In vitro susceptibility to rhinovirus infection is greater for bronchial than for nasal airway epithelial cells in human subjects

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Background: Human rhinoviruses (HRVs) characteristically cause upper respiratory tract infection, but they also infect the lower airways, causing acute bronchitis and exacerbating asthma.
Objective: Our purpose was to study *ex vivo* the differences in the response to HRV infection of nasal and bronchial epithelial cultures from the same healthy and asthmatic individuals using conditions favoring development of fully differentiated, pseudostratified mucociliary epithelium.
Methods: Cells from the inferior turbinates and bronchial tree of 5 healthy and 6 asthmatic individuals were cultured at an air-liquid interface. Cultures were infected with HRV-16, and after 48 hours, the degree of infection was measured.
Results: Baseline median transepithelial resistance was lower in human bronchial epithelial (HBE) cell cultures than in human nasal epithelial (HNE) cell cultures (195 $\Omega.cm^2$ [95% CI, 164-252] vs 366 $\Omega.cm^2$ [95% CI, 234-408], respectively; $P < .01$). Virus replicated more easily in HBE cells than in HNE cells based on virus shedding in apical wash (log tissue culture infective dose of 50%/0.1 mL = 2.0 [95% CI, 1.0-2.5] vs 0.5 [95% CI, 0.5-1.5], $P < .01$) and on a 20- to 30-fold greater viral load and number of infected cells in HBE cell cultures than in HNE cell cultures. The increases in expression of RANTES and double-stranded RNA-dependent protein kinase were greater in HBE cell cultures than in HNE cell cultures, as were the concentrations of IL-8, IL-1α, RANTES, and IP-10 in basolateral medium. However, no significant differences between asthmatic and healthy subjects (including IFN-β1 expression) were found.
Conclusions: Differentiated nasal epithelial cells might have mechanisms of increased resistance to rhinovirus infection compared with bronchial epithelial cells. We could not confirm previous reports of increased susceptibility to HRV infection in epithelial cells from asthmatic subjects. (J Allergy Clin Immunol 2009;123:1384-90.)

Key words: *Human rhinovirus, nasal and bronchial airway epithelial cells, air-liquid interface*

Infection with human rhinovirus (HRV) characteristically causes common colds but might also provoke acute bronchitis in healthy persons and exacerbate airway disease in persons with asthma, cystic fibrosis, or chronic obstructive pulmonary disease.[1-4]

The primary site of HRV infection *in vivo* is the airway epithelial cell.[5-7] The involvement of the lower airways is likely due to direct infection of the bronchial mucosa because HRV RNA has been detected in tissues and secretions from the lower airways by means of application of PCR and *in situ* hybridization.[7-9] Infection of epithelial cells with HRV *in vitro* induces secretion of a variety of cytokines and chemokines, including IL-1, IL-6, IL-8, IL-11, interferon-inducible protein 10 (IP-10), GM-CSF, RANTES,[10,11] and IFN-β1.[12] Because minimal or no discernible epithelial necrosis or sloughing is found in airway mucosal biopsy specimens taken from persons infected with HRV or in cultures of airway epithelium infected *in vitro*, the symptoms of HRV infection are thought to reflect activation of host defense and inflammatory responses.[7,13]

In vivo only a small proportion of cells are infected with HRV. Small and scattered foci of cells are infected in the upper[6,14,15] and lower[7] airways. In primary bronchial and adenoidal epithelial cells cultured on solid supports and infected *ex vivo*, again only a small subset of cells are infected.[7,16]

Reasoning that the severity of the response to HRV infection might be related to the number of cells infected and the number of HRV virions produced, we examined the hypothesis that susceptibility to HRV infection might differ between nasal and bronchial epithelial cells from the same individual and between cells from asthmatic and healthy subjects.

Asthmatic subjects have similar upper respiratory tract symptoms but more severe lower respiratory tract symptoms than healthy subjects during acute respiratory tract viral infections.[17] A recent study reported that bronchial epithelial cells from asthmatic patients differ from those from healthy patients in supporting markedly greater viral replication and cell lysis, which was attributed to an impairment of virus-induced IFN-β1 production

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Abbreviations used
HBE: Human bronchial epithelial
HNE: Human nasal epithelial
HRV: Human rhinovirus
ICAM-1: Intercellular adhesion molecule 1
PKR: Double-stranded RNA-dependent protein kinase
Rte: Transepithelial resistance
STAT1A: Signal transducer and activator of transcription 1A
Vte: Transepithelial potential difference

and of induction of apoptosis.[18] This study, like virtually all *in vitro* studies of the effects of HRV, used undifferentiated human airway epithelium, in which cells are grown to form a single cell layer submerged in medium. We have previously shown that poorly differentiated tracheal and nasal airway epithelial cells produced more infectious virus particles, a 7-fold higher proportion of cells were infected, a 30- to 130-fold greater replication of HRV was supported, and a 16-fold greater production of RANTES was induced than when the same cells were grown at an air-liquid interface.[19] The air-liquid interface induces differentiation into a pseudostratified mucociliary epithelium closely resembling the *in vivo* appearance of native epithelium. Differentiation of airway epithelium therefore increases resistance to rhinovirus infection.[19]

Because of the importance of the level of differentiation of airway epithelial cells as a determinant of their *ex vivo* susceptibility to HRV infection, we undertook our study of differences in the response of nasal and bronchial epithelial cells from healthy and asthmatic subjects using only culture conditions favoring the development of a normal-appearing mucociliary epithelium.

METHODS

Study subjects

Six subjects with allergic asthma and 5 healthy nonallergic subjects were studied (Table I). Allergic status was based on the presence of a positive skin prick test response (wheal 3 mm greater than the negative control with erythema) to common allergens.[20] Asthmatic subjects had a diagnosis of asthma and bronchial hyperresponsiveness (PC_{20} methacholine, <8 mg/mL),[20] were allergic to at least 1 allergen, and had mild-to-moderate disease, as defined by the National Institutes of Health guidelines.[21] None had used inhaled corticosteroids for at least 4 weeks before enrollment. The healthy subjects had no previous history of lung disease, had normal spirometric results and bronchial responsiveness to methacholine, and had no positive responses to skin prick tests. All subjects were nonsmokers. The study was approved by the University of California, San Francisco's, Committee on Human Research, and written informed consent was obtained from all subjects.

Human airway cell culture and cell infection

Nasal scrapings were performed as previously described.[22] Bronchial brushings were obtained with a flexible Pentax bronchoscope (Model FB-18X; Pentax, Orangeburg, NY) by using standard guidelines.[23] The human nasal epithelial (HNE) and human bronchial epithelial (HBE) cell cultures were processed in parallel, and they were cultured at an air-liquid interface, as described previously (see also the Methods section of this article's Online Repository at www.jacionline.org).[19,22]

HRV-16 passage 3 (grown from a stock sample from E. Dick and W. Busse, University of Wisconsin) suspension with 1×10^6 tissue culture infectious dose of 50% per milliliter was added to the apical surface (multiplicity of infection, 1.0) in triplicate. After 24 hours, viral suspension (or medium in control cells) was removed, and the apical surface was washed 3 times with PBS. Cells were then allowed to recover for 24 hours, after which the apical surface was washed with 200 to 300 μL of PBS, and this wash fluid was retained for titration of virus shedding. HRV was titrated in half-log dilutions of apical washes by using confluent human fetal diploid lung cells (VRDL, Richmond, Calif) and standard virologic techniques.[24] Transepithelial resistance (Rte) and transepithelial potential difference (Vte) were measured, and basolateral medium was collected and stored at −80°C. Filters with attached cells were cut in half. One half was placed in RLT buffer (QIAGEN Inc, Valencia, Calif), vortexed for 10 seconds, and frozen at −80°C for future RNA isolation. The other half was fixed in buffered 3.5% formaldehyde for 10 minutes and stored in PBS at 4°C for future histology.

LINCOplex human cytokine kits (Linco Research, St Charles, Mo) were used to measure levels of cytokines and chemokines in basolateral medium samples.

TABLE I. Baseline characteristics of the subjects

	Asthmatic group	Healthy group	*P* value
No.	6	5	NA
Sex (% male)	67	60	.40
Age (y)	33 (24 to 50)	37 (32 to 46)	.43
FEV_1 (% predicted)	70 (59 to 84)	104 (90 to 124)	<.01
PC_{20} (mg/mL)	0.509 (−0.03 to 1.75)	>64	<.01

Values are displayed as the median and 95% CI. Allergy skin tests (to cat, house dust mites, local pollens, and molds) showed that no healthy subject was allergic and all asthmatic subjects were allergic to at least 4 allergens. Groups were compared by using the Mann-Whitney rank test for continuous variables and the Fisher exact test for proportions.

Gene expression and immunocytochemistry

A 2-step real-time RT-PCR method and immunofluorescence for HRV-16 were performed as described previously (for more details, see the Methods section in this article's Online Repository).[25]

Statistical analysis

The Shapiro-Wilk test for normality showed that the data were not normally distributed, even if log transformed. Therefore we used nonparametric statistics to analyze the data. We compared baseline characteristics in Table I by using the Mann-Whitney rank sum test for continuous variables and the Fisher exact test for sex. We analyzed data on inflammatory mediators by using the generalized estimating equations method, which is a multivariate statistical test that allows simultaneous comparison of all characteristics of each culture (asthmatic or healthy group, nasal or bronchial site, and rhinovirus or sham infection) and also takes into account the subjects as sources for several replicate cultures. Differences were considered significant if the *P* value was less than .05 with 2-tailed tests. Data were analyzed with Stata 8 software (StatCorp, College Station, Tex).

RESULTS

Epithelial cell cultures

After 2 to 3 weeks of growth at an air-liquid interface, all HNE (n = 70) and HBE (n = 84) cell cultures appeared fully confluent under the inverted microscope and showed properties resembling those of native epithelium, including an apical mucus layer and ciliated pseudostratified epithelium (Fig 1), as well as a permeability barrier, as demonstrated by a "dry" apical surface and the development of Rte, which is indicative of tight junction formation.[22] Cultures also showed vectorial transport of ions, as demonstrated by the Vte and equivalent short-circuit current values.