Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Rhinovirus 39 infection in allergic and nonallergic subjects

William J. Doyle, PhD,* David P. Skoner, MD, Philip Fireman, MD,** James T. Seroky, MA,* Israel Green, MD,* Frederick Ruben, MD,*** David R. Kardatzke, MPH,**** and Jack M. Gwaltney, MD*******

Pittsburgh, Pa., and Charlottesville, Va.

To determine if individuals with allergic rhinitis are hyperresponsive to upper respiratory tract viral infections, 20 allergic and 18 nonallergic, susceptible, adult volunteers were challenged and infected with rhinovirus type 39 before the pollen seasons. Before challenge and on each of 6 days of cloister, all volunteers were interviewed for symptoms and completed a test battery consisting of evaluations of secretion production by weighed tissues, nasal patency by active posterior rhinomanometry, nasal clearance by the dyed saccharin technique, pulmonary function by spirometry, eustachian tube function by sonotubometry, and middle ear status by tympanometry. The symptomatology and pathophysiology resulting from the rhinovirus infection were consistent with those reported in previous studies with this challenge system. Between-group comparisons revealed no differences in symptom presentation, nasal secretion production, or overall pathophysiologic response. However, for decreased mucociliary clearance rate, increased nasal congestion, eustachian tube dysfunction, and symptoms of sneezing, the allergic group demonstrated an earlier onset compared with that of the nonallergic group. The biologic significance of the differences in onset of dysfunction is tempered by the observation that the temporal pattern of responses in the allergic group was similar with that of nonallergic subjects in previous studies. The results of the present study do not support the hypothesis of a physiologic hyperresponsiveness to rhinovirus type 39 infection in allergic subjects during nonallergy seasons. (J ALLERGY CLIN IMMUNOL 1992;89:968-78.)

Key words: *Rhinovirus, allergy, eustachian tube, URI, nasal work*

In patients with AR, a nasal hyperresponsiveness to intranasal challenges with a variety of substances, including inflammatory mediators, has been reported.[1-4] A number of these substances have been implicated as causal in the development of the diverse pathophysiologies accompanying a viral URI.[4-8] Also, in patients with AR, a functional abnormality in the autonomic nervous system and, specifically, a hypersensitivity of the cholinergic nervous system have been demonstrated.[2, 9, 10] These findings could affect the response of subjects with AR to URI in which the production of secretions, rhinorrhea, and other nasal symptoms have been related to activation of the parasympathetic-cholinergic nervous system.[11-13] These observations suggest that patients with AR may experience a more pronounced symptomatology/patho-

Abbreviations used

AR:	Allergic rhinitis
URI:	Upper respiratory infection
RV-39:	Rhinovirus type 39
CHP:	Children's Hospital of Pittsburgh
FAST:	Fluoroallergosorbent test
ANOVA:	Analysis of variance
SPT:	Skin prick test
ET:	Eustachian tube

From the *Department of Otolaryngology and **Department of Pediatrics, Division of Allergy, Immunology, and Rheumatology, Children's Hospital of Pittsburgh and the University of Pittsburgh School of Medicine; the ***Department of Medicine, University of Pittsburgh School of Medicine, ****Department of Biostatistics, School of Public Health, University of Pittsburgh, Pa.; and the *****Department of Internal Medicine, University of Virginia, Charlottesville, Va.

Supported in part by National Institutes of Health Grant AI 19262, and General Clinical Research Centers Grant MO/RR 000 84.

Received for publication June 14, 1991.

Revised Nov. 26, 1991.

Accepted for publication Jan. 7, 1992.

Reprint requests: William J. Doyle, PhD, Dept. of Pediatric Otolaryngology, One Children's Place, 3705 Fifth Ave. at DeSoto St., Pittsburgh, PA 15213-2583.

1/1/36125

physiology when they are compared with individuals without AR during a URI episode. This relationship has been implicitly recognized by researchers, as evidenced by the frequent use of AR as an exclusion/stratification criterion in controlled studies of therapies for URI. One recent study evaluated the response of subjects with and without AR to experimental infection with coronavirus 229E. The investigators reported significantly higher clinical cold scores in subjects with measurable IgE in their nasal secretions and trends favoring higher scores in subjects with high levels of serum IgE (>150 IU/ml) or with positive skin tests to a panel of allergens.[14] These investigators suggested that immunologic imbalances, either hormonal or cell mediated, which have been associated with atopy, cause the increased severity of cold symptoms in the group with AR.[15-17] If this finding is confirmed, this mechanism may explain the purportedly higher incidence in the allergic population of disease entities commonly associated with URI, such as otitis media, sinusitis, and acute asthma.[18-20] In this study, the responses of subjects with and without AR to RV-39 URI are compared for evidence of physiologic hyperresponsiveness in the former with subjective symptom scores and objective assessments of pathophysiology as outcome measures.

MATERIAL AND METHODS

Population

Potential subjects were recruited from the University of Pittsburgh and surrounding community by newspaper advertisements. Also, subjects enrolled in previous intranasal allergen challenges conducted at our center were invited to participate. In a telephone interview, applicants were questioned as to general health and allergic sensitivities. Subjects with a history of asthma or perennial rhinitis were excluded from consideration. All interested subjects reported to the CHP Allergy Clinic, and a history was taken. Subjects had an ear, nose, and throat and general physical examination, and blood was obtained. Sera was first screened for neutralizing antibody to RV-39, and those samples with titers of <2 were submitted for assay of specific IgE antibody titer to common inhalant allergens (RAST). All subjects with serum-specific antibody titers to RV-39 of <2 had SPTs with ragweed mix, grass mix, tree mix, *Alternaria, Aspergillus,* and *Hormodendrum* extracts. A positive diagnosis of AR was made on the basis of positive history, positive skin tests to inhalant allergens, and elevated specific serum-IgE antibodies. Control subjects had a negative history for allergies, negative skin tests to the panel of allergens, and no evidence of elevated specific serum-IgE antibodies. Eighteen subjects without AR (five men and 13 women) and 20 subjects with AR (12 men and eight women, 18 to 44 years of age), were enrolled after providing an informed consent. The protocol was reviewed and approved by the Institutional Review Board at CHP.

Experimental plan

Subjects were randomly assigned to one of two cohorts with cohort 1 (N = 18; eight subjects without AR and 10 with AR) studied 2 weeks before cohort 2 (N = 20; 10 subjects without AR and 10 with AR). The study of both cohorts was conducted before the pollen seasons (March). Challenge virus, methods of evaluation, and duration of follow-up were identical for the two cohorts. For the period of study, the subjects were asked to refrain from taking any over-the-counter or prescription medication with the exception of birth control pills. Two days before viral inoculation (study day 2), subjects reported to the laboratories of the CHP during the morning or afternoon hours. At that time, a blood sample was obtained and submitted for assay of total serum IgE and specific IgE titers (FAST, 3M, Santa Clara, Calif.) to a panel of allergens, including short ragweed, grass, oak, house dust mite, and molds. Preinfection, baseline symptom scores were obtained, and a battery of physiologic tests was administered. A nasal lavage was performed for viral culture. These data for symptoms and physiologic function were also collected immediately before viral inoculation that was administered between 5 PM and 9 PM on study day 0. Forty to 48 hours after challenge, the subjects reported to a hotel located within 5 miles of the CHP and were cloistered in individual rooms for a 6-day, 5-night period (study days 2 through 7). During cloister, a nasal lavage was performed each morning. Daily data for symptoms, secretion production, and physiologic functions were collected. Twenty-one days after release from the cloister, the subjects reported to the laboratory for an exit interview, and bloods were obtained for assay of convalescent antibodies to RV-39.

Viral challenge

The challenge virus pool was a safety tested, clinical isolate RV-39, passaged twice in WI-38 human embryonic lung fibroblasts. Viral challenges were performed as previously described.[21] Briefly, with the subjects sitting erect and their neck hyperextended, 0.25 ml of inocula per nostril was administered intranasallay by pipet as coarse drops. The subjects were instructed to perform a lateral head sway to insure more complete distribution to the nasal mucosa and to refrain from sneezing or nose blowing for 30 minutes. The procedure was repeated after a 15- to 30-minute waiting period for a total dose of 100 $TCID_{50}$.

Assessments of infection

For viral culture, nasal lavages were performed on days −2 and 2 during the afternoon or evening hours, and on days 3 to 7 between 6 and 7 AM. With the subject sitting erect and neck hyperextended, 5 ml of sterile saline was instilled into each nostril while the subject closed the oral-nasal port. After a period of approximately 20 seconds, the subject expelled the wash fluid into a cup. Recovered fluids varied in quantity but averaged approximately 6 ml (total) and were immediately transferred to collection tubes, placed on ice, and submitted to the virology laboratories for culture. Methods for virus culture were previously described.[21]

Preinoculation and convalescent serum samples were tested for neutralizing antibodies to RV-39 as previously described.[21] Infection was defined as active shedding on any day of cloister and/or seroconversion to a titer of at least 8.

Assessments of illness

On the two baseline test days (day −2 and 0) and on each day of cloister (days 2 to 7), subjects had an ear, nose, and throat examination and were interviewed regarding the presence and extent of symptomatology. During cloister, these interviews and examinations were conducted between 12 PM and 1 PM. Eight specific symptoms, including sneezing, nasal discharge, nasal congestion, malaise, headache, chilliness, sore throat, and cough were rated by the subject on a 0 to 3 scale corresponding to none, mild, moderate, or severe. On the day of release from cloister (day 7), the subjects were asked if they believed that they had a cold. For data presentation, the criteria for a cold were modified from that of Jackson to require a total interview symptom score for the period of cloister of >5 and either symptoms of nasal discharge for more than 2 days or the subject's impression that they had a cold.[5]

To provide a more complete temporal description of symptomatology, all subjects maintained a 14-day symptom diary (days 0 to 13). For the diary data, the same eight symptoms were scored on a scale of 0 to 5 at 6 PM on each day. To provide a measure of secretion production during the period of cloister, subjects expelled all nasal secretions into preweighed tissues and sealed expended tissues in plastic baggies. These were collected at 10 AM on each day and weighed. Secretion weights were determined by subtraction.

Physiologic assessments

Physiologic testings included assessments of middle ear pressure, nasal patency, pulmonary function, ET function, and nasal clearance function. Middle ear pressures were measured with a commercially available, automatic digital tympanometer (model TA-7A, Teledyne Avionics, Charlottesville, Va.). Instrument output consisted of external canal volume, tympanic membrane compliance, and middle ear pressure. Nasal patency was measured by active posterior rhinomanometry with a custom-built, computer-assisted rhinomanometer previously described.[22] For testing, subjects breathed normally into a mask sealed against the face and serially aligned to a flow sensor. Upstream mask pressure was referenced via a differential transducer to that in the oral cavity. Voltages from the sensors were digitized and routed to the memory of an IBM AT computer for editing and analysis. Software programs computed the work performed in moving a liter of air on inspiration. This variable (work/liter) is a direct and linear measure of congestion, and its value was recorded at each test session. Pulmonary function was measured by a commercial spirometer (Multispiro-PC, Medical Equipment Designs, Inc., Laguna Hills, Calif.) interfaced to an IBM AT personal computer. Forced expiratory maneuvers were performed three times at each testing, and the maximum FEV_1 referenced to a panel of normal subjects (percent FEV_1) was recorded as the test value. ET function was evaluated with the technique of sonotubometry and a custom instrument developed in our laboratories.[22] Briefly, a white noise of known sound pressure was supplied by a high-volume speaker to the nose via a coupling at the nostril. Insert microphones were placed in the external auditory canals and shielded from ambient noises with padded earphones. The individual was asked to swallow, a maneuver triggering activity of the tubal dilatory musculature, and the changes in sound pressure in the ear canals were monitored. Transient (50 to 500 milliseconds) increases in canal-sound pressures of at least 5 dB were recorded as tubal dilations. At each session, the test was performed a total of four times. A tubal dilation on any of the four swallowing attempts was considered to be a positive test. Nasal-clearance function was assessed with the dyed saccharin technique as previously described.[22] For this test, 15 μl of a test solution was placed bilaterally in the anterior part of the nasal cavity on the mucosa just behind the internal ostium. The test solution consisted of 8 mg/ml of indigo carmine, 3 mg/ml of saccharin, and 45 mg/ml of sorbitol (pH adjusted to 7.4). The subjects swallowed as required and were asked to report the first occurrence of a sweet taste; then the pharyngeal cavity was examined for the appearance of the blue dye. Repeat inspections of the pharynx were made at 1-minute intervals to a total of 30 minutes or until the dye was observed unilaterally. The time between administration of the dye and the subjects' reporting a sweet taste was recorded as the nasal mucociliary-clearance time.

Before each test session, the recording instruments were calibrated. Nasal clearance and pulmonary function were assessed on each of the baseline test days and once daily for the period of cloister. Rhinomanometry, tympanometry, and ET function tests were performed once daily on days −2, 0, 2, and 7, and three times per day in morning, afternoon, and evening test sessions on days 3 to 6. This test battery and sequence has been used in previous studies of experimental rhinovirus infection without incident or demonstrable effect on measured responses.[22, 23]

Statistical methods

For analysis, secretion weight, work per liter, clearance time, percent FEV_1, the combined symptom score (sum of eight interview symptom scores), and nasal symptom score (sum of rhinorrhea, congestion, and sneezing interview scores) were considered to be continuous variables. Middle ear pressures were categorized as abnormal if they were $> +30$ or < -80 mm H_2O. ET function test results were classified as normal if dilation occurred on any of four deglutition attempts within a 3-minute period. The average values for the continuous variables of the groups with and without AR recorded at baseline testings were compared with a Student's t test (two-tailed; evaluated at $P < 0.05$). Means of the postinfection values for the continuous variables were calculated over all study subjects and postinfection means were compared to baseline with a Student's t test (two-tailed; evaluated at $P < 0.05$). For dichotomous variables, the chi-squared statistic (with Yates' correction for continuity) was used for these comparisons. To deter-

TABLE I. Summary data

	Clearance time (min)	Mucus weight (gm)	FEV_1 (%)	Work (MJ)	Symptom score	
					Total	Nasal
Baseline*						
AR	8 ± 5	ND	96 ± 9	169 ± 76	0.5 ± 0.9	0.4 ± 0.2
Without AR	7 ± 4	ND	94 ± 7	169 ± 53	0.5 ± 1.2	0.2 ± 0.5
Status†						
AR	12 ± 6	38 ± 37	0 ± 4	74 ± 75	3.2 ± 1.7	1.7 ± 0.9
Without AR	8 ± 5	35 ± 32	1 ± 5	50 ± 56	3.5 ± 2.6	2.0 ± 1.6
Session†						
Cohort 1	11 ± 7	37 ± 32	0 ± 5	66 ± 80	4.4 ± 1.8	2.6 ± 1.1
Cohort 2	10 ± 5	37 ± 36	2 ± 4	59 ± 54	2.4 ± 2.1	1.0 ± 1.1
F values‡						
Status	3.2§	0.1	0.4	1.1	0.4	1.0
Session	0.1	0.0	1.4	0.1	0.6‖	17.5‖

ND, Not done.
*Measured variable on baseline testing.
†Postinfection response variable (see text).
‡Postinfection data comparisons.
§$p < 0.1$.
‖$p < 0.01$.

mine if allergic status affected the pathophysiologic response to rhinovirus infection, the following response variables were calculated for each study subject: total weight of secretions expelled during the period of cloister and the difference between the postchallenge and baseline values averaged across test session of nasal clearance time, inspiratory work per liter, and combined symptom score and nasal symptom score. For each variable, the contributions to total variance associated with allergic status and cohort assignment were determined and evaluated for statistical significance with ANOVA (Table I).

RESULTS

Allergic status

All subjects considered to be allergic had positive SPTs to at least one of the seasonal inhalant allergens tested. In all subjects with AR, at least one of the inhalant sensitivities was confirmed by RAST. Eight of these subjects also had positive skin reactions to one of the two molds evaluated. Total IgE antibody ranged from a low of 20 to a high of 900 IU/ml (mean, 207 ± 220; median, 128). Elevated specific serum-IgE antibodies (>3 IU/ml) to at least one of the three inhalant allergens was documented by FAST in 17 of the 20 subjects. Also, seven subjects had elevated IgE antibody against dust mite, but none had elevated IgE antibodies to the two molds tested. Of the 18 subjects classified as nonallergic, none had positive skin tests to the panel of allergens or positive RAST results. Total IgE antibody ranged from 1 to 300 IU/ml (mean, 36 ± 70; median, 13). One of the 18 subjects had a low specific serum-IgE antibody titer ($0.3 < IgE <$ 0.6 IU/ml) to three inhalant allergens and two other subjects had low specific IgE antibody titers ($0.3 < IgE < 3$ IU/ml) to dust mite when IgE was measured by FAST.

Infection

All 38 subjects were infected and shed virus. The average number of days of viral shedding was not different for groups with AR (4.6 ± 0.8) and without AR (4.7 ± 0.8). Eight of 20 subjects with AR (40%) and 10 of 18 subjects without AR (56%) had convalescent antibody titers to RV-39 of 8 or higher that was considered to be indicative of seroconversion.

Illness

Fifteen of 18 subjects without AR (83%) and 17 of 20 subjects with AR (85%) had a cold on the basis of the modified Jackson criterion.[5] All six subjects without a cold were in cohort 2. The averages of the combined symptom score and nasal symptom score for subjects with and without AR were not significantly different at baseline. Both the combined and nasal symptom scores demonstrated a significant increase during the postinfection cloister period. ANOVA documented a significant effect of cohort assignment but not allergic status on these two summary scores (Table I). Data for each of the eight symptoms are presented in Table II as the average of the total scores (summed across days) collapsed across cohorts for groups with and without AR. None of these specific symptom scores was significantly different

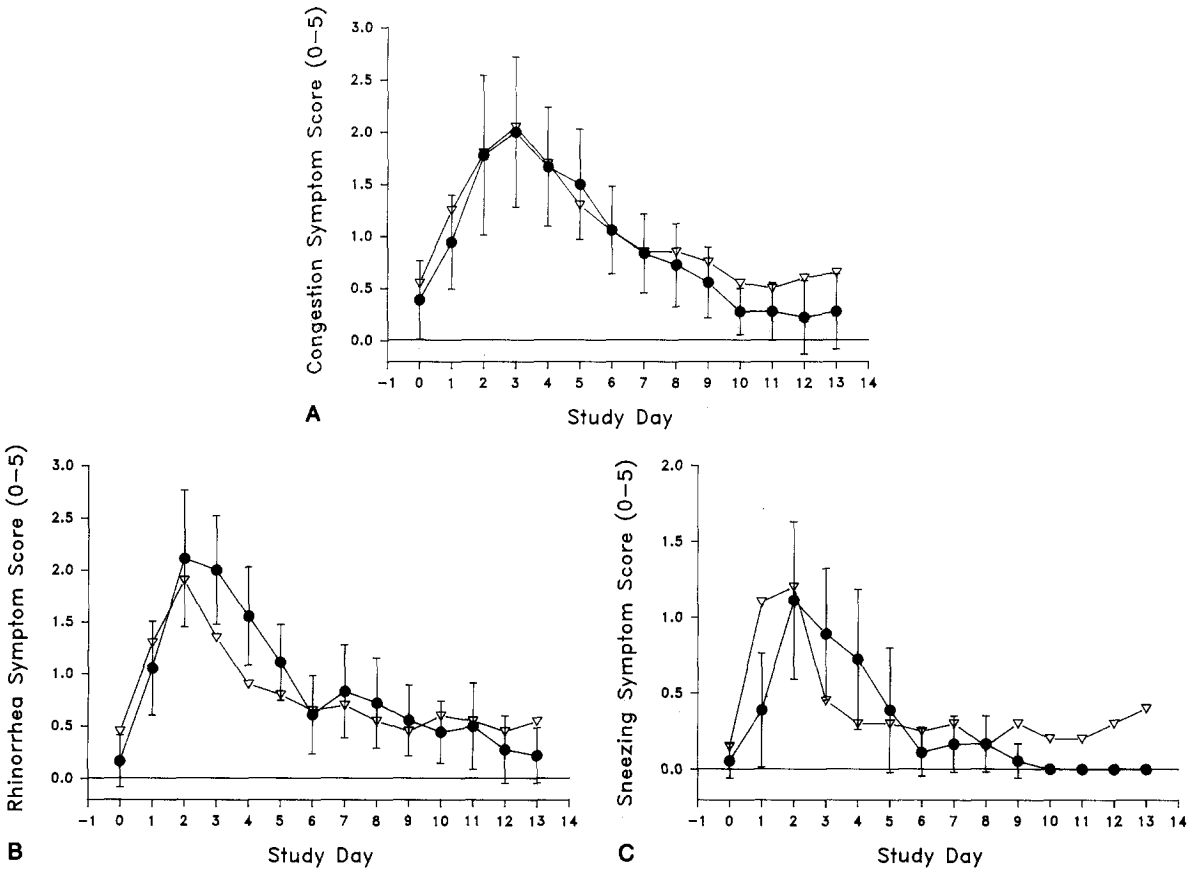

FIG. 1. Means of diary-symptom scores as a function of study day of groups with AR (△) and without AR (●). **A,** For nasal congestion. **B,** For rhinorrhea. **C,** For sneezing. *Vertical bars,* 95% confidence interval means for group without AR.

between groups with and without AR. Examination of the 14-day, diary-symptom data demonstrated that with the exception of sneezing, the onset, magnitude, and extent of each of the eight symptoms were similar in groups with and without AR. These longitudinal data are illustrated for the nasal symptoms of congestion, rhinorrhea, and sneezing in Fig. 1, *A* to *C*. In general, symptoms revealed a slight increase on day 1, a peak on day 2 or 3, and a return to the respective baseline values by days 6 to 10. For sneezing, groups with and without AR demonstrated similar magnitudes (maximum score, 1.2) and extent (approximately 4 days), but the subjects with AR had an earlier onset (day 1 versus day 2).

The total secretion weight expelled during the period of cloister was not significantly influenced by cohort assignment (cohort 1, 37 ± 32 versus cohort 2, 37 ± 36 gm) or allergic status (with AR, 38 ± 37 versus without AR, 35 ± 32 gm). There were no differences between subgroups with and without AR in the temporal pattern or amount of secretion produced on any study day. Corresponding average values for subjects with AR versus subjects without AR are 6.3 ± 6.4 versus 5.6 ± 6.9; 11.9 ± 12.8 versus 12.5 ± 12.6; 7.6 ± 8.7 versus 6.6 ± 5.8; 7.1 ± 7.7 versus 5.8 ± 6.2; and 5.2 ± 6.0 versus 4.5 ± 5.2 gm for days 2, 3, 4, 5, and 6, respectively.

Physiologic testings

Nasal work per liter inspired is a direct and linear measure of nasal congestion. Before challenge, the average values of the work for the group with AR (169 ± 76 mj) and the group without AR (169 ± 53 mj) were not different. As illustrated in Fig. 2, average postinfection values were significantly increased for both groups. The increase occurred earlier and was of greater magnitude in the subjects with AR. However, ANOVA did not identify significant effects of either cohort (66 ± 80 versus 59 ± 54 MJ) or subgroup assignment (with AR, 74 ± 75 versus without AR, 50 ± 56 mj) for this variable. Also, between-group comparisons of the average values for each of the test days revealed no statistically signficiant differences (Student's *t* test, two-tailed).

The nasal-clearance time is an inverse measure of clearance rate. Before challenge, the clearance times

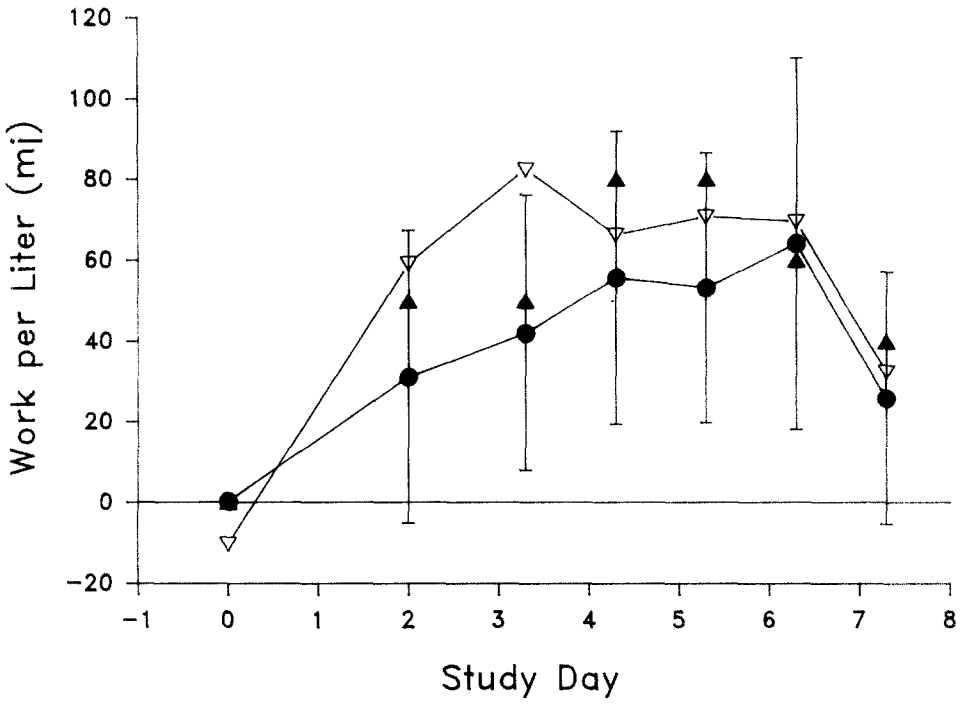

FIG. 2. Average inspiratory work per liter corrected for baseline of subjects with AR (△) and without AR (●) as a function of study day; historical average data for this variable from a group of untreated subjects without AR[22] (▲). *Vertical bars,* 95% confidence interval for group without AR.

were not significantly different for the groups with AR (8 ± 5 minutes) and without AR (7 ± 4 minutes). Significant prolongations in clearance time were observed after infection, with subjects with AR demonstrating an earlier and more extreme response when they were compared with subjects without AR (Fig. 3). No significant effect of cohort assignment was associated with this variable (11 ± 7 versus 10 ± 5 minutes). A trend favoring the subgroup without AR ($p = 0.08$) was documented by ANOVA. Collapsing the data for the two cohorts, average values for the groups with and without AR were 8 ± 6 versus 8 ± 5; 8 ± 6 versus 6 ± 4; 10 ± 8 versus 6 ± 4; 17 ± 10 versus 12 ± 9; 21 ± 9 versus 15 ± 9; 25 ± 7 versus 19 ± 8; 23 ± 9 versus 20 ± 8; and 24 ± 9 versus 20 ± 9 minutes for days −2, 0, 2, 3, 4, 5, 6, and 7, respectively. The between-group differences were significant on postchallenge days 2, 4, and 5 (Student's *t* test, two-tailed; $p < 0.05$).

Pulmonary function measured by the variable, percent FEV_1, was not different between subjects with and without AR at baseline. No significant changes in this variable were observed for either group consequent to the rhinovirus infection. Longitudinal data for each individual were examined and confirmed the lack of response.

On baseline testing, the frequency of ears with documented ET dilations during swallowing was not different for the groups with AR (71%) and without AR (69%). After infection, the frequency of ET dilations during the test sessions decreased and varied about a mean frequency of 38% for subjects with AR and 44% for subjects without AR. The difference in response between groups was not significantly different for test sessions conducted on days 3 to 7. However, on day 2, a significantly lesser number of ears in the group with AR (N = 12; 30%) tested positive for tubal opening compared with that of the group without AR (N = 22; 61%) (chi-squared, 6.21; $p < 0.02$). A similar pattern was observed when individuals were considered rather than ears as the experimental unit. Before challenge, 83% of individuals in the groups with and without AR had at least unilateral tubal dilations. This finding was decreased to an average frequency of 55% for the subjects with AR and 65% for the subjects without AR during the period of active infection. Significant between-group differences were only observed on day 2 in which seven subjects with (35%) and 13 subjects without AR (72%) had at least unilateral tubal dilations (chi-squared, 3.88; $p < 0.05$).

As previously reported, both positive and negative abnormal middle ear pressures were observed after infection. On baseline testing, approximately 10% of the ears in groups with AR and without AR were categorized as abnormal observations. The frequency

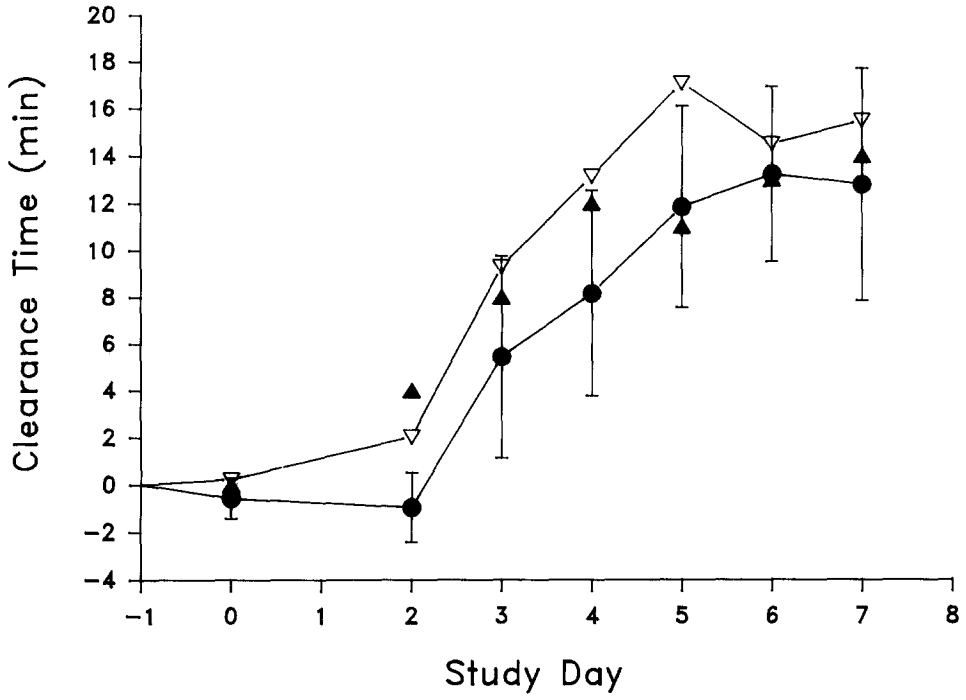

FIG. 3. Average clearance times corrected for baseline of subjects with AR (△) and without AR (●) as a function of study day; historical average data for this variable from a group of untreated subjects without AR[22] (▲). *Vertical bars,* 95% confidence interval for group without AR.

of abnormal observations increased to about 27% in the group with AR and 33% in the group without AR during days 3 to 6. Lesser frequencies of abnormal observations were observed for both groups on day 7. This pattern of response was unchanged when individuals were considered as the experimental unit. Specifically, before challenge, 17% of subjects with AR and 14% of subjects without AR demonstrated abnormal pressures in at least one ear. These frequencies increased to about 32% for subjects with AR and 38% for subjects without AR for days 3 to 6. There were no significant differences between groups in the frequency of abnormal middle ear pressures.

DISCUSSION

Previous studies of nonallergic, adult volunteers challenged with rhinovirus reported that infection results in an early and acute period of sneezing and secretion production with primary expression of symptoms as nasal congestion, rhinorrhea, sneezing, and malaise.[8] More recent studies documented an increased nasal work of breathing, decreased nasal ciliary-clearance rate, and increased frequencies of functional ET obstruction and abnormal middle ear pressures during periods of active infection.[22, 23] In the present study, these symptomatic and pathophysiologic responses were reproduced after RV-39 infection in subjects with AR and without AR. In general, the responses of the subjects in this study were similar in magnitude, extent, and frequency to those previously reported. Additionally, in the present study, pulmonary function (percent FEV_1 predicted) was measured daily during the course of the infection and was demonstrated not to be changed from the preinfection baseline.

Because of limitations imposed by the extensive testing protocol, the study population was divided into two cohorts that were studied sequentially within a 30-day period. The challenge protocol, viral inocula, location of cloister, and timing were identical for the two challenge sessions. The results reported in the Table I reveal that, for the objective measures of clearance time, secretion weight, and work per liter, no differences between cohorts in the response of the subjects were documented. However, all 18 subjects (100%) enrolled in cohort 1 had a cold by the modified Jackson criterion,[5] whereas only 14 of 20 (70%) subjects enrolled in cohort 2 had a cold. This finding most likely reflected the significantly lower symptom scores reported by subjects in cohort 2. A comparison with published studies with this challenge system demonstrates that the results for cohort 2 are more representative of previous experiences with RV-39.[22-24] Also, one recent study reported widely divergent fre-

TABLE II. Average (standard deviation) for specific interview symptoms

	Total symptom score		Total minus baseline score*	
	AR	NAR	AR	NAR
Congestion	6.6 (3.9)	7.0 (4.2)	4.8 (3.2)	5.3 (4.6)
Rhinorrhea	4.6 (2.7)	5.3 (2.8)	3.7 (2.0)	4.5 (3.3)
Malaise	3.2 (3.4)	2.4 (2.8)	2.9 (3.3)	1.8 (2.1)
Sore throat	2.7 (2.9)	3.9 (3.5)	2.5 (2.9)	3.3 (3.7)
Sneezing	2.3 (2.0)	2.3 (2.4)	1.7 (2.0)	2.0 (2.9)
Cough	2.0 (2.4)	2.8 (2.6)	1.8 (2.3)	1.8 (2.7)
Headache	1.9 (2.7)	2.2 (3.3)	1.7 (2.5)	1.6 (3.6)
Chills	0.2 (0.5)	0.6 (1.2)	0.2 (0.5)	0.6 (1.2)

NAR, Without AR.
*Average values after subtraction of baseline values.

quencies of colds in infected volunteers for two sequential cohorts (33% versus 73%), although other measures, including secretion weight, were consistent for the two groups.[25] These and other data suggest that the quantification of perceived symptoms is highly variable among individual subjects despite a similar underlying pathophysiologic extent. Therefore, we believe the differences in symptoms observed for the two cohorts result from chance subject assignment and do not reflect a systematic effect associated with differences in the challenge sessions.

Early research demonstrated that cytopathology was not extensive in rhinovirus colds, leading to the suggestion that the symptoms and pathophysiologies associated with rhinovirus infections are mediated by the release of inflammatory substances.[8] A nasal hyperresponsiveness of subjects with AR to intranasal challenge with a variety of inflammatory substances has been reported.[1-4] This finding appears to be most pronounced for provoked secretion production and frequency of sneezing and has been interpreted as revealing a cholinergic hyperreactivity of the nasal mucosa in patients with AR.[9, 10] Because these same substances have been implicated as mediators of inflammation during viral URI and because activation of the cholinergic nervous system is associated with disease expression,[11-13] we hypothesized that subjects with AR would develop more symptoms and more extensive pathophysiologies compared with those of subjects without AR when they were infected with rhinovirus. One recent study reported trends favoring higher clinical scores in allergic subjects with induced coronavirus infections when scores were compared with scores of nonallergic subjects. However, in that study, assignment of allergic status was made post hoc in a study population in which the recruitment procedures were designed to exclude subjects with manifest allergic disease.[14] Moreover, the significant difference in clinical scores between subjects with and without IgE in nasal secretions could be explained as a greater transudation of serum proteins, including IgE in subjects with more severe cold symptoms (e.g., rhinorrhea).

The results of the present study demonstrated that, for symptoms, a hyperresponsiveness of subjects with AR could not be supported. In our rhinovirus-infected subjects, the combined symptom load was almost identical, for subjects with and without AR. Also, the nasal symptom scores and average scores for individual symptoms were similar and not statistically different for the two groups. Indeed, seven of the eight average individual symptom scores were higher in the group without AR. Examination of the daily scores for the various symptoms demonstrated that, with the exception of sneezing, their temporal patterns were identical in onset, magnitude, and extent for the two groups. Sneezing demonstrated an earlier onset in the group with AR resulting in a 1-day left shift in the temporal profile.

When the objective measures of pathophysiology are considered, an interpretation of the data is more ambiguous. Although secretion production was identical for the two groups, both when secretion was expressed as a total weight summed across study days or weights for each individual study day, the response curves for other measures were shifted 1 day later in the group without AR. This was true, as noted above, for symptoms of sneezing and for measures of nasal patency, mucociliary clearance rate (inverse of clearance time), and ET function. For these variables, the summary response descriptors entered into the ANOVA were not significantly different between the groups, but for the latter two functions, significant differences were detected on individual study days early in the course of the infection. These data are interpretable as documenting an earlier onset of the

deficit function in the group with AR compared with the subjects without AR.

To evaluate the biologic significance of the differences in onset of dysfunction, these data from the present study were compared with data reported previously. Unfortunately, experience with the objective measures of nasal patency, nasal clearance, ET function, and middle ear pressure within the context of the rhinovirus challenge system is less extensive than for the other measures. However, in one recent study, results were presented for 40 untreated subjects challenged with RV-39 and evaluated with a protocol identical to that used in the present study.[22] The longitudinal data reported in that study for clearance time and work per liter are overlaid on the corresponding data for the present study in Figs. 2 and 3. For both measures, the results for the earlier study document an impairment as early as day 2 after infection that agrees better with the observed onset for the group with AR than for the group without AR in the present study. For ET function, a 40% decrease in ears with good tubal function was observed on day 2 that is comparable to the 41% decrease observed on that day in the group with AR (versus 9% decrease in the patients without AR). For all functions, the magnitudes of the responses were similar in the group with and without AR and historical control subjects. Thus, although the subjects with AR in the present study demonstrated an earlier onset for some physiologic dysfunctions when they are compared with concurrent subjects without AR, this difference is probably not attributable to allergic status of the subjects.

These results do not support the hypothesized physiologic hyperresponsiveness of patients with AR to rhinovirus infections. However, this interpretation is dependent on three conditions that we believe are satisfied. These are (1) that the assignment of subjects to comparison groups is based on an accurate diagnosis of allergic status, (2) that the statistical techniques have sufficient power for identifying reasonable differences in response between groups, and (3) that the induced colds provoked in this study reproduce the illness associated with their natural counterparts. In regards to the former, all allergic subjects had a positive history for seasonal allergies, positive SPTs to at least one of the inhalant allergens tested, and elevated specific IgE antibody titers measured by RAST. These data were confirmed with FAST assay. Also, the distribution of total IgE for the allergic group was consistent with that reported in the literature. Therefore, we are confident with the accuracy of assignments to the allergic group. Because accurate assignment to the control group constitutes proof of a negative condition, this is perhaps more equivocal. However, all the control patients had a negative history for allergy, had no positive skin tests to the panel of allergens tested, and displayed no specific serum-IgE elevations when IgE antibodies were measured by RAST. With the exception of two subjects (J. M. B. and P. J. H.), total IgE titers for control patients were low (<50 IU/ml) and specific IgE titers measured by FAST were negligible. Extended-panel FAST testing of the two subjects with elevated total IgE antibodies revealed low specific antibody titers to ragweed, grass, and oak in one case, and dust mite in the other case. However, elimination of these subjects from the data set did not affect the results of the study. Moreover, these negative results for between-group comparisons were robust to analysis with different criteria for allergic status.

Confidence in original group assignment is also gained from the results of a companion study with these subjects. In that study conducted 2 months before viral challenge, all subjects were challenged intranasally with histamine, and symptomatic and pathophysiologic responses were quantified. The defined allergic group had significantly more secretion production (3.1 ± 2.1 versus 1.4 ± 0.9 gm; mean ratio, 2.2), higher rhinorrhea symptom score (5.5 ± 3.9 versus 2.3 ± 2.3; mean ratio, 2.4) and sneeze count (10.0 ± 9.1 versus 4.9 ± 4.6; mean ratio, 2.0), and an elevated congestion symptom score (6.6 ± 2.7 versus 5.1 ± 2.6; mean ratio, 1.3) when the allergic group was compared with the control group (Doyle WJ. Unpublished data). These data reproduce previous work that reported increased sneezing and secretion production in subjects with nasal allergies, when they were challenged with histamine, and confirm that our assigned allergic subjects were hyperresponsive to that inflammatory mediator.[1-4]

With these data for the subjects without AR in the present study, the increase in AR responsiveness required for statistical significance at 80% power was determined. These calculations demonstrated that between-group differences of approximately 80% of the standard deviation for measured variables could be detected, assuming equal sample sizes of 20 and equivalent standard deviations for the comparison groups. None of the between-group differences in study variables approached the required value, and contrary to our hypothesis, the mean score for most symptoms was higher in the subjects without AR. The predicted mean ratios at statistical significance for most functions hypothesized to be hyperresponsive in subjects with AR with URI varied about 1.6, a value much less than the mean ratios of approximately 2 documented for the histamine-challenge study de-

scribed above. Thus, we do not believe that the failure to detect significant differences in the response of subjects with and without AR to a URI was related to an insufficient statistical power.

In numerous past studies with this challenge model and RV-39, >95% of the susceptible subjects were infected with rhinovirus. Also, a reproducible severity of illness has been documented with approximately one third of the subjects having little to no symptoms, one third reporting mild symptoms, and one third reporting moderate to severe illness. Similar proportions of illness severities were observed in this study. Overall, the average symptom scores are relatively low in comparison with scores reported by subjects with a "natural cold." This difference most likely reflects a presentation bias in studies of "natural colds." Assuming that the spectrum of symptom presentation documented in the model system is accurate, the approximately one third of patients with few symptoms and a proportion of patients with mild symptoms would not be included in the population of subjects with a "natural cold" since they would not present with sufficiently intense diagnostic symptoms. When this presentation bias is considered, a comparison of the symptoms for "natural cold" presenters with the two thirds of the subjects in the model who believed that they had a cold demonstrates good agreement. Moreover, the variability in subjective response to infection documented for the model system is prerequisite to the testing of the primary hypothesis that can be paraphrased as "allergic subjects with URI develop more extreme symptoms than nonallergic subjects with a URI." This cannot be evaluated in a population restricted to the most highly symptomatic individuals since the presentation bias would exclude the hypothesized less symptomatic patients without AR.

In summary, a physiologic hyperresponsiveness of patients with AR to rhinovirus infection was not supported by our data. This was particularly notable for sneezing and secretion production, which in previous studies were consistently demonstrated to be more intense in patients with AR compared with that in subjects without AR when they were challenged with inflammatory substances. However, nasal reactivity in subjects with AR was reported to increase with previous or continuous allergen exposure, a phenomena termed the "priming effect" by Connell,[26] Skoner et al.,[27] and van Wijk et al.[28] Consequently, the reactivity of subjects with AR may cycle seasonally with a maximum responsiveness during and immediately after seasonal pollen exposure. The juxtaposition of the natural ragweed (August to September) and rhinovirus (September to November) seasons in the United States affords a potential for priming of subjects with AR before viral infection. Because we disqualified subjects with symptoms of perennial rhinitis and performed this study well outside of the period for seasonal pollen exposure, the subjects with AR were not "primed" and therefore may not have been physiologically hyperresponsive at the time of viral challenge. The effect of allergen priming on the physiologic response of subjects with AR to rhinovirus infection will be evaluated in a future experimental trial.

REFERENCES

1. van Wijk RG, Dieges PH. Comparison of nasal responsiveness to histamine, methacholine and phenolamine in allergic rhinitis patients and controls. Clin Allergy 1987;17:563-70.
2. Asakura K, Enomoto K, Ara H, Azuma E, Kataura A. Nasal responsiveness to methacholine stimulation in allergic rhinitis patients. Arch Otorhinolaryngol 1984;239:273-8.
3. Okuda M, Ohtuska H, Sakaguchi K, Watase T. Nasal histamine sensitivity in allergic rhinitis. Ann Allergy 1983;51:51-5.
4. Doyle WJ, Boehm S, Skoner DP. Physiologic responses to intranasal dose-response challenges with histamine, methacholine, bradykinin, and prostaglandin in adult volunteers with and without nasal allergy. Allergy Clin Immunol 1990;86:924-35.
5. Doyle WJ, McBride TP, Skoner DP, Maddern BR, Gwaltney JM, Uhrin M. A double-blind placebo-controlled clinical trial of the effect of chlorpheniramine on the response of the nasal airway, middle ear and eustachian tube to provocative rhinovirus challenge. J Pediatr Inf Dis 1988;7:229-38.
6. Sperber S, Hendley JO, Hayden FG, Riker DK, Sorrentino JV, Gwaltney JM. Beneficial effects of naproxen in experimental colds [submitted]. J Infect Dis.
7. Naclerio RM, Proud D, Lichtenstein LM, et al. Kinins are generated during experimental rhinovirus colds. J Infect Dis 1988;157:133-42.
8. Turner RB, Hendley JO, Gwaltney JM. Shedding of infected ciliated epithelial cells in rhinovirus colds. J Infect Dis 1982;145:849-53.
9. Kaliner M. The cholinergic nervous system and immediate hypersensitivity. I. Eccrine sweat responses in allergic patients. J ALLERGY CLIN IMMUNOL 1976;58:308-15.
10. Smith LJ, Shelhamer JH, Kaliner M. Cholinergic nervous system and immediate hypersensitivity. II. An analysis of pupillary responses. J ALLERGY CLIN IMMUNOL 1980;66:374-8.
11. Jackson RT, Teichgraeber J. Low-dose topical atropine for rhinorrhea. Arch Otolaryngol 1981;107:288-9.
12. Gaffey MJ, Gwaltney JM, Dressler WE, Sorrentino JV, Hayden FG. Intranasally administered atropine methonitrate treatment of experimental rhinovirus colds. Am Rev Respir Dis 1987;135:241-4.
13. Gaffey MJ, Hayden FG, Boyd JC, Gwaltney JM. Ipratropium bromide treatment of experimental rhinovirus infection. Antimicrob Agents Chemother 1988;32:1644-7.
14. Callow KA, Tyrrell DAJ, Shaw RJ, Fitzharris P, Wardlaw AJ, Kay AB. Influence of atopy on the clinical manifestations of coronavirus infection in adult volunteers. Clin Allergy 1988;18:119-29.
15. Katz DH. Regulation of the IgE system: experimental and clinical aspects. Allergy 1984;39:81-106.
16. Leung DYM, Geha RS. Regulation of IgE synthesis in man. Clin Immunol Rev 1984;3:1-24.
17. Haeney M. An introduction to allergic disease. JR Coll Physicians Lond 1985;19:147-53.

18. Pelikan Z, Plikan-Filipek M. Role of nasal allergy in chronic maxillary sinusitis—diagnostic value of nasal challenge with allergen. J ALLERGY CLIN IMMUNOL 1990;86:484-91.
19. Bernstein JM, Lee J, Conboy K, Ellis E, Li P. The role of IgE mediated hypersensitivity in recurrent otitis media with effusion. Am J Otol 1983;5:66-9.
20. Lemanske RF, Dick EC, Swenson CA, Vrtis RF, Busse WW. Rhinovirus upper respiratory infection increases airway hyperreactivity and late asthmatic reactions. J Clin Invest 1989;83:1-10.
21. Gwaltney JM, Jordan W. Rhinovirus and respiratory illness in university students. Am Rev Respir Dis 1966;93:362-71.
22. Doyle WJ, McBride TP, Swarts JD, Hayden FG, Gwaltney JM. The response of the nasal airway, middle ear and eustachian tube to provocative rhinovirus challenge. Am J Rhinol 1988;2:149-54.
23. McBride TP, Doyle WJ, Hayden FG, Gwaltney JM. Alteration of eustachian tube function, middle ear pressure and nasal patency in response to an experimental rhinovirus challenge. Arch Otol 1989;115:1054-9.
24. Hayden FG, Gwaltney JM. Prophylactic activity of intranasal enviroxime against experimentally induced rhinovirus type 39 infection. Antimicrob Agents Chemother 1982;21:892-97.
25. Hsia J, Goldstein AL, Simon GL, Sztein M, Hayden FG. Peripheral blood mononuclear cell interleukin-2 and interferon production, cytotoxicity, and antigen-stimulated blastogenesis during experimental rhinovirus infection. J Infect Dis 1990;162:591-7.
26. Connel JT. Quantitative intranasal pollen challenge. III. The priming effect. J Allergy 1969;43:33-44.
27. Skoner DP, Doyle WJ, Boehm S, Fireman P. Priming of the nose and eustachian tube to intranasal challenge. Am J Rhinol 1989;3:53-7.
28. van Wijk RG, Dieges PH, van Toorenenbergen AW. Seasonal variability in nasal sensitivity to house dust mite extract. Rhinology 1987;225:41-8.

Role of sodium in mediator release from human basophils

Thomas F. Smith, MD,* Fernando Sanchez-Legrand, MD, PhD,* Lawrence P. McKean, MD, PhD, Michael H. Kutner, PhD,*** Edward J. Cragoe, Jr., PhD, and Douglas C. Eaton, PhD****** *Atlanta, Ga.*

We studied the effect of extracellular sodium concentration on histamine release (HR) from human basophils initiated by immunologic and nonimmunologic stimuli. We found that lowering extracellular sodium markedly enhances HR induced by an immunologic stimulus from these cells. In buffer in which sodium had been replaced with univalent ions of strong bases, enhancement of HR increased as extracellular sodium decreased. Enhancement was the result of increased duration of release. When sucrose was used for replacement of sodium, we also observed that enhancement of HR increased as extracelluar sodium decreased, but there was some lessening of enhancement at $[Na^+]_e$ between 5 and 10 mmol/L. Ouabain, which is an inhibitor of the Na^+/K^+ adenosine triphosphatase, and bumetanide and furosemide, which are inhibitors of Cl^--dependent Na^+-K^+ cotransport, caused small increases in enhancement of HR by sodium-deficient buffers; 4,4'-diisothiocyanostilbene-2-2'-disulfonic acid, an anion transport inhibitor, caused some inhibition of enhancement of HR. Analogues of amiloride, such as 5-(N-N-hexamethylene) amiloride (HMA) and 5-(N-4-chlorobenzyl)-2'-4'dimethylbenzamil (CBDMB), inhibit Na^+/H^+ exchange, Na^+/Ca^{++} exchange, and Na^+ channels. Interestingly, at higher doses, HMA and CBDMB caused marked enhancement of HR in both normal and sodium-deficient buffers. These results suggest that several cellular regulatory mechanisms potentially are important for normal basophil secretion. The most likely are pH regulatory mechanisms that include Na^+/H^+ exchange and anion exchangers that transport alkaline equivalents. Our finding enhancement of basophil HR by HMA and CBDMB is particularly noteworthy in light of the recent interest in use of amiloride by inhalation for therapy of lung disease in patients with cystic fibrosis. (J ALLERGY CLIN IMMUNOL 1992;89:978-86.)

Key words: *Histamine release, basophils, sodium*

From the *Division of Allergy, Immunology, and Respiratory Medicine, the **Division of Cystic Fibrosis, Department of Pediatrics, the ***Department of Biostatistics, and the ****Department of Physiology, Emory University School of Medicine, Atlanta, Ga.

Supported in part by grants from the Emory University Research Committee, the American Lung Association, the American Lung Association of Georgia, Biomedical Research Support Grant SO7RRO5364, and United States Public Health Service Grant AI21072.

Received for publication March 21, 1991.
Revised Oct. 23, 1991.
Accepted for publication Jan. 7, 1992.

Reprint requests: Thomas F. Smith, MD, Department of Pediatrics, 2040 Ridgewood Drive, NE, Atlanta, GA 30322.

1/1/36124