

Short Communication

Analysis of tracheal secretions for rhinovirus during natural colds

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Rhinoviruses (RV) cause 50% of common colds and are frequently isolated from children and adults hospitalized for asthma exacerbations. Although colds may trigger severe coughing and wheezing, it is not known whether these symptoms are a result of lower airway infection with RV. Previous efforts to address this question by sampling lower airway secretions during experimental RV infections have been complicated by the possibility of contamination of the bronchoscope with nasopharyngeal cells and secretions. To further test the hypothesis that RV infections involve the lower airways, tracheal and nasal secretions were obtained from 23 pediatric tracheostomy patients, including seven with cold symptoms and 16 asymptomatic controls. RV was detected by reverse-transcription polymerase chain reaction from the nasal and tracheal secretions of three of the seven children with cold symptoms. In the 16 well children, RV was not detected in any samples of nasal secretions, but was isolated from four samples of tracheal secretions. These results demonstrate the presence of RV in the lower airways of children with tracheostomies during community-acquired colds, without the possibility of nasal contamination. In addition, these findings suggest that children with tracheostomies carry subclinical viral infections in their tracheas, rather than their noses.

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Rhinoviruses (RV) cause approximately 50% of common colds and are responsible for the majority of respiratory illnesses during the spring and fall. Furthermore, these viruses are isolated in up to 80% of pediatric patients during asthma exacerbations (1, 2). Although the mechanism by which an upper respiratory infection can trigger severe lower respiratory symptoms remains controversial, a leading theory is that RV replicates in lower airway cells, causing cellular damage or secretion of cytokines and mediators with chemotactic and inflammatory effects.

Current evidence for the presence of RV in the lower respiratory tract is largely derived from studies involving experimental inoculation of volunteers, who then undergo bronchoscopy to retrieve specimens of lower airway cells and secretions for analysis (3, 4). Although these studies have been instructive, there are several

problems with their interpretation. Firstly, volunteers are typically infected with a larger inoculum of RV (100–10,000 infectious units) than is likely to initiate a natural inoculation. Secondly, the viral suspension is often atomized to ensure that the volunteer is infected, raising the possibility of direct infection of the lower airway by inhalation. Finally, during the process of bronchoscopy, lower airway secretions and cells may become contaminated with rhinovirus-containing upper airway secretions as the bronchoscope passes through the nasopharynx.

To test the hypothesis that naturally-acquired RV infections extend to the proximal lower airway, we obtained tracheal secretions from tracheostomy patients with direct access to their lower airways, minimizing the potential for contamination with virus from the upper airways.

Methods

Study population

Following approval from the University of Wisconsin Hospital and Clinics human subject committee, informed consent was obtained from parents and assent was obtained from children over 6 years old. Twenty-three children with tracheostomies were enrolled and classified into groups based on parental report of cold symptoms: 16 children were well, and seven children had cold symptoms (runny nose, sneezing, nasal congestion or cough). Children with active allergic rhinitis, known or suspected bacterial lower respiratory tract infection, and those whose parents refused consent were excluded from the study.

Sample collection

Tracheal suctioning was performed using the routine sterile technique for each patient and the secretions were collected in a sterile container. Nasal suctioning was performed using a nasal bulb syringe and the secretions were collected in a sterile container. If no nasal secretions were present, five to six sprays of nasal saline were administered before nasal suctioning. Secretions were stored in phosphate buffered saline with 0.5% albumin and were frozen at -70°C until analysis.

Reverse-transcription polymerase chain reaction

Total RNA was extracted from 100 μl of nasal or tracheal secretions, and cDNA was synthesized as previously described (5). The extracted product was then analyzed for the presence of RV RNA using a nested reverse-transcription polymerase chain reaction according to the method of Ireland et al. (6) with minor modifications (5). Specimens of nuclease-free water (negative control) and RV cDNA (positive control) were processed with each batch of test specimens.

Statistical analysis

The ages of the groups of children with and without colds were compared by the Kruskal–Wallis nonparametric test. The gender, reasons for tracheostomy, symptoms, and presence of RV in the noses and tracheas of children with and without colds were compared by Fisher's exact test.

Results

A total of 23 children with tracheostomies, ages 3 months to 15 years, were entered in the study,

Table 1. Characteristics of children with and without colds

	Cold (n = 7)	Well (n = 16)	p-value
Age (median) (yr)	2	2	0.8
(Range)	8 months to 4 yr	3 months to 15 yr	
Male gender [no. (%)]	4 (57)	7 (44)	0.7
Reason for tracheostomy [no. (%)]			0.2
Lung parenchymal	4 (57)	5 (31)	
Airway obstruction	2 (29)	6 (38)	
Neurological	1 (14)	4 (25)	
Musculoskeletal		1 (6)	

including 16 asymptomatic children and seven with cold symptoms. These children were not significantly different with respect to age, gender or reason for tracheostomy (Table 1). Compared with the asymptomatic children, the children with colds were significantly more likely to have rhinorrhea (86% vs. 6%, $p < 0.001$), congestion (43% vs. 0%, $p = 0.02$), sneezing (57% vs. 0%, $p = 0.004$) and cough (71% vs. 0%, $p = 0.001$). Two children with colds were reported to have recent wheezing. Sore throat is commonly associated with RV infections, but could not be reliably ascertained because of the young age of many subjects.

Of the seven children with colds, three were positive for RV. In all of these children, RV was detected in both the nasal and tracheal secretions. Children with cold symptoms were significantly more likely to have RV detected in nasal secretions ($p = 0.02$). Of the 16 well children, four had RV-positive tracheal secretions. None of the asymptomatic children had RV in their nasal secretions.

Discussion

Rhinovirus may induce lower respiratory tract symptoms by activation of systemic immune mechanisms through RV infection of upper airways, by direct RV infection of lower airways, or by a combination of these mechanisms. Direct lower airway infection with RV implies that virus should be detectable in the tracheal secretions as well as the nasal secretions of children with colds because of RV. In this study, RV was detected with equal frequency in upper and lower airway secretions of children with tracheostomies during natural colds. This study extends findings from previous studies (3, 4), because all children had community-acquired infections and samples of tracheal secretions were collected directly from the lower airways, reducing the risk of contamination with nasal secretions during sample collection.

In previous studies, the asymptomatic carriage rate of RV in nasal secretions has ranged

between 14% and 41% (1, 2). Of our 16 asymptomatic pediatric tracheostomy patients, four (25%) were RV-positive. Interestingly, all of these children had RV in their tracheal secretions but none had RV in their nasal secretions. Considering that children with tracheostomies ventilate primarily through their tracheostomies instead of their noses, these findings suggest that the infections were acquired through aerosol transmission that bypassed the nasal cavity. Direct inoculation with rhinovirus into the tracheostomy site during tracheostomy care is a less likely possibility, because all caregivers used sterile technique during tracheal suctioning.

In summary, this study shows that RV infections can involve the large lower airways of children with tracheostomies, and provides evidence that these children carry subclinical RV infections in their tracheostomies rather than in their noses. As the tracheostomies are the major avenue of ventilation, these findings suggest that the subclinical infections were obtained via the aerosol route. Previous experiments conducted *in vitro* and studies involving experimentally infected volunteers have suggested that RV can involve the proximal lower airways, and our results support this concept. Studies are now underway to perform quantitative assessments of RV replication in proximal and distal airways of experimentally and naturally infected

individuals. The recognition of RV as a lower airway pathogen may lead to new insights related to the pathogenesis of bronchitis and acute exacerbations of asthma.

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