys (Woodlands, TX); histamine was from Sigma-Aldrich (St.
Cells. L cells were grown in DMEM with 10% FBS, penicillin (100 μg/ml), and streptomycin (100 μg/ml). L cells transfected with the human histamine receptor H1 in the pcDNA3.1 vector and E-cadherin in the pHBAP-1-neo vector were grown as above with the addition of zeocin (1 mg/ml) and G418 (1.4 mg/ml) for selection but without penicillin and streptomycin.

Airway epithelial cells, generously provided by the laboratory of Joseph Zabner, were isolated from bronchial tissue obtained from normal people and people with cystic fibrosis and maintained as described previously (28). Cells were seeded onto collagen-coated, semi-permeable membranes (0.6 cm² Millicel-HA; Millipore, Bedford, MA) and grown at the air-interfaceliquid interface. Culture medium, a 1:1 mixture of Dulbecco’s modified Eagle’s medium and Ham’s F12 medium (DMEM/F12), was supplemented with 2% Ultroser G (Bio-Rad, Sepra, Villeneuve, France) and, initially, with 100 mU/ml penicillin, medium (DMEM/F12), was supplemented with 2% Ultroser G (Bio-Rad, Sepra, Villeneuve, France) and, initially, with 100 mU/ml penicillin, 100 μg/ml streptomycin, 15 μg/ml gentamicin, 125 μg/ml ceftazidime, and 2 μg/ml floconazole. Epithelia were studied at least 14 days after seeding, when they had differentiated.

Plasmid preparation and transfection. The cDNA for the human histamine receptor was extracted from ECV304 cells and inserted into the pcDNA3.1 expression vector as previously described (29). L cells expressing the histamine receptor in the vector pcDNA3.1 neo were transfected with the E-cadherin-pHBAP-1-neo plasmid using Lipofectamine Plus. Doubly transfected cells were selected with zeocin (1 mg/ml) and G418 (1.4 mg/ml). Clones were isolated based on expression of E-cadherin.

Analysis of protein expression. Protein was solubilized with CSK buffer (50 mM NaCl, 10 mM PIPES, pH 6.8, 3 mM MgCl₂, 1% Triton X-100, 300 mM sucrose, 5 mg/ml PMSF, 5 μg/ml aprotinin, 1 mg/ml leupeptin, 5 μg/ml pepstatin A, 4 mM Na-orthovanadate, 10 mM NaF, 10 mM Na₂PO₄), and cell proteins were separated on 8% PAGE gels and transferred to polyvinylidene difluoride membranes in 25 mM Tris, 192 mM glycine, 20% methanol, and 0.04% SDS buffer for 1 h at 10 V in a semi-dry transfer. The membranes were blocked with Sea Block Blocking Buffer (Pierce; http://www.piercenet.com). Blocked membranes were incubated with primary antibody in 1:1 blocking buffer-PBS for 1 h at room temperature, washed 4× with 1:1 blocking buffer-PBS, incubated with the secondary antibody, IRDye 800 goat anti-mouse IgG (Rockland, Gilbertsville, PA) for 1 h at room temperature, washed 4× with 1:1 blocking buffer-PBS, and then washed 2× with PBS. Blots were scanned using an LI-COR Odyssey infrared imaging system (LI-COR, Lincoln, NE).

Ussing chamber. For measurement of transepithelial electrical properties, epithelia were mounted in Ussing chambers and studied as described previously (28). Epithelia were bathed in symmetrical solutions containing (in mM) NaCl 135, K₂HPO₄ 2.4, KH₂PO₄ 0.6, CaCl₂ 1.2, MgCl₂ 1.2, dextrose 10, and HEPES 5, at pH 7.2, 37°C, and gassed with 100% O₂. Transepithelial resistance was measured before and after application of histamine by applying a 5-mV spike every 5 s and measuring the change in current to calculate the resistance. To independently assess the effect of agonists on the paracellular component of resistance, we first blocked the epithelial sodium channel of cystic fibrosis (CF) airway epithelia with 10 μM amiloride.

Adhesion of cells to cadherin-Fc. We used the adhesion assay described previously (29) to measure cadherin-dependent adhesion to cadherin-Fc-coated microtiter plate surfaces. Briefly, protein G-coated strip plates were rinsed three times with 0.05% Tween 20 in PBS, and then 100 μl 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. In the indicated experiments, r11-conditioned media or media alone were added to the wells and allowed to bind for 1 h and then washed with PBS before use.

Transfected L cells or primary airway epithelial cells were labeled by replacing the media with Hanks’ balanced salt solution with calcium, glucose, albumin (HBSSGA)-containing calcine AM (5 ng/ml) and incubating for 45 min at 37°C. The cells were then rinsed with PBS, harvested in lifting solution (137 mM NaCl, 4.2 mM NaHCO₃, 5.4 mM KCl, 5.6 mM glucose, 0.5 mM EDTA), pelleted by mild centrifugation, and resuspended in HBSSGA at a final concentration of 100,000 cells/ml. Two hundred microliters of the cell suspension were added to each well along with salmeterol or 8-CPT-cAMP in selected experiments. Cells were allowed to bind for 45 min at 37°C. Each eight-well strip was then treated with PAR2-AP (10 μM, 2 min) or histamine (100 μM, 1 min) and rinsed three times with HBSSGA to remove nonadherent cells. The fluorescence remaining in each well was then measured (Victor2; EG&G Wallac, Gaithersburg, MD) and used as an estimate of the relative number of adherent cells after background subtraction (29).

Statistical analysis. Changes were compared by analysis of variance, and individual group comparisons were done 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

PAR2-AP transiently reduces transepithelial resistance. Addition of the peptide SLIGRL (PAR2-AP) to both sides of a monolayer of primary human airway cells caused a rapid decrease in transepithelial resistance followed by a slower recovery back to baseline resistance over the next several minutes (Fig. 1). Both 100 and 10 μM PAR2-AP caused similar decreases. The response to 1 μM peptide was much smaller, causing only 20–50% of the decrease seen with 10 μM peptide. Addition of the reverse peptide had no effect on transepithelial resistance.

To determine the polarity of receptor localization, PAR2-AP was initially added to only one side of the monolayer and then added to the opposite side after sufficient time for the transient response to return to a stable baseline (20 min). Addition of 10 μM PAR2-AP to the apical side of the monolayer had no effect. However, adding the peptide to the basolateral side caused a transient drop in transepithelial resistance that was indistinguishable from the addition to both sides simultaneously (Fig. 2). Addition of activating peptide to the basolateral side of monolayers previously exposed to apically applied peptide also resulted in a response indistinguishable from addition to both sides.

PAR2-AP increases paracellular permeability. A decrease in transepithelial resistance may be due to either activation of transepidermal ionic conductances or increasing paracellular conductance. To determine whether PAR2 activation altered barrier function, we measured the flux of mannitol across the monolayer. Addition of 10 μM PAR2-AP to the basolateral side resulted in a 50 ± 12% increase in mannitol flux in the 20 min after treatment compared with the 20 min before addition (Fig. 3). This increase was not seen in monolayers treated with the reverse peptide. This indicates that activation of PAR2 decreases the barrier function of the monolayer.

An increase in paracellular permeability does not eliminate the possibility that PAR2 might activate Na or Cl conductances and thereby decrease transepithelial resistance. To eliminate the contribution of these ionic conductances, we used primary airway cells containing the homoygous deltaF508 mutation in the CF transmembrane conductance regulator (CFTR). This mutation prevents the protein from trafficking to the cell surface, disrupting the major pathway for chloride transport. In
addition, amiloride was added to block sodium transport. Amiloride alone caused a significant increase in transepithelial resistance, consistent with a basal Na conductance. In CF airway cells, PAR2-AP caused a large drop in resistance, even in the presence of amiloride (Fig. 4).

PAR2 activation alters E-cadherin binding. Having established that activation of PAR2 increased paracellular permeability of airway epithelium, we next examined the effect of activation of PAR2 on E-cadherin adhesion. A purified chimera of the extracellular domain of E-cadherin fused to the Fc portion of human IgG was bound to the bottom of 96-well plates, coated with protein G. E-cadherin-expressing L cells, labeled with a fluorescent marker, were bound to the immobilized E-cadherin chimera. L cells express the PAR2 receptor (Fig. 5). After addition of PAR2-AP, nonadhering cells were washed from the plate, and the relative number of bound cells was determined as described in MATERIALS AND METHODS. Addition of 10 μM PAR2-AP caused a significant decrease in L-cell binding compared with mock treatment (control) or addition of an equivalent amount of reverse peptide (Fig. 5).

Regulation of cell adhesion may be sensitive to cell type (3). Hence, in addition to the adhesion of E-cadherin-expressing L cells, the effect of PAR2-AP on E-cadherin adhesion was examined in a cell line lacking the PAR2 receptor.

Fig. 1. Time course of protease-activated receptor-2-activating peptide (PAR2-AP) response. Primary human airway cells grown as a monolayer on a permeable support are mounted in an Ussing chamber, and transepithelial resistance is measured as described in MATERIALS AND METHODS. Arrows indicate the addition of peptide (A). A: PAR2-AP or the reverse peptide is added to both sides of the monolayer, as indicated by the arrow. B: summary of dose response. Peak drop in resistance for PAR2-AP and 100 μM reverse peptide (RP) is reported as a fraction of the baseline resistance measured immediately before addition of peptide. For each monolayer, the minimum resistance measured after PAR2-AP addition is divided by the baseline resistance to calculate the fraction of baseline resistance. The mean and SE for at least 3 separate experiments are shown for each condition. *Significant difference from control binding.

Fig. 2. Polarity of PAR2 response. A: transepithelial resistance of 2 monolayers in Ussing chamber, measured simultaneously, is shown over time. In each monolayer, 10 μM PAR2-AP is added to both the apical or basolateral (BL) side. After an allowance of 20 min for any resistance changes to recover to baseline, PAR2-AP is added to the opposite side. B: summary comparing the maximum decrease in resistance caused by addition of 10 μM PAR2-AP to either side individually and both sides together. Fraction of baseline resistance is calculated as described in legend to Fig. 1. *Significant change from baseline resistance.

Fig. 3. Mannitol flux is increased by PAR2 activation. Ussing chamber experiments were performed as described in legend to Fig. 2, with 1 mM mannitol added to the bathing media and including trace amounts of [3H]mannitol on the apical surface only. The level of mannitol flux is calculated by comparing the amount of [3H]mannitol accumulated in the basolateral bathing media over 20-min time periods before the addition of PAR2-AP (10 μM) to the basolateral side of the monolayer with the amount that accumulates over the same time period after PAR2 activation. Results are the means and SE of 4 experiments. *Significant change from baseline flux.
cells, we measured adhesion of primary airway epithelial cells to the same E-cadherin-Fc immobilized to the 96-well plates. Adhesion of the primary airway cells to the immobilized E-cadherin extracellular domain was specific, as it was prevented by antibody to E-cadherin (Fig. 6). As with the E-cadherin-expressing L cells, addition of 10 μM PAR2-AP decreased the adhesion of primary airway epithelial cells to immobilized E-cadherin by ~60% (Fig. 6).

**E-cadherin binding regulates the paracellular pathway.** We previously demonstrated a link between cadherin-mediated cell-cell adhesion and the maintenance of airway epithelial barrier function (29). To determine whether disruption of E-cadherin-mediated binding is sufficient to decrease transepithelial resistance, an antibody to the extracellular domain of E-cadherin, known to prevent its homotypic binding, was added to the apical and basolateral compartments of monolayers of human airway epithelium in the Ussing chamber. Three to six hours after addition of antibody, transepithelial resistance fell to immeasurably low levels (Fig. 7). A similar antibody, previously demonstrated to interrupt VE-cadherin adhesion, had no effect on transepithelial resistance (14). Hence, interruption of E-cadherin adhesion alone is sufficient

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**Fig. 4. PAR2-AP response of cystic fibrosis (CF) cells.** Primary human airway cells homozygous for the deltaF508 mutation in CF transmembrane conductance receptor (CFTR), used in conjunction with amiloride, abrogate the contribution of transepolar ion transport in measuring changes in transepithelial resistance. A: time courses show that amiloride alone causes an increase in resistance but does not prevent the drop in resistance when PAR2-AP is added to the basolateral side. B: summary of the peak drop in resistances as a fraction of baseline resistance (described in legend to Fig. 1) in response to 10 μM PAR2-AP in CF cells in the presence and absence of amiloride. PAR2-AP caused a statistically significant drop in resistance in both cases, with no difference in the decrease detected with the addition of amiloride.

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**Fig. 5. PAR2 activation decreases cell binding.** A: Western blot of PAR2. Cells were lysed, and proteins were separated, transferred to nitrocellulose, and labeled with anti-PAR2 antibody as described in MATERIALS AND METHODS. MW marks the lane with molecular mass markers (as indicated, in kDa), and the remaining lanes are labeled by cell type. B: fluorescently labeled L cells expressing E-cadherin are allowed to adhere to an E-cadherin-Fc fusion protein immobilized on a protein G-coated surface. After a 2-min treatment with PAR2-AP (AP), the reverse peptide (RP), or mock treatment (None), nonadherent cells are washed off, and fluorescence measured in peptide-treated wells is compared with that in mock-treated wells. *Significant decrease in binding relative to mock-treated cells.

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**Fig. 6. PAR2 activation decreases cell binding of airway cells.** The binding of primary human airway cells and response to PAR2-AP were measured as described in legend to Fig. 5, with some modification. Background binding (Bkgnd) was measured by omitting Ecad-Fc in some wells. Wells were additionally treated with either antibody to E-cadherin (rr1)-conditioned media or unconditioned media before cell binding, as described in MATERIALS AND METHODS. Binding is calculated relative to control binding in mock-treated wells. Background binding and binding in rr1-treated wells are statistically indistinguishable. *Significant change in binding relative to control.
to compromise the transepithelial resistance (TER) of the airway epithelium.

Effects of a β-agonist on the response of E-cadherin adhesion to activating H1 and PAR2 receptors. The data above demonstrate that PAR2 activation interrupts the homotypic adhesion of the E-cadherin-expressing L cells to immobilized E-cadherin. We had previously reported that activation of the histamine type 1 receptor (H1) in L cells expressing E-cadherin and the H1 receptor (L-H1-E-cad cells) similarly interrupts adhesion of the L-H1-E-cad cells to immobilized E-cadherin (29). cAMP enhances cadherin and integrin adhesion (16, 18). We examined the effects of the β-agonist salmeterol on the effect that activating the H1 or the PAR2 receptor has on the adhesion of L-H1-E-cad or L-E-cad cells, respectively, to immobilized E-cadherin/human Fc chimeric protein. Pretreatment of L-H1-E-cad cells with salmeterol prevented the interruption of adhesion of L-H1-E-cad cells to immobilized E-cadherin/human Fc chimeric protein when they were exposed to histamine. Similarly, pretreatment of L-E-cad cells with salmeterol prevented interruption of the adhesion of the L-E-cad cells to immobilized E-cadherin/human Fc chimeric protein when the cells were exposed to PAR2-AP (Fig. 8).

Effects of 8-CPT-cAMP on the response of E-cadherin adhesion to activating H1 and PAR2 receptors. The cAMP analog, 8-CPT-cAMP, specifically activates an E-pac-Rap1 pathway that enhances integrin and cadherin adhesion (16, 18). L cells express Epac by immunoblotting (data not shown). Pretreatment of L-H1-E-cad cells or L-E-cad cells with the cAMP analog, 8-CPT-cAMP, increased adhesion of the cells to immobilized E-cadherin/human Fc chimeric protein (Fig. 8). Pretreatment of the cells with 8-CPT-cAMP also prevented activation of the H1 receptor in L-H1-E-cad cells, or activation of the PAR2 receptor in L-E-cad cells, from interrupting adhesion of the cells to immobilized E-cadherin/human Fc chimeric protein (Fig. 8). Hence, 8-CPT-cAMP blocked the interruption of E-cadherin adhesion initiated by activating either of the two receptors.

Effects of a β-agonist on response of airway epithelium to activating H1 and PAR2 receptors. Activation of the type 1 histamine receptor or activation of the PAR2 receptor causes a transient decrease in the transepithelial resistance of primary human airway cells (29). We pretreated primary human airway epithelium with the long-acting β-agonist salmeterol and then examined the response of the epithelium to histamine or PAR2-AP. Pretreatment of human airway cells with 100 nM salmeterol prevented the decrease in resistance caused by histamine (Fig. 9). The same dose of salmeterol had a marginal effect on the response of the epithelium to PAR2-AP. However, when the dose of salmeterol was increased to 1 μM, it significantly limited the decrease in transepithelial resistance caused by activation of PAR2 (Fig. 9). In similar experiments, primary human airway cells pretreated overnight with forskolin (10 μM) and IBMX (100 μM) did not show a decrease in

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**Fig. 7.** Primary human airway cells are mounted in an Ussing chamber, and a stable baseline is established. At time 0, conditioned medium containing the rr1 antibody to E-cadherin or a similar antibody to VE-cadherin was added to both sides of the chamber at a 1:10 dilution, and transepithelial resistance was monitored for several hours. Time courses shown are typical representatives of several separate experiments.

**Fig. 8.** Salmeterol and 8-(4-chlorophenylinthio)-2'-O-methyl-cAMP (8-CPT-cAMP) block loss of binding. The loss of cell binding caused by PAR2-AP (10 μM, 2 min) and histamine (100 μM, 1 min) of L-H1-Ecad cells to E-cad-Fc was measured as described in MATERIALS AND METHODS. During the 45-min binding period, cells were pretreated with vehicle, salmeterol (100 nM), or 8-CPT-cAMP (100 μM). Salmeterol and 8-CPT-cAMP prevented the loss of E-cadherin adhesion in response to histamine and PAR2-AP. *Significantly different from corresponding controls.

**Fig. 9.** Primary human airway cells are mounted in an Ussing chamber, and resistance is measured as in legend to Fig. 1. Monolayers are pretreated with vehicle or salmeterol (dose indicated) for 30 min before addition of PAR2-AP (10 μM) or histamine (100 μM). The peak drop in resistance caused by either agonist is compared with the baseline resistance to calculate the fraction of baseline resistance. Results are the means of at least 3 experiments. *Significantly different from corresponding controls.
resistance when subsequently exposed to histamine (data not shown).

Epithelial cells may have signaling pathways different from those in L cells (3). We examined the adhesion of primary airway epithelium to immobilized E-cadherin-Fc to confirm that β-agonists preserved the TER by enhancing E-cadherin adhesion. Our primary airway epithelium did not express Epac by Western blotting (Fig. 10A). Therefore, we pretreated the airway cells with salmeterol before exposing them to histamine or PAR2-AP and measuring the adhesion of the airway cells to immobilized E-cadherin-Fc. Salmeterol pretreatment prevented histamine and the PAR-AP from causing a significant decrease in adhesion of primary airway epithelium to immobilized E-cadherin-Fc (Fig. 10B).

DISCUSSION

PAR2 is expressed on airway epithelium. Its expression is increased in asthmatics (9). Activation of PAR2 enhances airway reactivity and inflammation in mice (6). Mice deficient in PAR2 have reduced airway reactivity and inflammation in response to ovalbumin sensitization (20, 23). Hence, activation of PAR2 appears to contribute to some of the phenotypic changes found in inflamed, allergic airways. The airway epithelium serves many functions, including acting as a barrier between the environment and subepithelial tissues. This barrier limits the dose of environmental substances delivered to subepithelial cells. The barrier also partitions the epithelium itself into basolateral and apical compartments that respond differently to receptor ligands and to infectious agents. The apical surface liquid of airway epithelium contains mitogens that can activate basolaterally restricted receptors to stimulate cell proliferation (24). The basolateral surface also expresses receptors for infectious agents, and when the normal barrier is disrupted, infection of airway epithelial cells is facilitated (29). The receptors for some cytokines that would be released by macrophages within the airways are also restricted to the basolateral surface of the airway epithelium, and these cytokines cannot activate the airway epithelium from the apical surface unless the barrier of the airway epithelium is compromised (laboratories of Dwight Look and Michael Shasby, unpublished data). Hence, the integrity of the airway epithelial barrier is an important determinant of airway inflammation and the phenotype of the airway epithelium.

Activation of the PAR1 receptor in the endothelium is a well-established pathway for interruption of VE-cadherin adhesion and disruption of the endothelial barrier (11). We hypothesized that some of the augmentation of airway inflammation associated with activation of the PAR2 receptor in airway epithelium might be related to compromise of the airway epithelial barrier by activation of PAR2. We found that activation of PAR2 with the peptide SLIGRL caused a rapid, transient decrease in transepithelial resistance of primary, differentiated, human airway epithelium (28). This decrease in resistance could not be explained by activation of sodium or chloride conductances, since cells defective in CFTR and treated with amiloride also responded to the peptide with a decrease in transepithelial resistance. Additionally, the diffusion of mannitol across monolayers of the same primary human airway epithelium increased when the monolayers were exposed to SLIGRL. A control peptide did not alter transepithelial resistance or mannitol flux.

Adherens junctions are important to the integrity of epithelia. E-cadherin is the cell-cell adhesion molecule anchoring most of the adherens junctions in airway epithelium. We hypothesized that activation of PAR2 might compromise the airway epithelial barrier by interrupting E-cadherin adhesion. To directly assess E-cadherin adhesion, we measured the adhesion of mouse L cells expressing E-cadherin to an immobilized extracellular domain of E-cadherin (29). Activation of PAR2 with the SLIGRL peptide caused an ~40% decrease in the number of E-cadherin-expressing L cells adherent to the immobilized E-cadherin extracellular domain, whereas the control peptide had no effect on cell adhesion. The physiology of cell adhesion may be specific to cell type (3). To be certain the observations with the E-cadherin-expressing L cells were relevant to airway epithelium, we measured adhesion of primary airway epithelium to the immobilized extracellular domain of E-cadherin. As with E-cadherin-expressing L cells, SLIGRL interrupted homotypic E-cadherin adhesion of primary human airway epithelial cells. Approximately 60% of the cells fell off the plates. Hence, activation of PAR2 interrupts E-cadherin adhesion in airway epithelium.

We next asked what the functional consequences were of selectively interrupting airway epithelial E-cadherin adhesion. We used a function-blocking antibody to E-cadherin to selectively interrupt E-cadherin adhesion. The antibody was administered to both the apical and basolateral sides of the epithelium, the latter making it possible for the antibody to diffuse to

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Fig. 10. A: Western blot of Epac1 expression. Cells were lysed, and proteins were separated, transferred to nitrocellulose, and labeled with anti-Epac1 antibody as described in MATERIALS AND METHODS. Epac1 migrates at 110 kDa as indicated. B: fluorescently labeled primary human airway cells are allowed to adhere to immobilized E-cadherin-Fc as described in FIG. 8 legend. Wells were treated with either 100 nM salmeterol or vehicle while binding and then challenged with either PAR2-AP (10 μM, 2 min) or histamine (100 μM, 1 min) as indicated. Nonadherent cells were rinsed off, and the fraction of cells remaining bound was measured relative to mock-treated cells. *Significantly different from mock-treated controls.
sites of cadherin adhesion that are basolateral to the tight junction. Antibody to E-cadherin eliminated almost all of the TER of the airway epithelium, demonstrating the importance of E-cadherin adhesion to the integrity of the airway epithelial barrier. Although these data clearly demonstrate that interruption of E-cadherin adhesion compromises the integrity of the airway epithelial barrier, our data do not eliminate the possibility that PAR2 activation initiates signaling that directly affects tight junction function.

Su et al. (22) reported that infusion of PAR2-AP into the airways of mice increased permeability of lung endothelial and epithelial barriers. Approximately one-half of the increase in permeability was dependent on neuropeptide release, confirming earlier observations by Steinhoff et al. (21). Our data would be consistent with a paradigm in which direct activation of PAR2 on endothelial and epithelial cells interrupts cadherin adhesion. Interruption of these barriers might provide the PAR peptide access to sensory neurons that augment the effects of PAR2 by activating neuropeptide release.

Cadherin adhesion is enhanced by activation of Rap1 by cAMP (10, 16). We examined the effects of a long-acting β-agonist, salmeterol, and the cAMP analog 8-CPT-cAMP, which more specifically activates the E-pac-Rap1 pathway, on the interruption of E-cadherin adhesion caused by activating the type 1 histamine receptor (H1) or the PAR2 receptor. We found that both salmeterol and 8-CPT-cAMP prevented interruption of E-cadherin adhesion in L cells when either the H1 or PAR2 receptor was activated. In primary airway epithelium, salmeterol prevented compromise of the airway epithelial barrier when the H1 or PAR2 receptor was activated. In confirmation of the important role of E-cadherin adhesion in preserving the TER of the airway epithelium, salmeterol pretreatment also preserved E-cadherin adhesion of airway cells to immobilized E-cadherin-Fc. Our primary human airway epithelium did not express Epac, and hence 8-CPT-cAMP would not prevent the effects of activation of the H1 and/or PAR2 receptors on the epithelial barrier.

The concentration of salmeterol necessary to prevent a compromise of the airway epithelial barrier after activation of PAR2 was greater than that required to preserve L-cell binding and greater than that required to prevent the effects of histamine on the epithelial barrier. The L cells express Epac, and the airway cells do not. This may affect the sensitivity to salmeterol of the airway cells relative to the L cells. Histamine activates both the H1 and the H2 receptors in airway cells. The H2 receptor activates adenylyl cyclase, which increases cell cAMP. Hence, a lower amount of supplemental cAMP may be necessary to protect against the effects of histamine vs. PAR2-AP. Some of our prior work in the endothelium supports this explanation (12). Endothelial activation of PAR1 causes an approximate threefold longer decrease in cell-cell adhesion than does activation of H1. Supplemental cAMP shortens the duration of decreased cell-cell adhesion due to activation of PAR1 to the much shorter duration of decreased cell-cell adhesion observed after activation of H1.

In summary, activation of the PAR2 receptor interrupts E-cadherin adhesion and the integrity of the airway epithelial barrier. Increases in cell cAMP through activation of β-agonists preserve cadherin adhesion and prevent the effects of PAR2 activation on the airway epithelial barrier.

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