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Respiratory pathogens and toxins often assault the lung from the airway lumen. Airway epithelia may initiate and amplify inflammation in response to these attacks, but under certain conditions confinement of inflammation to the airway lumen may be beneficial to the host. Accordingly, we hypothesized that airway epithelial polarity allows different responses to basolateral vs apical stimuli that may modulate inflammation. Using primary human airway epithelial cells differentiated at an air-liquid interface in culture, we found that responses to several cytokines required basolateral mediator application. In contrast, responses to Haemophilus influenzae occurred after either basolateral or apical interaction with airway epithelia. Experiments focused on IFN-γ receptor polarity confirmed its predominant basolateral location in cultured airway epithelia as well as in normal human airway tissue. Furthermore, physical and pharmacologic disruption of barrier function in airway epithelia allowed responses to apical application of IFN-γ and other cytokines. These in vitro studies directly correlated with experiments in mice in which an airway epithelial response to IFN-γ injected into the airway lumen was seen only after disruption of barrier function. The results indicate that airway epithelia with intact barrier function restrict inflammatory responses by limitation of cell activation through requiring interaction of selected mediators with the basolateral surface. However, loss of barrier integrity allows epithelial responses to these mediators if located in the airway lumen to amplify airway defenses. *The Journal of Immunology*, 2007, 178: 6395–6403.
cell culture and in vivo animal models were used to examine airway epithelial responses to basolateral vs apical interaction with specific cytokines and bacteria. The results indicate that selected cytokine receptors segregate to the basolateral surface of airway epithelium. This polarized restriction of receptors governs airway epithelial defense responses depending on epithelial paracellular permeability and the apical vs basolateral location of stimuli. Insensitivity of epithelial responses to some stimuli located only in the apical compartment would avoid inflammation in the airway epithelium and allow confinement of the host response within the airway lumen. However, loss of airway epithelial barrier function, reflecting a greater threat to the host, would result in increased epithelial capacity to participate in and amplify airway defenses.

**Materials and Methods**

**Airway epithelial cell isolation, culture, and treatments**

Human tracheobronchial epithelial cells from 20 individuals without lung disease were obtained under a protocol approved by the University of Iowa Institutional Review Board. Airways were dissected from lung tissue, and primary airway surface epithelial cells from tracheal and bronchial mucosa were isolated by enzymatic dissociation. These cells were grown as epithelial colonies at a density of 5 × 10⁶/cm², collagen-coated, semipermeable, polycarbonate membranes (Millipore). Epithelial cells under these culture conditions were grown in 49% DMEM, 49% Ham’s F12 medium, 2% Ultraser (Sepacor), 100 U/ml penicillin G, 100 µg/ml streptomycin, 50 µg/ml gentamicin, 2 µg/ml fungazole, and 1.25 µg/ml amphotericin B as described previously (11–15). Medium was removed from the apical chamber 24 h after plating to establish air-liquid interface culture conditions, and basolateral culture medium was changed every 2–4 days. Epithelial cells were maintained at 37°C in a humidified atmosphere of 5% CO2 and air and were studied at least 14 days after isolation and seeding to allow for redifferentiation and establishment of baseline inflammatory gene expression (11). Cells were treated on the apical or basolateral surface with the following mediators: 100 U/ml recombinant human IFN-γ (a gift from Genentech) for 30 min to induce Stat1 phosphorylation, 1000 U/ml recombinant human IL-4 (R&D Systems) for 30 min to induce Stat6 phosphorylation, or 100 U/ml recombinant human TNF-α (R&D Systems) for 24 h to induce IL-8 release and NF-κB activation. Some cells were treated on the apical or basolateral surface with equivalent inclusions (10⁻¹⁰ M). Epithelial cells of centimetric-treated nonmalignant H. influenzae strain 12 for 24 h to induce NF-κB activation as described previously (16). For epithelia treated at the apical surface, mediator in a medium volume equal to that at the basolateral surface was added. For epithelia left untreated or treated at the basolateral surface, an equal volume of control medium was added to the apical surface. Mechanical injury to epithelia was accomplished using a standardized wounding apparatus that scraped a ring of cells of consistent size off of culture membranes as described previously (17). Epithelial cell tight junction integrity was also altered by addition of 1 µg/ml anti-E-cadherin mAb or 10 mM sodium caprate. Experiments were repeated using cells from different individuals to assure reproducible and generalizable results.

**Primary Abs**

Rabbit polyclonal IgG 9171 against phosphorylated human and mouse Stat1, rabbit polyclonal IgG 9172 against total human Stat1, and rabbit polyclonal IgG 9361 against phosphorylated human Stat6 were from Cell Signaling Technology; rabbit polyclonal IgG 07-224 against phosphorylated human Stat2, rabbit polyclonal IgG 06-502 against total human Stat2, and rabbit polyclonal IgG 06-778 against total human Stat6 were obtained from Upstate Biotechnology; goat polyclonal IgG AF73 against human IFN-γ receptor 1 (IFNGR1), mouse mAb IgG2a clone 25463 against human IL4Rα, and mouse mAb IgG1 clone 33423 against human IL9Rα were from R&D Systems; mouse mAb IgG2b clone G1R-94 against human IFNGR1 was from Biologend; rabbit polyclonal IgG 61-7300 against human ZO-1 was purchased from Invitrogen Life Technologies; mouse mAb IgG2a clone AC-74 against human β-actin was obtained from Sigma-Aldrich; mouse mAb IgG1 clone r1 against human E-cadherin was obtained from the Hybridoma Studies Bank at the University of Iowa (18).

**Immunoblot analysis**

Selected epithelial cell protein levels were assessed by immunoblot analysis using specific Abs as described previously (19–21). Whole-cell protein extracts were prepared by lysis of cell monolayers in 50 mM Tris (pH 7.4), 150 mM NaCl, 1% Nonidet P-40, a protease inhibitor mixture (Roche Biosciences), and a phosphatase inhibitor panel (Calbiochem). Protein concentrations were determined using a Coomassie brilliant blue G-250-binding assay (Bio-Rad) and equal amounts of cell protein were subjected to SDS-PAGE in 7.5% polyacrylamide. Resolved proteins were electrophoretically transferred to nitrocellulose membranes (Hybond; Amersham Biosciences), exposed to 5% nonfat milk in TBS with 0.1% Tween 20 to block nonspecific Abs, and then incubated with Abs against a specific cell surface protein. Primary Abs binding was detected using goat anti-rabbit IgG, goat anti-mouse IgG, or donkey anti-goat IgG conjugated to HRP (Santa Cruz Biotechnology) and an ECL detection system (Amersham Biosciences). Reprobing of membranes with a different primary Ab was done after washing in Restore buffer (Pierce) for 15 min at 37°C.

**Enzyme-linked immunosassay**

IL-8 protein concentrations in equal volumes of medium incubated on the apical or basolateral surface of epithelia were determined using a commercial sandwich enzyme-linked immunosassay kit (R&D Systems) as described previously (10, 11, 16). According to the manufacturer, the sensitivity of this assay system for IL-8 is <10 pg/ml.

**NF-κB activation assay**

NF-κB activation was determined using a reporter gene assay system as described previously (16). Epithelia were cultured in medium without antibiotics for 4 days and challenge was applied to the basolateral surface in the inverted position at multiplicity of infection = 50 for 1 h with the recombinant adenoviral vector Ad-NFκBLuc that expresses a luciferase gene driven by four tandem NF-κB enhancer sequences (a gift from P. McCray, University of Iowa, Iowa City, IA) (22). Epithelia were allowed to recover in the upright position for 18 h and then incubated for an additional 24 h without or with TNF-α or H. influenzae applied to the apical or basolateral surface. Photonus pyralis tissue culture activity was determined using a commercial luciferase reporter assay kit (Promega) and a Lumat LB 9501 luminometer (Berthold Bad Wildbad).

**Transepithelial electrophysiology**

Transepithelial conductance was determined in most experiments using an ohmmeter (Millipore). The mean of five measurements was used after correction for background conductance by subtracting the measurement taken from a filter membrane without cells. In experiments in which epithelial tight junction integrity was assessed with anti-E-cadherin Abs, epithelia were mounted in modified Ussing chambers (Jim’s Instruments) and studied as described previously (11, 18). Epithelia were bathed in symmetrical solutions containing a 50:50 mixture of DMEM and Ham’s F12 medium at pH 7.2 and 37°C and continuously gassed with hydrated 100% O2. Transepithelial conductance was calculated by applying a 5 nA spike every 5 s and measuring the change in current.

**Immunofluorescence microscopy**

For human tissue samples, normal human trachea was fixed in 4% paraformaldehyde and embedded in paraffin. Serial 5-µm sections were deparaffinized using xylene, rehydrated in graded ethanol solutions, and one section was stained with H&E and viewed on a light microscope (BX-51; Olympus) with image acquisition using a digital charge-coupled device camera system (SPOT; Diagnostic Instruments) interfaced with SPOT software version 2.2. For detection of IFNGR1, other sections were treated with a proteinase K (DakoCytomation) for 30 min and then specific Abs were blocked by exposure to 37% egg proteins in water for 10 min, 5% milk in water for 10 min, and 10% rabbit serum in PBS for 1 h at 25°C. Slides were then incubated with 0.25 mg/ml primary or control goat IgG plus 1% rabbit serum in PBS, followed by streptavidin conjugated to Alexa 647 (Molecular Probes) in PBS. Tissue sections were mounted for microscopy using VectaShield (Vector Laboratories), and images were acquired using a laser scanning confocal system (MRC-1024; Bio-Rad) mounted on a Nikon E600 microscope interfaced with ImageJ software (National Institutes of Health).

For cultured airway epithelia, cells were fixed and permeabilized in 100% methanol for 20 min at –20°C. Nonspecific Abs were blocked by exposure to 2% BSA in Superblock (Pierce) for 1 h at 25°C. Epithelia were
corresponding surface of untreated epithelia is indicated by an asterisk.

incubation with the same treatment is indicated by an asterisk.

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cultured human airway epithelia that were initially infected with an adenoviral vector expressing a luciferase gene driven by four tandem NF-

FIGURE 2. Airway epithelial response to TNF-α requires exposure to the basolateral surface. A. Released IL-8 protein levels were determined using an enzyme-linked immunosorbant assay of apical or basolateral medium from cultured human airway epithelia that were left untreated or incubated on the apical (Ap) or basolateral (Ba) surface with TNF-α. Values are expressed as mean ± SD (n = 3), and a significant increase from medium levels on the corresponding surface of untreated epithelia is indicated by an asterisk. B. NF-κB activation was assessed using luciferase activity assays of extracts from cultured human airway epithelia that were initially infected with an adenoviral vector expressing a luciferase gene driven by four tandem NF-κB sites. Epithelia were then either left untreated or incubated on the apical or basolateral surface with TNF-α or H. influenzae. Values are expressed as mean fold change in luciferase activity compared with untreated epithelia ± SD (n = 3), and a significant difference in levels induced by apical vs basolateral incubation with the same treatment is indicated by an asterisk. C. Transepithelial conductance was determined for cultured human airway epithelia using an ohmmeter and epithelia treated as in B. Some epithelia were infected with Ad-NFκBBluc to allow monitoring of NF-κB activity. Conductance was measured in samples without epithelial treatments, after incubation with control medium on both epithelial surfaces (Ctl), or after incubation with TNF-α or H. influenzae on the indicated surface with control medium on the opposite surface for 24 h. Values are expressed as means (n = 2–10).

Surface protein isolation

The basolateral or apical surfaces of airway epithelia were selectively treated with 1 mg/ml N-hydroxysulfosuccinimidobiotin (Pierce) in PBS for 30 min at 25°C as described previously (15). The epithelial surface was washed and then incubated with 100 mM glycine in PBS for 20 min at 25°C to quench unreacted biotin. Epithelial protein lysates were prepared by sonication in a buffer consisting of 0.1% Triton X-100 in PBS and biotinylated proteins precipitated using neutravidin covalently linked to immobilized diaminopropylamine (Pierce). Biotinylated proteins were released in 8% SDS-containing loading buffer, incubated at 100°C for 5 min, and then underwent immunoblot analysis.

Mouse airway injury model

Experiments using mice to assess airway cytokine responses were performed under a protocol approved by the University of Iowa Institutional Animal Care and Use Committee. Six-week-old C57BL/6J mice (Harlan Breeders) were anesthetized by i.p. injection of 87.5 mg/kg ketamine HCl and then underwent airway injection of a 25-gauge i.v. catheter, and lungs were inflated under a 20 inH2O pressure and fixed with 2% paraformadehyde for 18 h. After fixation, lungs were reinfact and incubated with cryogenic embedding medium (Optimal Cutting Temperature Compound; Sakura Finetek). After freezing at −80°C, 7-μm serial lung cryosections were prepared on microscope slides, and some sections were stained with H&E (to assess lung architecture). Other sections were immunohistochemically stained for phosphorylated Stat1 expression by first treating with 10 mM sodium citrate (pH 6.0) at 95°C for 10 min. Slides were cooled and incubated with 3% hydrogen peroxide at 25°C to quench endogenous peroxidases. Non-specific Ags were blocked by exposure to 5% goat serum in 0.1% Tween 20 in TBS for 1 h at 25°C. Slides were then incubated with primary Ab against phosphorylated Stat1 in TBS with 0.1% Tween 20 and 5% goat serum for 18 h at 4°C. Primary Ab binding was detected by incubation with a polymer conjugated to anti-rabbit Ab and HRP (Envision+; DakoCytomation) for 30 min at 25°C, with subsequent development using a commercial kit that uses 3,3’-diaminobenzidine (Vector Laboratories). Tissue sections were mounted for microscopy using an aqueous mounting medium (Faramount; DakoCytomation) and images were acquired using a charge-coupled device camera system (DFC 300FX; Leica) mounted on a Leica DM5000 B light microscope interfaced with Leica Application Suite version 2.3.3 R1 software.

Statistical analysis

Enzyme-linked immunosorbant assays and luciferase activity assays were analyzed for statistical significance using a one-way ANOVA for a factorial experimental design. The multicomparison significance level for the ANOVA was 0.05. If significance was achieved by one-way analysis, post-ANOVA comparison of means was performed using Bonferroni’s multiple comparison test (24).
Results

Responses to IFN and IL-4 require exposure to the epithelial basolateral surface

To assess airway epithelial responses to selected mediators, a well-characterized system was used in which primary human airway epithelial cells are cultured at an air-liquid interface (12, 13). These airway epithelia exhibit multiple markers of airway epithelial cell differentiation, including formation of adherens and tight junctions, polarized expression of proteins, establishment of transepithelial resistance, transcellular transport of electrolytes, and organized expression of characteristic cellular organelles such as cilia (12–15, 18, 25). In initial experiments, epithelia were selectively exposed on the basolateral or apical surface to IFN-γ, and phosphorylation of the IFN-γ-activated transcription factor Stat1 was detected using immunoblot analysis of cell lysates. We found that Stat1 was activated in airway epithelia treated on the basolateral surface with IFN-γ, while no effect was detected with apical application (Fig. 1A). A similar requirement for basolateral application of mediators to airway epithelia was observed with IFN-α-induced phosphorylation of Stat2 (Fig. 1B) and IL-4-induced phosphorylation of Stat6 (Fig. 1C). Thus, airway epithelial responses to IFNs and IL-4 are restricted under baseline conditions to interaction of these mediators with the basolateral epithelial surface.

Response to TNF-α requires exposure to the epithelial basolateral surface

Based on initial results using IFNs and IL-4 as stimuli, we questioned whether other airway epithelial responses that do not require STAT proteins display a similar polarized sensitivity to stimulation. Airway epithelial release of IL-8 is induced by epithelial exposure to a variety of cytokines, bacteria, and other inflammatory stimuli, and production of this neutrophil chemoattractant is regulated by the transcription factor NF-κB (11, 16, 26). Basolateral exposure of airway epithelia to TNF-α resulted in increased IL-8 release from both surfaces, while minimal effect was observed following apical application (Fig. 2A). To detect NF-κB activation, epithelia were infected with an adenoviral vector that imparts NF-κB-dependent luciferase expression. In parallel with results for IL-8 release, this system detected no NF-κB activation after exposure of airway epithelia to TNF-α on the apical surface, but a significant response was seen after basolateral exposure (Fig. 2B). To assure airway epithelia could respond to stimuli at the apical surface, epithelial responses to a bacteria were also tested. This choice was based on reports that the bacterial product receptor TLR2 is expressed on both the apical and basolateral surfaces of airway epithelia (27, 28). In contrast to the restriction of airway epithelial responses for selected cytokines to interaction with the basolateral surface, NF-κB activation was observed following exposure of airway epithelia to the respiratory pathogen H. influenzae at either surface, with no clear differences between the two epithelial sides (Fig. 2B). Airway epithelial responses to bacterial interaction with the apical surface were not due to loss of epithelial integrity, as no significant difference in transepithelial conductance was observed among control-exposed, TNF-α-treated, and bacteria-exposed epithelia (Fig. 2C). The small decrease in epithelial conductance seen with TNF-α and bacterial treatment in comparison to untreated samples was likely the result of application of additional fluid to the apical surface of epithelia, as a similar effect was seen in control-exposed samples. Taken together, these results indicate that polarized airway epithelia have the capacity for responses to apical stimuli, but under baseline conditions restrict some responses to TNF-α by requiring contact of this mediator with the basolateral surface, similar to responses observed with IFNs and IL-4.

Selected airway epithelial receptors are polarized to the basolateral surface

For subsequent experiments, we primarily focused on airway epithelial responses to type II IFN, as sensitive reagents for studying both receptor detection and activation are available, and the majority of IFN-γ is released in vivo by lymphocytes but not epithelial cells, thereby allowing precise control of stimulation conditions (29). Photomicrographs of normal human airway tissue
following immunofluorescence staining for IFN-γ receptor (IFNGR)1 suggested that there was greater expression of this IFN-γ receptor component on the basolateral surface of conducting airway epithelia (Fig. 3A). Polarized expression of the IFN-γ receptor was also suggested by the observation that IFNGR1 was consistently expressed on the basolateral side of the tight junction protein ZO-1 in confocal photomicrographs of the cultured airway epithelia that underwent immunofluorescence staining (Fig. 3B). To confirm a polarized distribution of IFNGR1 in differentiated epithelia, selective apical or basolateral surface biotinylation with subsequent precipitation of labeled proteins was performed. Immunoblot analysis of precipitated proteins confirmed that IFNGR1 is expressed in greater abundance on the basolateral surface of airway epithelia (Fig. 3C). Similar predominant basolateral expression was seen with the α component of the IL-4R in these samples. In contrast, the α component of the IL-9R was more abundant on the apical surface of airway epithelia as previously reported (17), and this finding also confirmed the selectivity of biotinylation in these experiments. The results indicate that the restriction of airway epithelial responses to basolateral application of IFN-γ directly correlated with the predominant expression of IFNGR1 on the basolateral surface of airway epithelia.

**Airway epithelial injury allows responses to mediators located at the apical surface**

One corollary to our hypothesis is that epithelial integrity plays a critical role in maintaining restricted epithelial responses to some stimuli. To confirm this corollary, several conditions that alter epithelial barrier function were tested. We first used a simple and well-characterized device for uniform wounding of the surface of cultured epithelia (30). IFN-γ was then applied to either the basolateral or apical surface and Stat1 activation was assessed using immunoblot analysis of cell lysates. Airway epithelia that were wounded in this manner lost restriction of type II IFN effects and responded to either apical or basolateral exposure to IFN-γ (Fig. 4A). As a second method of altering epithelial barrier function, we applied an Ab to airway epithelia that blocks the homotypic binding of the essential adherens junction protein E-cadherin (18, 31). An increase in transepithelial conductance in cells treated with the Ab against E-cadherin was verified by monitoring this parameter in Ussing chambers (Fig. 4B). Cells treated with anti-E-cadherin Ab developed IFN-γ induced Stat1 phosphorylation in response to apical application of IFN-γ, and this finding correlated with a large increase in transepithelial conductance (Fig. 4C). A similar loss of restricted responses to other mediators after epithelial injury was observed, as epithelia that were wounded developed the capacity for Stat2 activation in response to IFN-α applied to the apical surface (Fig. 5A) and Stat6 activation in response to apical IL-4 (Fig. 5B). The effects of epithelial wounding on activators of NF-κB were more difficult to interpret due to the fact that the wounding process itself caused a variable increase in NF-κB activation, as detected by luciferase gene activity in epithelia infected with the adenoviral reporter vector (Fig. 5C). Addition of TNF-α to the apical surface of injured epithelia increased luciferase levels.

**FIGURE 4.** Airway epithelial injury allows a response to IFN-γ located at the apical surface. A, Stat1 phosphorylation was assessed using immunoblot analysis of extracts from cultured human airway epithelia that were either left uninjured or mechanically injured. Epithelia were then either left untreated or incubated on the apical (Ap) or basolateral (Ba) surface with IFN-γ. B-Actin levels were also assessed to verify equivalent protein isolation and loading. B, Transepithelial conductance was determined for cultured human airway epithelia using Ussing chambers and cells were treated on both sides of the epithelia with control Ab or Ab against E-cadherin for the indicated duration. C, Stat1 phosphorylation was assessed using immunoblot analysis of extracts from cultured human airway epithelia that were either left untreated or incubated on the apical or basolateral surface with IFN-γ. Lanes 3 and 4 used extracts from epithelia in B in which increased conductance was verified following treatment on both sides of the epithelia with Ab against E-cadherin (E-cad), but not control Ab (Ctl). Epithelia that underwent Ab treatment were also incubated on the apical surface with IFN-γ for the duration of Ab exposure.

**FIGURE 5.** Airway epithelial injury allows responses to other mediators located at the apical surface. A, Stat2 phosphorylation was assessed using immunoblot analysis of extracts from cultured human airway epithelia that were left uninjured or mechanically injured. Epithelia were then either left untreated or incubated on the apical (Ap) or basolateral (Ba) surface with IFN-α. B, Stat6 phosphorylation was assessed using immunoblot analysis of extracts from cultured human airway epithelia that were left uninjured or mechanically injured. Epithelia were then either left untreated or incubated on the apical or basolateral surface with IL-4. C, NF-κB activation was assessed using luciferase activity assays of extracts from cultured human airway epithelia that were initially infected with an adenoviral vector expressing a luciferase gene driven by four tandem NF-κB sites. Epithelia were left uninjured or mechanically injured, and then either left untreated or incubated on the apical or basolateral surface with TNF-α. Values are expressed as mean fold change in luciferase activity compared with uninjured and untreated epithelia ± SD (n = 3), and a significant difference in corresponding levels without or with injury was not observed.
of airway epithelia, mice underwent airway luminal application of and allowing a response to type II IFN applied to the apical surface efficacy of sodium caprate in modulating paracellular permeability (Fig. 6). However, animals treated with both sodium caprate and C shows detectable airway epithelial staining for activated Stat1 (Fig. 6 A). Furthermore sodium caprate treatment resulted in airway epithelial conductance, indicating loss of epithelial barrier integrity (18, 35). For cytokine receptors, it appears that erbB receptor ligand heregulin-α and severe acute respiratory syndrome coronaviruses infect human airway epithelia at higher efficiency from the apical surface, and this correlates with predominant apical expression of their receptors, amniogenic peptide N (CD13) and angiotensin-converting enzyme 2 (15, 33). In contrast, adenoviruses infect human airway epithelia best from the basolateral surface, which correlates with Incovirus receptor segregation to the basolateral surface (34). Indeed, adeno viral infection of airway epithelium from the apical surface is evaluated by subsequent lung isolation followed by immunohistochemical staining of the tissue using Abs against phosphorylated Stat1. Mice treated with sodium caprate or IFN-γ alone did not show detectable airway epithelial staining for activated Stat1 (Fig. 6C). However, animals treated with both sodium caprate and IFN-γ had epithelial cells in the airways with nuclear staining for phosphorylated Stat1. These in vivo results support the concept that airway epithelia respond poorly to IFN-γ on the apical surface (i.e., in the airway lumen) unless there is increased epithelial permeability.

**Discussion**

In addition to providing a simple physical barrier to environmental insults, airway epithelia actively participate in airway defense by directly responding to potential respiratory assaults and also by communicating with other detector and modulating cells in the airway (8, 10, 11). Using well-differentiated human airway epithelia to study epithelial responses to mediators important in a wide variety of host responses, we found that intact airway epithelia react to selected cytokines only after basolateral application. For IFN-γ and IL-4, this correlated with the predominant expression of their specific receptors on the basolateral surface of airway epithelial cells. Based on these results, we conclude that an intact epithelial barrier restricts responses to several cytokines while the erbB receptor ligand heregulin-α is released.
into the apical compartment (30). This physical separation of ligand and receptor allows for immediate erbB receptor activation when epithelial integrity is disrupted. In contrast, the α component of the IL-6 receptor has also been reported to be distributed chiefly on the apical surface of airway epithelial cells (36). Similarly, IL-9 receptors are expressed on the apical surface of airway epithelia, but these receptors respond to IL-9 only when the epithelia are actively differentiating (17). Restricted responses are not limited to respiratory epithelia, as increased expression of the adhesive glycoprotein for leukocytes ICAM-1 in differentiated colonic epithelial cells occurs after basolateral, but not apical, application of IL-1β and TNF-α (37). These observations have now been extended by our report to critical components of the airway defense system, revealing a physiologic pattern of segregating signaling complexes with pivotal effects on organ function.

Although epithelial cells actively participate in airway defense responses, maintenance of barrier function is of primary importance in preventing airway injury and preserving pulmonary function. In our studies, physical and pharmacologic disruption of epithelial barrier function allowed epithelial responses to apical application of IFN-γ and other cytokines using polarized airway epithelium in vitro. In this epithelial system, epithelial cells are cultured for at least 14 days to allow establishment of polarity and transepithelial resistance and to assure stable, low-level inflammatory signaling pathway activation that is representative of epithelia in vivo (11). Experiments were performed both without and with washing of epithelial cell surfaces before mediator addition (results not shown), and the lack of effect of washing suggests that mucus or other barriers susceptible to mechanical removal did not inhibit cell responses to apical application of mediators. However, it was also important to test polarized epithelia using an in vivo system to assure results were relevant and could be generalized for airway epithelial cell behavior. In mice, an epithelial response to IFN-γ in the airway lumen was seen only after disruption of barrier function, and this observation paralleled results with cultured airway epithelia. These findings indicate that airway epithelial activation by specific cytokines placed at the apical surface can occur after loss of barrier function. Although increased epithelial paracellular permeability likely allowed mediator diffusion to the basolateral surface, we have not excluded the possibility that loss of barrier integrity leads to receptor relocation at the apical surface.

Evolution of respiratory pathogens has generated virulence factors that overcome airway epithelial barrier function, providing evidence of the importance of this barrier for lung defense. For example, LPS from Gram-negative bacteria compromises the integrity of the airway epithelial barrier (38). Elastase, exotoxin A, and rhamnolipids from Pseudomonas aeruginosa also disrupt airway epithelia to promote paracellular invasion (39, 40). The Der p1 cysteine proteinase allergen from house dust mites increases epithelial permeability, likely by activation of the protease-activated receptor 2 (41, 42). It has been speculated that the capacity of respiratory pathogens to breach the airway epithelial barrier provides the microbe with advantages over the host, including access to receptors, diversion of nutrients, and protection from the immune system (43). Therefore, nonmicrobial factors, such as ozone, that decrease epithelial barrier function could assist respiratory pathogens in establishing infection in the lung (44). In addition, patients with lung diseases such as asthma and smoking-induced bronchitis demonstrate increased airway epithelial permeability, and this may also affect bacterial infection and inflammatory responses (45–48). Conversely, airway epithelia may provide an important “watchtower” function by amplifying inflammation when the airway mucosal barrier is breached. This could explain the paradoxical observation that selected inflammatory mediators may directly lower airway epithelial barrier function, as has been demonstrated when histamine, protease-activated receptor 2-activating peptide, or both TNF-α and IFN-γ are applied to the basolateral surface (18, 25, 42, 49).

Given the variety of stimuli tested in our experiments, responses that require mediator interaction with the basolateral surface of airway epithelia represent a wide range of different inflammatory and immune responses. Type I IFNs (IFN-α and -β) are produced by a wide variety of cell types and are potent antiviral agents (29). Type II IFN (IFN-γ) is primarily produced by certain T cells and NK cells and has been shown to regulate immunity and inflammation, particularly in Th1-type responses (50, 51). IL-4 is also produced by selected T cells and is a key regulator of humoral and adaptive immunity, particularly in Th2-type responses (52, 53). TNF-α is produced by many different cell types and has numerous effector functions in acute inflammatory responses (54, 55). The finding that initiation of epithelial signaling by each of these mediators is normally restricted to basolateral application indicates that epithelial amplification of antiviral, Th1, Th2, and acute inflammatory responses is triggered through these mediators when there is loss of barrier function, or in situations in which mediators are generated basolateral to the epithelium. Although restriction of some responses to mediator interaction with the basolateral surface of airway epithelia is directly related to receptor location, other undefined mechanisms may participate in this phenomenon. For example, our results indicate airway epithelia restrict some responses to TNF-α by requiring contact of this mediator with the basolateral surface, but TNFR1 expression has been reported on the apical surface of airway epithelial cells (28, 56).

Our results indicating that nontypeable H. influenzae-activated NF-κB in airway epithelia after interaction with either the apical or basolateral surfaces are similar to reported airway epithelial cytokine responses to P. aeruginosa (32). These findings suggest that at least a subset of the cellular receptors for this bacteria or bacterial components reside on or can be mobilized to both sides of airway epithelial tight junctions, as has been reported for TLR2 (27, 28). However, they do not exclude other possibilities such as expression polarity of downstream signaling components or bacterial internalization from either surface allowing access to intracellular receptors (57). These observations do suggest that responses to bacteria are initiated at both cell surfaces and may allow direct epithelial cell detection of pathogens that could potentially be located at either site.

Polarized cytokine release from airway epithelia may also be important for modulation of the host response. For example, greater initial apical release of IL-8 after apical exposure of respiratory epithelial cells to ozone has been reported (58). Similarly, airway epithelial cell treatment with IFN-γ results in preferential apical release of the cytokine RANTES (59). For epithelial cell activation by bacteria, application of Staphylococcus aureus to the apical surface of human airway epithelia results in more IL-8 release into the apical compartment (60). We observed that basolateral exposure of airway epithelia to TNF-α resulted in higher levels of IL-8 release into the basolateral compartment. These results suggest that airway epithelia have the ability to establish a gradient of chemotraactants, although the mechanisms and importance of polarized cytokine release to specific airway defense situations are unclear.

The combination of epithelial barrier function and restricted responses to basolateral inflammatory mediators allows airway epithelial defense to be conceptually separated into three categories of host responses. For the first, a modest assault on the airway from the apical surface that does not alter airway epithelial barrier function results in minimal epithelial cell activation by cytokines. In this case, the host response is primarily handled by constitutive
mechanisms or by other resident cells, and inflammation remains confined to the airway lumen. In the second situation, an attack from the airway lumen that results in disruption of airway epithelial integrity provokes a stronger epithelial response that amplifies defense (e.g., neutrophil recruitment) at the site. Finally, if the threat activates elements of the immune system basolateral to the epithelium, a strong response is rapidly generated to defend against the insult, initiating a specific defense to a selected pathogen, and participating and/or coordinating an appropriate host response through communication with other airway cells that may also detect assault on the lung.

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