

Prechallenge Antibodies Moderate Disease Expression in Adults Experimentally Exposed to Rhinovirus Strain Hanks

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This double-blind study determined the influence of serum neutralizing antibody titers on the rate of infection and magnitude of disease expression after experimental exposure of adult volunteers to rhinovirus strain Hanks (RV-H). A total of 133 healthy volunteers were tested for antibody status, cloistered for a 6-day period, and challenged with RV-H at the end of the first cloister day. On these days, response to viral challenge is assessed with symptom diaries and physical examinations. The low-titer infected group was significantly different from the intermediate-titer infected and the uninfected groups in terms of postchallenge nasal and throat symptoms, expelled secretion weights, nasal mucociliary clearance rates, and frequency of negative middle ear pressures. A similar trend held for the infected high-titer vs. low-titer group comparisons. These data show that high homotypic serum neutralizing antibody titers are associated with protection from infection and lessened signs and symptoms following experimental RV-H exposure.

Upper respiratory infection (URI) is the most common disease in both children and adults. URIs are associated with nasal, throat, and systemic signs and symptoms and contribute greatly to morbidity in the human population. Moreover, URIs often precede and predispose to complications such as sinusitis, otitis media, pneumonia, and exacerbations of asthma, which further contribute to the morbidity and economic costs resulting from that disease [1–7]. The majority of URIs are caused by viruses, with the rhinoviruses contributing to >30% of these infections [8, 9]. Unfortunately, an effective treatment for rhinovirus colds is not available at present, and the high degree of antigenic variation in the rhinoviruses limits the feasibility of effective prevention by vaccination [10].

Past studies have shown that antibodies to rhinovirus are highly serotype specific, demonstrating little or no cross-neutralization of heterologous serotypes. The mechanism of antibody-mediated neutralization of rhinoviruses is not completely understood but has been suggested to involve alterations in the structure of the capsid, interference with viral attachment to cellular receptors, or altered kinetics of viral uncoating by intracapsid cross-linking [11]. Recently, we completed a large study of adults that was designed to define those factors that influence susceptibility to infection and the degree of manifestation of

symptoms, signs, pathophysiologies, and complications following experimental exposure to two rhinovirus strains. Because it was previously suggested that preexisting homotypic antibody titer affects susceptibility to infection and/or disease expression, the first analysis of these data was performed to evaluate the moderating role of that factor. Results for 147 subjects challenged with rhinovirus type 39 were presented in an earlier report [12]; this report focuses on subjects challenged with rhinovirus strain Hanks (RV-H). Similarities and differences between these virus strains in the level of protection afforded by homotypic antibodies are discussed.

Materials and Methods

Population. Subjects were recruited with use of a newspaper advertisement from the population and surrounding community of the University of Pittsburgh (Pittsburgh). Potential subjects were excluded if they had histories of previous nasal or otologic disease. A general physical examination was performed, as were a urinalysis; a blood assay for markers of hepatic and renal function; and assays of serum antibodies to HIV, hepatitis virus, and RV-H, with use of standard methods [12]. Subjects were excluded if they presented with findings or histories suggestive of systemic illness or recent URIs, if they had marked elevations in the assayed parameters indicative of hepatic or renal impairment, if they required prescription medication for any condition other than birth control, or if they had antibodies to HIV. The study was approved by the Human Rights Committee at the University of Pittsburgh, and all subjects provided written informed consent for HIV screening and study participation.

The study population for this report consisted of 133 healthy adult volunteers (age range, 18–54 years; mean age, 29.6 years)

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assigned to one cohort studied in November 1994 ($n = 50$) or two cohorts studied in March 1995 ($n = 41$; $n = 42$). There were 62 males (46.6%); 20 of the subjects (15%) were black, two (1.5%) were Asian, and the remainder (83.5%) were white. Protocols, procedures for monitoring the subjects, viral challenge strain, cloister site, and study personnel were identical for the three cohorts. All subjects and investigators were blinded to prechallenge antibody levels.

Protocol. After entry, the subjects were cloistered in separate rooms of a local hotel for a 6-day period (study days 0–5). On each day of cloister, symptoms were scored by the subjects; signs were evaluated by a physician; temperatures and vital signs were recorded; and objective measurements of nasal mucociliary transport rate, airway patency, secretion production, and middle ear pressures were made. In addition, nasal lavages were performed, and samples were submitted for quantitative rhinovirus culture. Twenty-four hours after admission to cloister (end of study day 0), all subjects were intranasally inoculated with ~ 300 TCID₅₀ of a safety-tested clinical isolate of RV-H as previously described [1, 2]. At the end of study day 5, the subjects were dismissed from the cloister but returned to the laboratory on days 19–21 for phlebotomy to assess convalescent antibody titers.

Methods of assessment. On arriving at the cloister site, the subjects were provided with preweighed tissues and were instructed to expel their nasal secretions into the tissues before they performed the rhinomanometric tests, and at other times as needed. Expended tissues were sealed in plastic bags of known weight. At the end of each day, the bags were weighed, and the total secretion weight expelled during the day was determined by subtraction.

During the morning of each day, subjects were given general physical examinations, vital signs were recorded, pulmonary function was evaluated by spirometry, and nasal lavages were performed for viral culture by using standard methods for isolation and quantification of rhinovirus [8, 13]. At that time, and then in the afternoon and early evening hours, oral temperatures were recorded. In the morning and evening of each day, nasal airway patency was evaluated by active anterior rhinomanometry with use of a microcomputer-assisted rhinomanometer developed in our laboratories. Inspiratory nasal conductance (liters per second per centimeter of water) was determined from the data collected over a period of 20 seconds while subjects were breathing in a relaxed manner [2]. In the morning, afternoon, and evening hours, middle ear status was assessed by tympanometry with use of a commercially available clinical instrument (Teledyne Impedance Screener; Teledyne, Earlysville, VA). At each testing, middle ear pressures and compliance were recorded bilaterally.

In the afternoon hours, eight specific symptoms previously identified as characterizing a cold were scored by subjects on a five-point scale corresponding to none, mild, moderate, moderate-to-severe, and severe degrees [2]. These symptoms included sneezing, headache, malaise, chilliness, nasal discharge,

nasal congestion, cough, and sore throat. In addition, the subjects were questioned as to whether or not they believed that they had colds on that day. At those times, the subjects underwent nasal examinations with a headlight and nasal speculum. Nasal patency was scored from 0 to 4, corresponding to wide open, open, slightly open, moderately obstructed, and severely obstructed. Mucosal edema was scored from 0 to 3, corresponding to none, mild, moderate, and severe.

The color of the mucosa was coded as normal, pale, pink, or red. The observed quantity of rhinorrhea was scored from 0 to 4, corresponding to none, scanty, some, moderate, and profuse, and the quality of rhinorrhea was coded as none, serous, seromucoid, mucoid, or purulent. The color of rhinorrhea was coded as none, colorless, white, or yellow. Any observation of drainage from the middle meatus suggestive of sinus disease was recorded. Any other finding such as mucosal erosion or epistaxis was recorded. The nasal mucociliary transport times were then measured by using a modification of the dyed saccharin technique [14]. For that test, the time required from introduction of the dyed saccharin solution into the nose to that when the subjects reported a sweet taste, confirmed by visual observation of dye in the nasopharynx, was defined as the clearance time. For each test, subjects were observed for a maximum of 30 minutes. The mucociliary clearance times for the subjects who did not report a sweet taste within that period are considered as 30 minutes for the calculations. Homotypic neutralization tests were performed in batch for each cohort on specimens of serum collected before and 3 weeks after challenge by using a microtitration system [9].

Statistical methods. For analysis, the subjects were assigned to one of three groups according to their prechallenge RV-H neutralizing antibody titers. The subjects with reciprocal titers of <2 were assigned to the low-titer (LT) group; those with titers of 2, 4, and 8 were assigned to the intermediate-titer (IT) group; and those with titers of >8 were assigned to the high-titer (HT) group. Subjects were defined as being infected if RV-H was recovered from their nasal lavage fluids on any of study days 1–5 or if at least a fourfold rise in homotypic antibody was documented in convalescent-phase (vs. prechallenge) serum samples. Subjects were classified as having had symptomatic colds on the basis of a modification of the criteria described by Jackson and colleagues [15], i.e., a total baseline adjusted symptom score of ≥ 6 during the period of cloister and either symptoms of rhinorrhea for 3 days or subjects' belief that they had a cold [15]. Data for the frequency of infected subjects and the frequency of subjects with colds were compared among groups with use of a χ^2 square test.

Because of the significant differences among groups documented for the infection rate, comparisons of the other response variables were performed by using the data for infected subjects in each of the three titer (LT, IT, and HT) groups and for a fourth group (not infected [NI]), defined as all uninfected subjects, irrespective of the prechallenge antibody status. To gain a visual appreciation of the temporal pattern of expression, the

average values for each variable in these four groups were plotted as a function of time (figure 1A-I). To avoid problems associated with multiple comparisons at different time points, the statistical analyses of the differences among groups for the measured variables were based on comparisons of summary response variables, as defined below.

For each recorded measure, a summary response variable was defined from the data available for each individual [16]. The magnitude of the summary variables for symptoms, scaled nasal signs, mucociliary clearance time, nasal conductance, and nasal secretion weight was determined by summing the differences between the recorded value of the variable at each observation point and the baseline value of the respective variable (i.e., baseline adjusted area under the curve). For middle ear pressure, the response variable was defined as the number of observations considered to be abnormal according to criteria that were previously published ($-50 < \text{normal} < +20$ mm H₂O) [1, 2]. The significance of the differences among groups for parametric measures was determined by performing analysis of variance, with variance partitioned by group and subject. If significance was established at $\alpha \leq 0.05$, pairwise between-group testing was done using a least significant difference test.

For nonparametric variables, the significance of differences among groups was determined by using the χ^2 test with Yates' correction or, where appropriate, Fisher's exact test. The relationships between pairs of summary response variables were evaluated by using Pearson correlation coefficients. All analyses were performed on a microcomputer using the program CSS:STATISTICA (StatSoft, Tulsa, OK). Statistical significance was evaluated at the $P < .05$ level by using two-tailed significance tests.

Results

Infection and colds. Viral cultures of the day-0 nasal lavage fluids from three subjects yielded a wild virus (non-RV-H), and the antibody titer data were not available for one subject. These four subjects were excluded from the analysis. Of the remaining 129 subjects, 51 were assigned to the LT group, 46 to the IT group, and 32 to the HT group on the basis of their prechallenge antibody titers. Twenty-three subjects (72%) in the HT group, four (9%) in the IT group, and one (2%) in the LT group were not infected with RV-H after challenge ($P < .05$). All uninfected subjects were reassigned to the NI group. In the presentation to follow, group designation followed by the prime symbol (e.g., LT') indicates all infected members of that group. Summary data are reported for the frequency of viral shedding, seroconversion, and symptomatic colds in table 1. Viral shedding was documented in seven subjects (78%) in the HT' group, in 29 subjects (69%) in the IT' group, and in 48 subjects (96%) in the LT' group ($P < .05$, LT' vs. HT'; $P = \text{NS}$, IT' vs. HT'). A fourfold rise in convalescent RV-H antibody titer was documented in five subjects (55.5%) in the

HT' group, in 39 subjects (93%) in the IT' group, and in 41 subjects (82%) in the LT' group ($P < .05$, IT' vs. HT'). By definition, no subject in the NI group shed virus or had a fourfold rise in antibody titer. According to the modified criteria of Jackson et al. [15], three (10.7%) of the subjects in the NI' group were considered to have symptomatic colds, as compared with 1 (11.1%) in the HT' group, 17 (40.5%) in the IT' group, and 31 (62%) in the LT' group ($P < .05$, LT' vs. IT' or HT' and NI).

Symptoms, signs, and pathophysiologies. Figure 1 shows the average daily values for: viral titer (A), nasal symptoms (B), throat symptoms (C), systemic symptoms (D), secretion weights (E), nasal conductance (F), nasal clearance times (G), positive middle ear pressures (H), and negative middle ear pressures (I) in the four comparison groups defined by pre-existing serum titers of antibody to rhinovirus type 39 (RV-39) and the presence or absence of infection. For most of these measures, no changes after exposure to RV-H were observed in the NI group. The average log viral titers for the LT' and HT' groups (including the subjects who did not shed virus but seroconverted) increased to peak on day 2 or day 3 and then decreased by day 5. While similar in temporal pattern, this function for the LT' group had a higher peak magnitude when compared to that of the HT' and IT' groups. For the three infected groups, total nasal symptoms (sum of congestion, rhinorrhea, and sneezing) showed a temporal profile that was characterized by an early increase to peak on day 3 (LT' and IT') or day 4 (HT') and a noted decrease to approach baseline by day 5.

The magnitude of the response was greatest for the LT' group, intermediate for the IT' group, and least for the HT' group. Total throat symptoms (sum of cough and sore throat) showed a shallow increase in all infected groups, to plateau between days 2 and 5. The magnitude of the increase was greater for the LT' group and less but similar for the IT' and HT' groups. Systemic symptoms (sum of chills, headache, and malaise) showed an increase with time only in the LT' group. Average daily secretion weights showed an initial increase on day 2, a peak on day 3, and a decrease by day 5 in the LT' group. The response magnitude was less for the IT' group, while little change was noted for the HT' group. Average daily conductance decreased in the LT' and HT' groups but was relatively unchanged for the other groups, while average daily clearance times were increased in all infected groups. For the later variable, the response magnitude was greatest for the LT' group and least for the HT' group. The percentage of abnormal middle ear pressures observed on each day showed a parallel increase in all groups from between 7.3% (LT' group) and 13% (NI group) on day 0 to a maximum of between 27% (NI group) and 52.3% (LT' group) on day 4. The observed differences between groups was attributable to an increase in the frequency of abnormal negative pressures.

The averages, standard deviations, and significance of between-group differences for summary variables related to

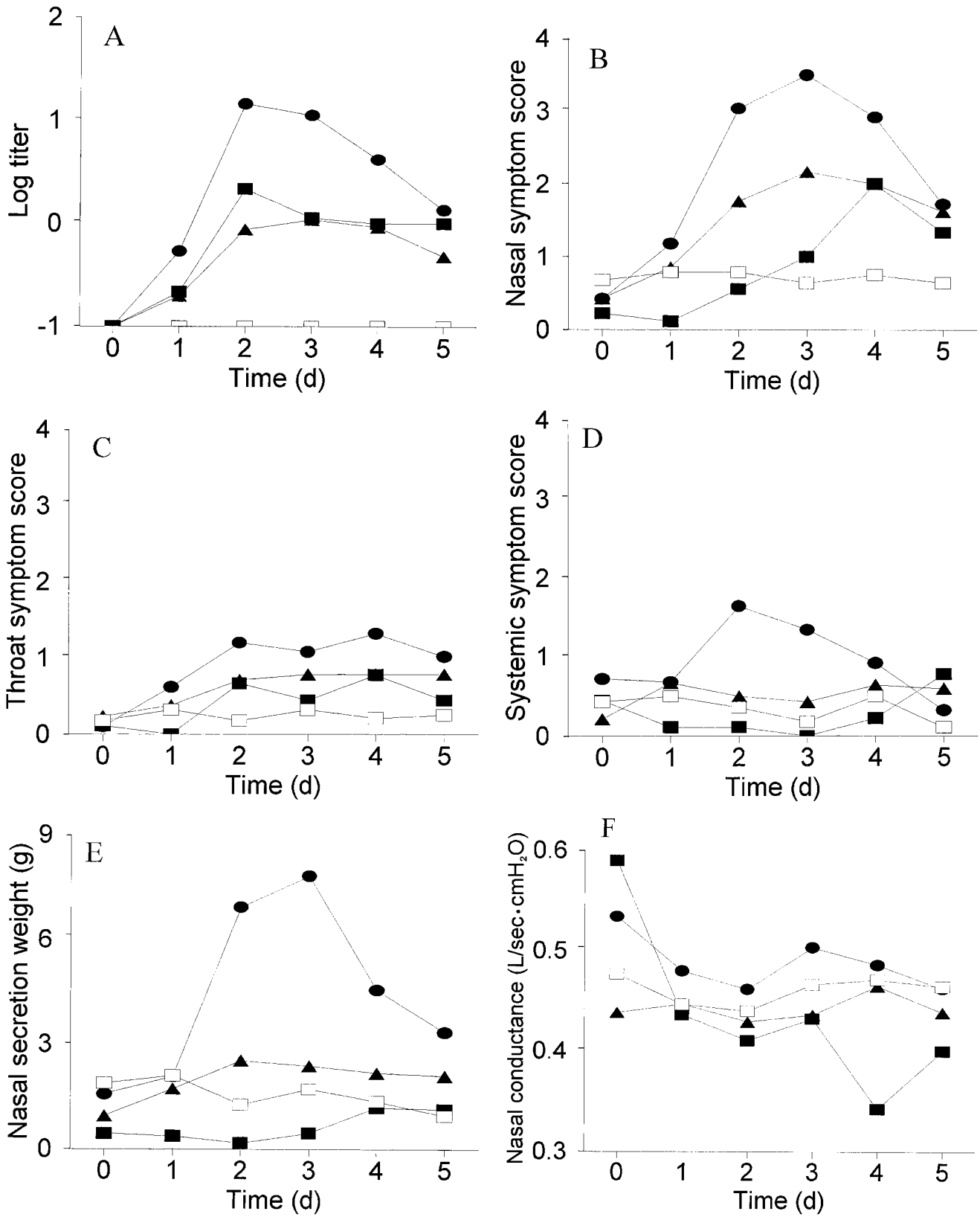


Figure 1.

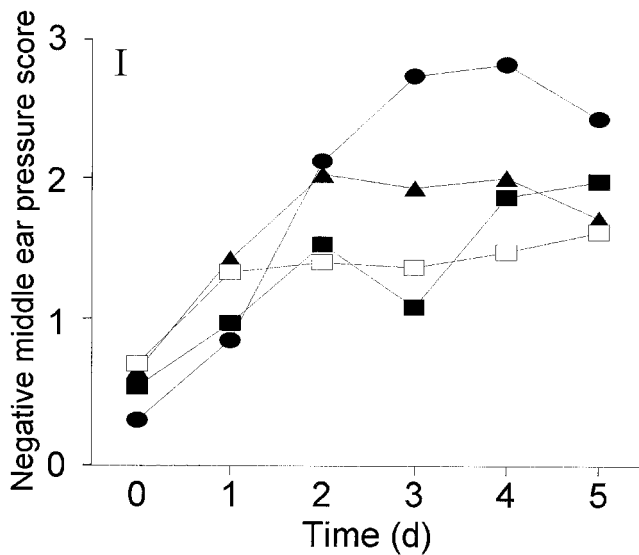
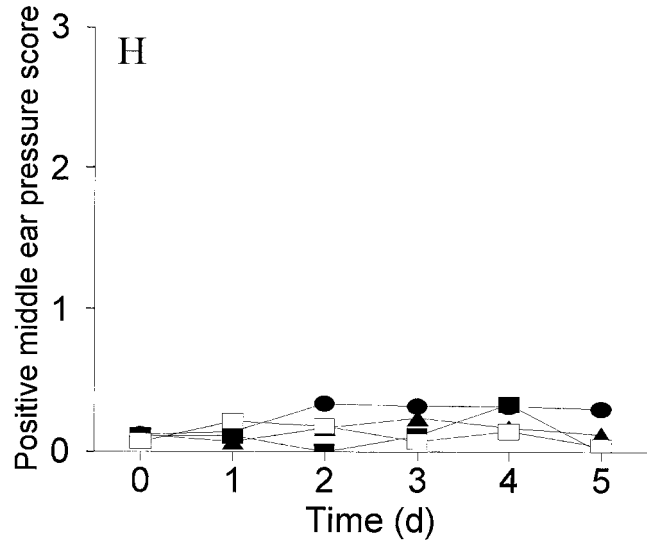
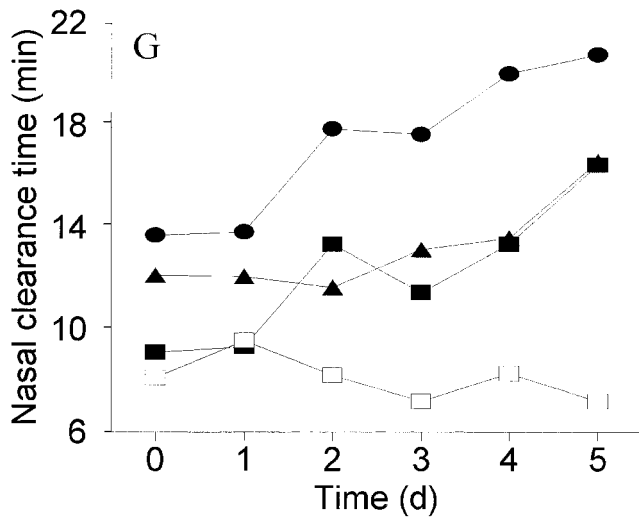


Figure 1. The average daily values for infected subjects in the high-titer (■), intermediate-titer (▲), low-titer (●), and noninfected (□) groups, defined by preexisting serum titers of antibody to rhinovirus strain Hanks and the presence or absence of infection for: virus titer (A), nasal symptoms (B), throat symptoms (C), systemic symptoms (D), secretion weights (E), nasal conductance (F), nasal clearance times (G), positive middle ear pressure score (H), and negative middle ear pressure score (I). The score for middle ear pressure is defined as the number of abnormal observations (two ears × three measurements = six per day).

symptoms, signs, and pathophysiologies are presented in table 2. For the eight individual subject-rated symptoms, the average summary symptom score was generally lowest in the NI group, followed by the IT' group and the HT' group and highest in the LT' group. The between-group differences in congestion, rhinorrhea, sneezing, cough, and sore throat achieved statistical significance. In general, pairwise comparisons for these measures between the LT' group and both the IT' and NI groups were significant. In all infected groups, the ear, nose, and throat examinations showed an early, progressive decrease in the area of the nasal airway, an increase in mucosal swelling, and an increase in the quantity of rhinorrhea with time. There were no obvious differences among groups in the temporal pattern or magnitudes of these physician-rated signs.

In general, summary response scores for all physician-rated signs were greatest in the LT' group and least in the NI group, but differences among groups were not statistically significant.

Neither the average value of the summary variable for nasal conductance nor that for abnormal positive middle ear pressure was significantly different among groups. In contrast, the value of the summary variable for expelled secretions, nasal clearance time, and abnormal middle ear pressure was greatest in the LT' group and least in the NI group ($P < .05$).

Some significant correlation between the study variables was noticed in the analysis. The variable defined for viral shedding was significantly correlated with most of the other variables including those measuring symptoms, mucociliary clearance, secretion weight, and the frequency of negative middle ear pressure. The variable defined for nasal mucociliary clearance was correlated with that for secretion weight and with the frequency of negative middle ear pressure. The variable reflecting secretion weight was correlated with a number of other variables including those measuring the frequencies of positive ($r = 0.53$) and negative middle ear pressures ($r = 0.27$). Among

Table 1. Frequency of subjects inoculated with rhinovirus strain Hanks or rhinovirus type 39 who shed virus, seroconverted, or developed colds, according to prechallenge antibody titers.

Variable	LT'	IT'	HT'	NI
Total no. of subjects in indicated group				
RV-H	50	52	9	28
RV-39	55	49	28	15
No. (%) who shed virus				
RV-H	48 (96)	29 (69)	7 (78)	0
RV-39	54 (98)	46 (94)	26 (93)	0
No. (%) who seroconverted				
RV-H	41 (82)	39 (93)	5 (55)	0
RV-39	28 (51)	39 (80)	14 (50)	0
No. (%) who developed colds				
RV-H	31 (62)	17 (40)	1 (11)	3 (11)
RV-39	32 (58)	22 (45)	5 (18)	3 (20)

NOTE. Data are from [12]. HT' = high titer; IT' = intermediate titer; LT' = low titer; NI = noninfected (prime denotes subjects with infection in each group); RV-H = rhinovirus strain Hanks; RV-39 = rhinovirus type 39.

the physician-rated nasal signs, the variable reflecting changes in mucosal edema was correlated with those for symptoms of congestion and rhinorrhea, mucociliary clearance, secretion weight, and the frequency of negative middle ear pressure. Among the symptom scores, the variables for congestion and rhinorrhea were the most highly correlated.

Discussion

The responses of our subjects to infection with RV-H were similar in magnitude and extent to those previously described for subjects with natural rhinovirus infections and experimental infections with other rhinovirus strains [12, 17]. Specifically, the infected subjects reported a number of symptoms consistent with those of the common cold; had physician-documented nasal signs of inflammation; and expressed increased amounts of nasal secretions, decreased nasal patency, and decreased nasal mucociliary clearance rates during the period of active viral shedding. Also similar to the results of earlier studies is the documented extension of the pathophysiology to the middle ear, which was expressed as the development of abnormal middle ear pressures. Because of the expense of conducting studies in adult volunteers as well as the possibility of nosocomial infections, the present study did not include subjects who were dummy-challenged with a control solution. This omission left open the remote possibility that some or all of the symptoms, signs, and pathophysiologies documented following challenge were artifactual and provoked by other factors associated with the cloistered environment.

In the current study, a relatively large number of rhinovirus-challenged subjects did not have evidence of infection but participated within the cloistered environment as if they were infected. The relative failure of these uninfected subjects to express symptoms, signs, or objective changes in the physio-

Table 2. Average and standard deviations of the response variables among subjects infected with rhinovirus strain Hanks, according to prechallenge antibody titers.

Variable	LT' (n = 50)	IT' (n = 42)	HT' (n = 9)	NI (n = 28)	Statistically significant*					
					L-I	L-H	L-N	I-H	I-N	H-N
Congestion	4.34 ± 3.23	2.26 ± 2.67	0.78 ± 2.04	0.14 ± 1.83	Yes	Yes	Yes	No	Yes	No
Rhinorrhea	3.24 ± 3.51	1.83 ± 2.83	1.22 ± 2.25	0.75 ± 2.97	Yes	No	Yes	No	Yes	No
Sneezing	2.94 ± 2.45	1.86 ± 2.59	1.89 ± 3.28	1.07 ± 2.05	Yes	No	Yes	No	No	No
Cough	2.44 ± 2.52	0.88 ± 2.28	0.22 ± 0.42	0.07 ± 1.79	Yes	Yes	Yes	No	No	No
Sore throat	2.30 ± 2.20	1.38 ± 2.36	1.56 ± 2.11	0.32 ± 0.71	Yes	No	Yes	No	Yes	No
Malaise	1.56 ± 6.80	0.98 ± 1.49	-0.44 ± 1.26	0.29 ± 0.84	No	No	No	No	No	No
Headache	-1.00 ± 4.41	0.57 ± 3.37	0.11 ± 2.60	0.68 ± 2.48	Yes	No	No	No	No	No
Chills	0.74 ± 1.92	0.21 ± 0.64	-0.67 ± 2.26	-0.11 ± 1.82	Yes	Yes	No	No	No	No
Nasal patency	2.44 ± 2.88	1.48 ± 2.23	3.67 ± 0.82	1.82 ± 2.17	No	No	Yes	No	No	No
Nasal secretion	2.56 ± 1.69	1.81 ± 1.95	2.00 ± 1.49	1.75 ± 2.03	No	No	No	No	No	No
Quantity of rhinorrhea	4.28 ± 4.92	3.76 ± 3.67	5.67 ± 4.06	3.68 ± 4.80	No	No	No	No	No	No
Nasal conductance	-0.28 ± 1.13	0.02 ± 0.87	-0.93 ± 1.11	-0.10 ± 0.76	No	No	Yes	No	Yes	No
Nasal secretion weight	16.80 ± 28.07	6.09 ± 11.15	1.19 ± 2.70	-1.99 ± 11.26	Yes	Yes	Yes	No	No	No
Nasal clearance time	21.60 ± 43.41	6.38 ± 32.37	18.33 ± 18.74	-0.32 ± 26.19	Yes	No	Yes	No	No	No
Positive middle ear pressures	0.82 ± 4.49	0.17 ± 2.13	0.00 ± 1.25	0.29 ± 1.79	No	No	No	No	No	No
Negative middle ear pressures	9.42 ± 7.69	5.88 ± 5.23	4.78 ± 8.24	3.75 ± 5.69	Yes	No	Yes	No	No	No

NOTE. HT' = high titer; H-N = high titer to noninfected; I-H = intermediate to high titer; I-N = intermediate titer to noninfected; IT' = intermediate titer; L-H = low to high titer; L-I = low to intermediate titer; L-N = low titer to noninfected; LT' = low titer, NI = noninfected (the prime denotes subjects with infection in each group).

* $P < .05$.

logical measurements during the cloister period strongly supports the position that the effects reported by or observed in the infected subjects were indeed caused by the viral infection.

A number of factors have been implicated in the moderation of susceptibility to viral infection and the magnitude of disease expression during a viral URI. These factors include size of the viral inoculum, allergic status, nutritional adequacy, personality characteristics, extent of support networks, general stress levels, and other factors related to immunologic competence [18–25]. Of these factors, the presence of specific serum antibodies is recognized as a primary moderator of susceptibility to infection, although controversy exists as to the role that these antibodies play in limiting disease expression. High viral-challenge titers may override the protection afforded by antibodies [21, 23]. In the present study, the effect of preexisting specific antibodies on the rate of viral infection and the degree of disease expression was evaluated in a large population of adults experimentally exposed to a well-characterized rhinovirus strain, RV-H.

Before challenge with RV-H, a large number of the subjects in this randomly chosen adult population had high (>8 ; 24.8%) and intermediate ($\geq 2, 4, 8$; 35.7%) titers of serum antibody to RV-H. This most likely reflects prior infection with RV-H, since seroconversion has been reported for $\sim 60.5\%$ of infected subjects, and serum antibodies to rhinovirus are extremely type specific [8–10, 13, 18, 19]. In the present study, 82% of the infected subjects in the LT group had at least fourfold increases in RV-H-specific serum antibody titers. A higher percentage of subjects (92.8%) in the IT group had fourfold increases in titers of antibody to RV-H, perhaps reflecting an amnesic response of the immune system to the previously encountered RV-H virus.

While only 55% of the subjects in the HT group showed antibody responses, this relatively low frequency is an artifact of the assay methods, where the highest titer reported was 64, thereby limiting the potential for a fourfold increase to the subset of subjects with preexisting titers of 16. Indeed, among all the subjects with preexisting high titers (including those in the NI group), 16% seroconverted, whereas 26% had a onefold increase in titers, and 29% could have had an undetected response, leaving only 29% with a known failure to seroconvert. Unfortunately, insufficient samples were available for reanalysis of these unknown titers at the termination of the study.

The results of past studies suggest that high titers of specific serum antibody are associated with resistance to both natural and experimental, homologous-type rhinovirus infections [19–23]. In the present study, this association was supported, in that 71.9% of the subjects with high antibody titers were protected from infection, while only 8.7% and 2% of the subjects with intermediate and low serum titers of antibody to RV-H, respectively, were protected. For those subjects with documented infection, high prechallenge serum antibody titers were also associated with a significantly reduced viral load but not an abbreviated duration of viral shedding. It is of interest that,

these results show that even at the relatively high viral challenge titers in this study, the effect of antibody titer on infection was demonstrable and similar to that reported previously for natural exposures.

An effect of preexisting serum antibody to RV-H was also demonstrated for disease expression. Overall, while 38% and 16.7% of infected subjects with low and intermediate RV-H antibody titers developed clinical colds (on the basis of the modified criterion of Jackson et al. [15]), this was true of only one infected subject (11%) with high RV-H antibody titers. This effect was not attributable to the higher frequency of uninfected and presumably asymptomatic subjects in the group with high antibody titers, since uninfected subjects were excluded from the analysis. While the majority of the differences between the groups of subjects with low and intermediate titers of antibody were statistically significant, differences between the subjects with low and high titers of antibody were not, although the expected trend was maintained. This finding is explained by the low power of the statistical tests, which was afforded by a sample size of only nine infected subjects in the HT group.

Of interest is the low magnitude of nasal and throat symptoms and the apparent lack of systemic symptoms reported by infected persons with preexisting high RV-H antibody titers. If these symptoms contribute greatly to an individual's assessment of his/her degree of illness, an infected individual with a high titer of specific antibodies may be less likely to absent himself/herself from social interactions and thereby represents a source for dissemination of the virus to other members of the population [12]. However, because of the high inoculation dose of virus used in this study, this conclusion may not be extrapolated directly to natural colds, but it remains an interesting hypothesis that can be evaluated in future studies.

The moderating effect of high antibody titers on disease expression was also supported by the data for the objective measures of secretion weight, nasal mucociliary clearance times, and the frequency of abnormal negative middle ear pressures, which showed a rank ordering of response magnitude inversely related to antibody titer. The failure to observe a similar effect for the objective measure of nasal patency can be explained by the rather low magnitudes of the responses in all groups and by the large intragroup variability resulting from the variable presence of secretions in the nasal airway. While physician-rated signs showed a trend favoring less severe disease in the subjects with high antibody titers, the between-group comparisons were not statistically significant. This finding is consistent with the results of earlier studies in which physician-assessed signs were reported to be poor indicators of the degree of illness perceived by the subjects [12, 15].

The effect of prechallenge specific serum antibodies on these outcome variables was previously reported for a large group of subjects who were challenged with RV-39 [12]. The volunteer subjects in that study and the present study were: (1) exposed

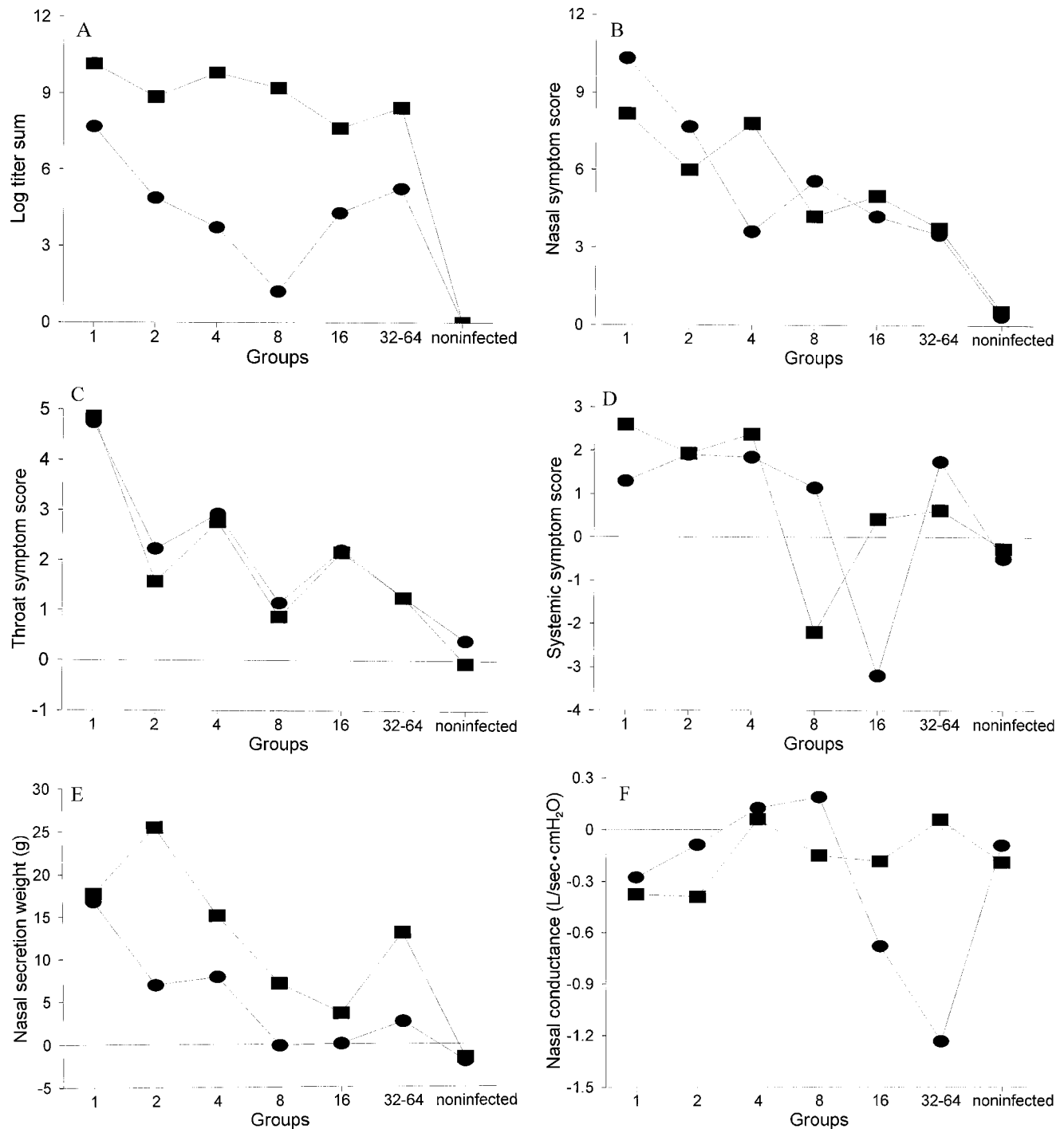


Figure 2.

to exactly the same cloister environment; (2) naturally stratified by titers of type-specific, preexisting serum antibodies; and (3) evaluated by the same investigators using identical methods. Thus, the two studies differed only by the rhinovirus type and the subject population. Comparative data for the results of experimental exposures to RV-39 and RV-H viruses in unin-

fected subjects and in infected subjects stratified by prechallenge antibody titers are reported in table 1 and figure 2A-I. For the LT' subgroup and the LT uninfected subgroup, the paired values for the two rhinovirus types with respect to the frequencies of subjects who shed virus and reported having colds, as well as the average values of the various response

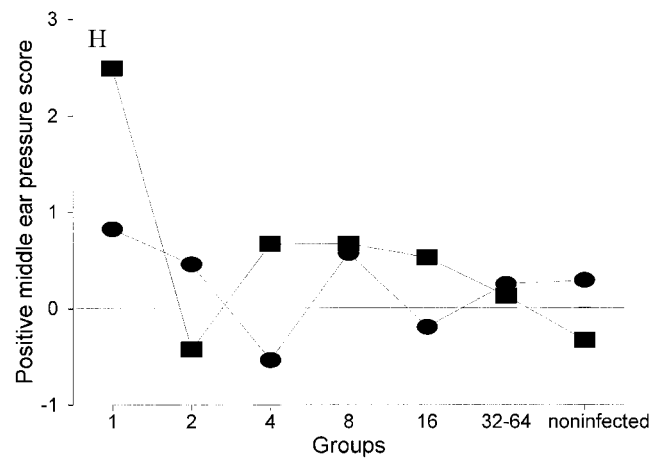
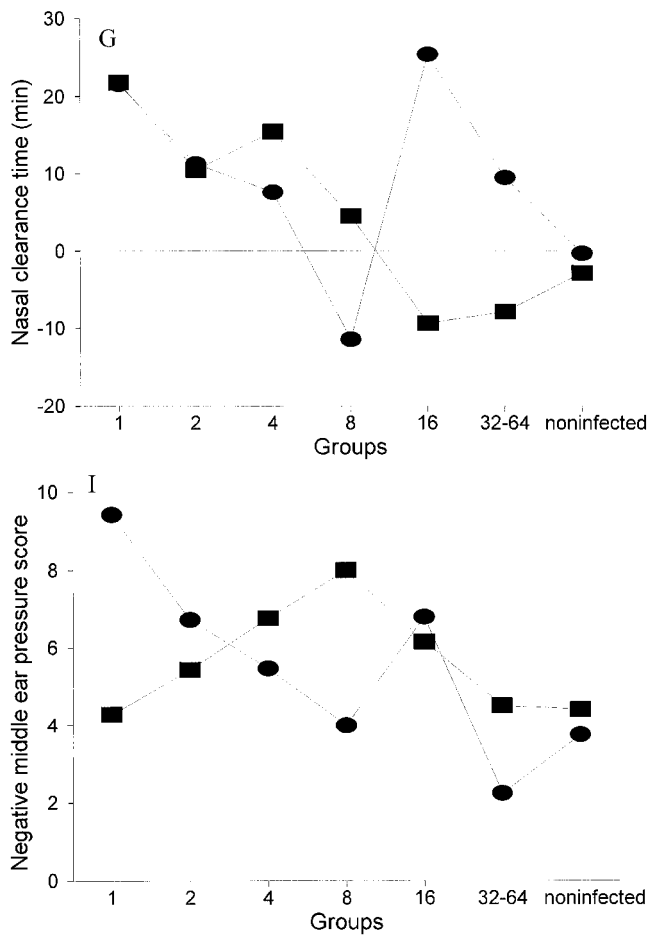


Figure 2. Comparison of the summary response variables for rhinovirus strain 39 (■) and rhinovirus strain Hanks (●) for: viral shedding (A), nasal symptoms (B), throat symptoms (C), systemic symptoms (D), secretion weights (E), nasal conductance (F), nasal clearance times (G), positive middle ear pressure score (H), and negative middle ear pressure score (I), defined by preexisting serum antibody titers. The score for middle ear pressure is defined as the total number of abnormal observations corrected by baseline. The noninfected subjects with all titers are presented as a separate group.

variables, were similar. In that case, only the rates of seroconversion and the average log virus titers were appreciably different between rhinoviruses.

With regard to the effect of prechallenge, specific antibody status, RV-39 and RV-H infections were characterized by similar patterns of decreasing symptom and sign expression with increasing antibody titers for: frequency of colds; nasal, throat, and general symptoms; expelled secretions; nasal patency (conductance); and nasal mucociliary clearance function. However, while the presence of those antibodies appeared to decrease the viral load in subjects infected with RV-H, no similar effect was noted for RV-39. Moreover, the effect of specific serum antibodies on the frequency of abnormal negative middle ear pressures was dissimilar for the two rhinovirus types, suggesting that other factors moderate the development of middle ear complications after viral infections. The imprecision of the assay for neutralizing antibody titers and assay variability between the cohorts may have led to a nonlinear difference in response between the titer groups.

In the previous study [12], interresponse variable correlations were used to develop possible causal relationships between paired responses. For example, the frequency of positive middle

ear pressures was correlated with the variable representing expelled secretions, suggesting nose-blowing as a possible cause for those pressures, while the frequency of negative middle ear pressures was correlated with the degree of impairment in mucociliary clearance function, suggesting “sniffing” as a possible cause of those pressures. These pairs of variables were also correlated in the present study, lending some support to those hypotheses.

Conclusion

Our study confirms the results of a previous study in which adults were exposed to a different rhinovirus type [12]. Both studies found a moderating effect of specific serum antibodies on rates of infection and symptomatic expression of colds among volunteers exposed by coarse drops to a homologous rhinovirus type. For subjects with documented infection by either virus, the presence of homotypic serum antibodies was correlated with a lessened expression of symptoms, signs, and pathophysiology. The reduced morbidity among infected subjects with high prechallenge serum antibody titers suggests that these individuals may not appreciate sufficient illness to absent

themselves from social activities, and thus they may serve as sources of viral infection within a community. Titers of specific serum antibody were reported to be well correlated with titers of specific secretory antibody; however, because the latter titers were not measured in either study, the specific mechanism by which protection is afforded cannot be determined with these data [21, 22]. Additional studies for measuring both types of antibodies are needed to address this question.

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References

- Doyle WJ, McBride TP, Swartz JD, Hayden FG, Gwaltney JM Jr. The response of the nasal airway, middle ear, and eustachian tube to experimental rhinovirus infection. *Am J Rhinology* **1988**;2:149–54.
- McBride TP, Doyle WJ, Hayden FG, Gwaltney JM Jr. Alterations of the eustachian tube, middle ear, and nose in rhinovirus infection. *Arch Otolaryngol Head Neck Surg* **1989**;115:1054–9.
- Lemanske RF Jr, 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.
- Hamory BH, Sande MA, Snyder A Jr, Seale DL, Gwaltney JM Jr. Etiology and antimicrobial therapy of acute maxillary sinusitis. *J Infect Dis* **1979**;139:197–202.
- Gregg I. Respiratory viruses and host factors: doubts and certainties concerning their roles in acute respiratory illness. In: Nicholson K, ed. *HIV and other highly pathogenic viruses*. London: Royal Society of Medicine Services Ltd **1988**:43–68.
- Henderson FW, Collier AM, Sanyal MA, et al. A longitudinal study of respiratory viruses and bacteria in the etiology of acute otitis media with effusion. *N Engl J Med* **1982**;306:1377–83.
- Casselbrant ML, Brostoff LM, Cantekin EI, et al. Otitis media with effusion in preschool children. *Laryngoscope* **1985**;95:428–36.
- Gwaltney JM Jr, Jordan WS Jr. Rhinovirus and respiratory illness in university students. *Am Rev Respir Dis* **1966**;93:362–71.
- Hendley JO, Gwaltney JM Jr, Jordan WS Jr. Rhinovirus infections in an industrial population. IV. Infections within families of employees during two fall peaks of respiratory illness. *Am J Epidemiol* **1969**;89:184–96.
- Hamparian VV, Colonna RJ, Cooney MK, et al. A collaborative report: rhinoviruses—extension of the numbering system from 89 to 100. *Virology* **1987**;159:191–2.
- Hamre D. Rhinoviruses. *Monogr Virol* **1967**;1:1–85.
- Alper CM, Doyle WJ, Skoner DP, et al. Pre-challenge antibodies moderate infection rate, and signs and symptoms in adults experimentally challenged with rhinovirus type 39. *Laryngoscope* **1996**;106:1298–305.
- Gwaltney JM Jr. Micro-neutralization test for identification of rhinovirus serotypes. *Proc Soc Exp Biol Med* **1986**;122:1137–41.
- Duchateau GS, Graamans K, Zuidema J, Merkus FW. Correlation between nasal ciliary beat frequency and mucus transport rate in volunteers. *Laryngoscope* **1985**;95:854–9.
- Jackson GG, Dowling HF, Spiesman IG, et al. Transmission of the common cold to volunteers under controlled conditions. I. The common cold as a chemical entity. *Arch Intern Med* **1958**;101:267–78.
- Doyle WJ, McBride TP, Skoner DP, Maddern BR, Gwaltney JM Jr, 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. *Pediatr Infect Dis J* **1988**;7:229–38.
- Rao SS, Hendley JO, Hayden FG, Gwaltney JM. Symptom expression in natural and experimental rhinovirus colds. *Am J Rhinol* **1995**;9:49–52.
- Skoner DP, Whiteside TL, Wilson JW, Doyle WJ, Herberman RB, Fireman P. Effect of rhinovirus 39 infection on cellular immune parameters in allergic and nonallergic subjects. *J Allergy Clin Immunol* **1995**;92:732–43.
- Hendley JO, Edmondson WP Jr, Gwaltney JM Jr. Relation between naturally acquired immunity and infectivity of two rhinoviruses in volunteers. *J Infect Dis* **1972**;125:243–8.
- Kellner G, Popow-Kraupp T, Binder C, Goedl I, Kundi M, Kunz C. Respiratory tract infections due to different rhinovirus serotypes and the influence of maternal antibodies on the clinical expression of the disease in infants. *J Med Virol* **1991**;35:267–72.
- Cate TR, Couch RB, Johnson KM. Studies with rhinoviruses in volunteers: production of illness, effect of naturally acquired antibody, and demonstration of a protective effect not associated with serum antibody. *J Clin Invest* **1964**;43:56–67.
- Cate TR, Rossen R, Douglas RG, Butler WT, Couch RB. The role of nasal secretion and serum antibody in the rhinovirus common cold. *Am J Epidemiol* **1966**;84:352–63.
- Mufson MA, Ludwig WM, James HD, et al. Effect of neutralizing antibody on experimental rhinovirus infection. *JAMA* **1963**;186:578–84.
- Cohen S, Tyrrell DA, Smith AP. Psychological stress and susceptibility to the common cold. *N Engl J Med* **1991**;325:606–12.
- Cohen S, Doyle WJ, Skoner DP, Rabin BS, Gwaltney JM. Social ties and susceptibility to the common cold. *JAMA* **1997**;277:1940–4.