

Respiratory pathophysiologic responses

Lower airway responses to rhinovirus-Hanks in healthy subjects with and without allergy

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A role for respiratory viruses in the pathogenesis of asthma is supported by several lines of evidence, including the results of epidemiologic and experimental rhinovirus (RV)-16 infection studies.^{1, 2} Although these studies suggested that allergic rhinitis (AR) may be a predisposing factor, the degree of generalizability of the findings to other viruses was unknown. Earlier studies conducted in our laboratory failed to detect changes in lower airway patency or methacholine responsiveness during either experimental influenza A or RV-39 infections, when the latter was assessed by using a maximum methacholine concentration of 25 mg/ml.^{3, 4}

The purpose of this study was to document the lower airway effects of experimental infection with a different rhinovirus serotype, the Hanks strain (RV-H), in healthy subjects with AR and in subjects without AR. Additionally, a higher maximum methacholine concentration (50 mg/ml) than that used in previous studies was used to assess lower airway responsiveness.³

METHODS

Healthy, nonasthmatic adult volunteers with AR and without symptoms at the time of study ($n = 56$) and volunteers without AR ($n = 75$) were identified as previously described.⁴ Subjects with a history of allergy and with at least one positive (wheal diameter >10 mm), relevant skin test for allergy by the puncture method were classified as AR subjects. Participants were not screened for the absence of preexisting serum neutralizing antibodies to RV-H. Consenting subjects were enrolled in the study, which was approved by Children's Hospital of Pittsburgh Human Rights Committee.

Subjects were cloistered in individual hotel rooms for a 6-day period. After baseline spirometry was performed, subjects were inoculated intranasally with 1 ml of a safety-tested viral pool of RV-H (100 to 300 median tissue culture infective dose) at the end of the first day. After inoculation, nasal washes were performed, and pulmonary function was assessed once daily for 5 mornings.^{3, 4} Additionally, nasal symptoms were scored (0 =

Abbreviations used

AR:	Allergic rhinitis
RV:	Rhinovirus
RV-H:	Rhinovirus-Hanks

no symptoms to 4 = severe symptoms), and nasal secretion weights were determined daily.^{3, 4}

Infection was defined as RV-H shedding on any of the postinoculation days or a fourfold increase in serum RV-H antibody titer at convalescence.^{3, 4}

A subset of eight subjects with AR and seven subjects without AR received a bronchial methacholine challenge with standard methods during the acute phase (days 5 to 6) and again, a minimum of 3 weeks after being free of symptoms (study days 26 to 28). The sequential methacholine concentrations of 0 (saline), 0.025, 0.25, 2.5, 10, 25, and 50 mg/ml were administered by a dosimeter, followed in 3 minutes by spirometry. The procedure was discontinued prematurely if the highest of the three FEV₁ values represented a decrease equal to or greater than 20% from baseline.

Viral effects on symptom scores were analyzed by the two-tailed paired Student's *t* test. Differences between subjects with and without AR were analyzed by using a repeated-measures analysis of variance with variance partitioned by group and study day. For methacholine responses, the majority of subjects did not have a measurable PC₂₀ value. Therefore within-subject differences between each postmethacholine FEV₁ value and the baseline FEV₁ value were calculated. To examine for a viral effect, the average differences were summed across methacholine challenge concentrations for each challenge session. The differences between the acute and convalescent challenge paired scores were averaged and compared with an expected difference of 0 under the null hypothesis of no effect with the two-tailed paired *t* test. Statistical significance was evaluated at a *p* value less than 0.05.

RESULTS AND DISCUSSION

Of the 131 subjects, 34 subjects with AR and 40 subjects without AR were infected. All infected subjects had serum neutralizing antibody titers to RV-H of 4 or less at baseline. The infected subjects experienced significant increases in nasal symptoms and secretion weights ($p < 0.05$), which peaked on days 2 to 3 and then lessened. None of the infected subjects had wheezing or other signs of lower respiratory tract disease during the study. No significant effects on allergy status were observed.

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TABLE I. Response variables for infected subjects with and without allergic rhinitis*

Response	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5
FEV _{1AR}	95 (13)	95 (12)	96 (11)	96 (12)	98 (15)	96 (13)
FEV _{1NAR}	97 (14)	96 (17)	97 (14)	95 (15)	96 (16)	96 (13)
FEF _{25-75AR}	94 (20)	97 (22)	96 (20)	97 (19)	99 (17)	98 (24)
FEF _{25-75NAR}	121 (36)	115 (35)	119 (33)	115 (31)	120 (40)	115 (34)
FVC _{AR}	94 (14)	93 (13)	94 (13)	93 (13)	95 (18)	92 (14)
FVC _{NAR}	91 (14)	89 (16)	90 (14)	89 (14)	89 (16)	89 (13)
SECWT _{AR}	1.4 (3)	2.2 (3)	6.7 (11)	6.6 (10)	4.7 (7)	3.6 (6)
SECWT _{NAR}	1.4 (3)	1.8 (3)	4.6 (6)	5.0 (6)	3.4 (4)	2.9 (4)
Symptom scores						
Nasal _{AR}	0.5 (.7)	1.3 (1)	3.3 (3)	3.4 (2)	3.0 (2)	1.8 (2)
Nasal _{NAR}	0.3 (.7)	1.0 (1)	2.4 (2)	3.2 (2)	2.7 (2)	2.0 (2)
Throat _{AR}	0.1 (.3)	0.5 (1)	1.0 (1)	0.8 (1)	1.1 (1)	0.8 (1)
Throat _{NAR}	0.2 (.5)	0.6 (1)	1.1 (1)	1.2 (1)	1.1 (1)	1.1 (1)

AR, Subjects with allergic rhinitis; NAR, subjects without allergic rhinitis; SECWT, secretion weight.

*Results are presented as mean (SD) values.

For the infected subjects, there were no significant postinoculation changes from baseline in FEV₁, forced vital capacity, forced expiratory flow, mid-expiratory phase, and FEV₁/forced vital capacity, and no allergy status effects were observed (Table I). Additionally, there were no significant viral effects on the degree of methacholine responsiveness in the subset of infected subjects with or without AR. During the acute phase, only two subjects, both in the AR subgroup, exhibited a measurable PC₂₀, 17.6 and 16.3 mg/ml. Both of these subjects had a lower PC₂₀ value during the convalescence phase, 6.27 and 14.1 mg/ml, respectively. None of the subjects without AR had a measurable PC₂₀ value.

The results of this study showed that a large number of healthy, nonasthmatic individuals did not experience detectable changes in the level of lower respiratory tract symptoms, pulmonary function, or sensitivity of the lower airways to methacholine during an experimental RV-H infection in a highly controlled environment. This was despite the fact that the maximum dose of methacholine (50 mg/ml) used to assess hyperresponsiveness was higher than that used in previous studies.^{3,4} Moreover, inhalant allergy was not identified as a predisposing factor for such changes. These findings are contrary to the results of several earlier studies in which experimental RV-16 infections were used^{1,2} but were similar to results reported during experimental infection with other rhinovirus serotypes and influenza A virus.³⁻⁵

Despite the conflicting results of studies with experimental inoculations, at least one common theme has emerged, which may implicate certain mechanisms or identify certain prerequisites for lower respiratory tract involvement. None of the viruses delivered in the experimental settings have been reported to trigger acute

asthma or to significantly alter routine spirometric parameters, even when the subject pool included patients with asthma.⁶ The lack of enhanced methacholine responsiveness in subjects with AR infected with RV-39, influenza A, and now RV-H may be due to a lack of viral deposition or infection in the lower airways. In all of these studies, the viral inoculum was delivered by coarse droplets. Such deposition, followed by infection, could be promoted by nebulization of the viral inoculum.^{1,2} Indeed, evidence that RV-16 can infect the lower airways under such conditions has been reported.⁷ A more remote possibility is viral strain specificity.

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