Clinical and Experimental Allergy, 1994, Volume 24, pages 457–464

Amplified rhinovirus colds in atopic subjects

P. G. BARDIN, D. J. FRAENKEL, G. SANDERSON, M. DORWARD, L. C. K. LAU, S. L. JOHNSTON and S. T. HOLGATE

Immunopharmacology Group, University Medicine, Southampton General Hospital, Southampton, UK

Summary

Evidence suggests that atopic individuals may be predisposed to more severe rhinoviral colds coupled to a worsening of existing airway disease than those with asthma. The role of atopy and IgE levels, as well as their relationship to clinical disease expression have not been defined. We hypothesized that an allergic diathesis modulates rhinoviral colds and have initiated studies of normal, atopic and asthmatic subjects employing experimental rhinoviral infection, with measurements of symptom scores, viral shedding and cultures, albumin in nasal washes and serological responses. Twenty-two subjects (11 normal, 5 atopic, 6 atopic and asthmatic) participated and were inoculated with human rhinovirus serotype 16 (HRV 16). Measurements of neutralizing antibody and viral culture were performed at screening, pre-inoculation, during the cold and at 8–10 weeks convalescence. Daily symptoms were noted, nasal washes done, IgE measured and atopy was diagnosed by skin tests. Seventeen volunteers developed clinical colds as assessed by symptom scores, virus shedding was demonstrated (with positive culture) in all subjects and a fourfold or higher seroconversion occurred in 11/22. Neutralizing HRV antibody developed unexpectedly in 10 subjects between screening and inoculation and the presence or absence of this pre-inoculation antibody determined subsequent severity of colds in normal but not in atopic subjects. Atopic antibody positive individuals developed severe clinical colds that were independent of preinoculation antibody in contrast to normal subjects who developed mild colds in the presence of a neutralizing antibody ($P=0{\cdot}01$). Both atopic and normal antibody negative subjects developed severe colds. This differential response was matched by nasal wash albumin levels which were significantly increased ($P=0{\cdot}01$) during the cold in atopic (but not in normal) volunteers with pre-inoculation antibody. Levels of IgE were not correlated with severity of clinical disease or viral shedding. Our studies of HRV disease in atopic subjects suggest heightened susceptibility to the detrimental effects of colds; additional studies are needed to clarify the relevant mechanisms.

Clinical and Experimental Allergy, Vol. 24, pp. 457–464. Submitted 18 October 1993; revised 31 December 1993; accepted 20 January 1994.

Introduction

Although the common cold is the most prevalent illness in humans, information on the pathogenesis of symptoms and relationship of the infection to atopic diseases such as asthma has been difficult to obtain. A recent study has implicated human rhinoviruses (HRV) as the major factor in association with up to 60% of exacerbations of asthma in children, a view based on studies employing a sensitive polymerase chain reaction for diagnosis [1]. Some investigators have suggested that worsening of atopic disease may be mediated by way of increased sensitivity of the atopic individual, manifesting as augmented airway responsiveness and the induction of late asthmatic reactions by HRV [2]; atopic subjects may thus intrinsically be more prone to develop severe cold symptoms including chest symptoms.

Early investigations into the epidemiology, spread, serological responses and infectivity of HRV were conducted in studies employing human experimental disease in volunteers [3,4]. More recently this model has been expanded to investigate the interrelationship between HRV and allergic diseases [5,6] and important aspects

Correspondence: Dr P. G. Bardin, University Medicine (Centre Block), Southampton General Hospital, Tremona Road, Southampton SO9 4XY, UK.

interacting with atopic status such as symptom scores, viral shedding and serological responses have been evaluated. No definite link was established and controversy remains as to whether atopic individuals are more susceptible to the consequences of rhinoviral infections.

We have postulated that clinical expression of rhinoviral colds is modulated by the allergic status of an individual. This report details the development of a programme for the induction of HRV serotype 16 (HRV 16) colds in adult volunteers and evaluates the clinical severity of infection, serological responses and associated factors that may determine the expression of disease in both normal and atopic volunteers.

Methods

Patients

The study was conducted in 11 normal non-smoking volunteers and 11 non-smoking atopic subjects (six asthmatics), who had no measurable antibody to HRV 16 in their serum (< 1:2 dilution) at initial screening. Asthmatic patients were controlled by the use of as required β_2-agonists only and had baseline $FEV_1 > 80\%$ predicted. Asthma was defined as a PEF variability of > 15% and histamine hyperresponsiveness (PC20 < 4 mg/ml). All subjects gave informed consent before enrolling in the study. The study had been approved by the Southampton Hospitals and University Ethical Subcommittee.

Study design

Subjects were studied in three phases. Phase 1: baseline observations were conducted on the 2 days prior to viral inoculation and included skin-prick testing to common allergens, total serum IgE, serum for repeat of neutralizing antibody and nasal washing for viral culture. This took place in the Infectious Diseases Unit (IDU) at Southampton General Hospital, where subjects were isolated for 7 days during the experimental HRV 16 infection. Phase 2: following HRV 16 inoculation, subjects had daily nasal washes, assessment of symptoms and repeat serology. Phase 3: all the above investigations were repeated at weeks 8–10.

Measurement of neutralizing antibodies

This was undertaken on serum (obtained 1–2 months prior to inoculation) from all volunteers before admission to the study (screening) as well as on the day prior to inoculation with HRV 16, day 3 of experimental disease (day 5 of admission) and finally 8–10 weeks later. The test could not be repeated in three volunteers who had moved elsewhere in the UK or overseas. A micro-neutralization test in 96 well tissue culture plates was used. Briefly, serial doubling dilutions of sera (0·05 ml) were made in diluent (HeLa maintenance, 2% fetal calf serum). This was added to an equal volume of virus at concentrations of 40–50 tissue culture infective doses-50%/ml (TCID50/ml) in a volume of 0·05 ml and incubated at room temperature for 1·5 h. To the serial dilutions were added freshly stripped HeLa cells at 3×10^5 cells/ml and the plates incubated at 33°C. Appropriate serum, cell and viral controls were included. Cytopathic effects (CPE) were read microscopically after 2–4 days' incubation and confirmed by fixing and staining plates. All assays were performed in duplicate.

Assessment of atopic status: (a) Skin-prick tests: the tests were performed using a standard set of allergens (*Dermatophagoides pteronyssinus*, mixed grass pollen, cat dander, dog hair, feathers, *Candida albicans* and *Aspergillus fumigatus*) with histamine used as a positive control (Bencard, Brentford, Middlesex, UK). Atopic individuals were defined as those with at least two positive tests (> 2 mm weal response than diluent control) to the allergens used. (b) IgE measurements: serum IgE values were determined by a double-antibody solid-phase ELISA (Enzygnost IgE, Calbiochem, Behring Corp., La Jolla, CA, USA).

Symptom assessment: all subjects completed a validated questionnaire daily for 2 weeks prior to, during and for 8–10 weeks after HRV 16 infection. Symptoms were graded 0–3 according to severity and the criteria for a cold were based on that proposed by Jackson *et al.* [7]. Clinical illness was considered present if a volunteer had a minimum cumulative symptom score of 14 over a 4-day period, and had a subjective impression that he had a cold, or if rhinorrhea was present on at least 3 of the 4 days of observation. Any baseline rhinitic symptoms for 4 days prior to inoculation were subtracted from new cold symptoms. Rhinitis was assessed as either absent, mild or severe on the basis of a history of nasal blockage, sneezing, and rhinorrhea over the preceding year. Because a recent report has suggested that cold symptoms are influenced by alcohol intake [8], all subjects were contacted and their intake at the time quantified as the average number of drinks per day [8].

Nasal lavage: nasal washing was undertaken by instilling 5 ml of pre-warmed Hanks' balanced salt solution (HBSS) with 0·5% gelatin into each nostril, holding for 5 s and then expelling into a sterile petri dish. Samples were aliquotted and frozen at −70°C until analysed.

Albumin measurement: measurements on coded samples were done by rocket immunoelectrophoresis [9]. Sensitivity of the assay was 1 μg/ml and the coefficient of variation of repeated measurements was < 5%.

Nasal inoculation with virus: nasal inoculation was performed by instilling 0·25 ml of HRV 16 suspension (kindly supplied by Drs E. Dick & W. Busse, Madison, Wisconsin, USA) into each nostril by pipette ($1{\cdot}5 \times 10^{2{\cdot}3}$ TCID50/ml) on two consecutive days in addition to spraying approximately the same amount into each nostril with an atomiser (No. 286; De Vilbiss Co., Heston, UK) using a hand-bulb (no. 5 bulb, De Vilbiss Co.) so that the spray coated the nasal activity [2].

Confirmation of viral infection: nasal washes were inoculated onto Ohio HeLa cell cultures and HRV shedding and infection diagnosed by means of the typical CPE on light microscopy. Cultures were examined daily and regarded as negative if they showed no CPE after two passes in cell culture. The presence of virus in nasal washes was determined by culture on HeLa cells, and confirmed by neutralisation by HRV 16 specific antiserum (ATCC, Cat. no. V105-501-558, Natl. Inst. Allergy, Infect. Dis., Bethesda, Maryland, USA). Positive infection was established by at least one identified viral isolate and/or a fourfold rise in titre of neutralizing antibody. Because quantification of viral shedding is difficult, a scoring system for viral culture was devised. For each of 3 days of lavage collection, the CPE observed in HeLa cultures were scored (0–4) by the same observer and then summated over the 3-day period of lavage collection, with a maximum cumulative score of 12 (3×4).

Statistical analysis: symptom scores, albumin levels and IgE levels in sub-groups were compared by Mann–Whitney *U*-test. Overall differences between the four groups were assessed by Kruskal-Wallis one-way ANOVA. For evaluation of associations between symptom scores and age, albumin or IgE values, we employed the Pearson correlation test. All other associations were examined using the Spearman rank correlation test. $P \leqslant 0{\cdot}05$ was considered significant.

Results

Patients and response to infection

A total of 64 volunteers were initially screened for the presence of serum neutralizing antibody; 33 (51%) had antibody present and nine were deemed unsuitable for personal reasons. The baseline characteristics of the 22 volunteers who participated in studies are shown in Table 1. The group consisted of 11 normal subjects (seven female) and 11 atopic individuals (two female), six of whom had asthma. No differences in age and sex distribution were noted; the atopic group had significantly higher IgE levels ($P = 0{\cdot}002$).

A definite clinical cold developed in 17/22 subjects as assessed by symptom scores. Negative by scoring were subjects 1, 2, 4, 8 and 11, all of whom were normal individuals. However, they all had either positive viral shedding and cultures or significant rises in neutralizing antibody to HRV 16. All atopic volunteers had clinical colds based on symptom scores calculated after baseline rhinitic symptoms had been subtracted. Two subjects in each group had baseline rhinitic scores that were taken into account (Table 1). Median symptom score was 15 in normal subjects (range 5–36) and 24 (range 14–36) in atopic volunteers. Comparison of scores was not significantly different ($P = 0{\cdot}17$), partly as a result of a few very high scores in the normal volunteers.

No associations were found between age and symptom scores, CPE scores or changes in serology. Also, no differences were noted in alcohol intake between normal and atopic volunteers and no influence on symptom scores was present.

Viral culture

Viral shedding and culture was positive in all subjects although low CPE scores were recorded in one subject (patient 15). Median CPE score was 12 in normal subjects and eight in atopics, a discrepancy likely related to the difference in preinoculation antibody present in the groups. No differences were noted between the groups in measurements such as first day of positive CPE and median number of days of viral shedding (data not shown).

Serological responses

Serology was performed 1–2 months before actual inoculation to screen subjects for admission (neutralizing antibody titre $< 1:2$) into the study. Repeat baseline serology on the day before inoculation yielded unexpected results. In the normal group four volunteers had developed an increase in antibody to 1:8 dilution or less and in the atopic volunteers this had occurred in six individuals. No rises greater than 1:8 were recorded. The presence of neutralizing antibody in concentrations equivalent to 1:8 dilution was correlated with the absence of any change in serology at 8–10 weeks after the cold in all cases (patients 4, 13, 15 and 18 in Table 1), as well as delayed viral shedding. Median CPE scores in them on the first day of sampling their cold was 0·5 as opposed to a median score of 4 for the other 18 subjects. The latter four subjects also tended to have low viral culture CPE scores (Table 1); median CPE score was calculated as 4 vs a score of 12 for the rest of the group.

Taking pre-inoculation antibody into account, a

Table 1. Baseline characteristics of study volunteers and response to infection

Subject no.	Age	Group*	EtOH score§	IgE	Rhinitis score	Cold symptom score†	Viral culture CPE score‡	Serology: titres
1	50	N	0	10	0	5	12	4–64
2	45	N	0	10	0	7	12	2–4
3	45	N	0·5	48	0	24	12	ND
4	20	N	0·3	11	0	10	4	8–8
5	21	N	0·4	54	2	28	12	0–8
6	20	N	0·4	10	4	36	12	0–8
7	29	N	0	10	0	15	12	0–4
8	49	N	0·5	32	0	6	12	2–4
9	24	N	U	10	0	15	10	ND
10	43	N	0	64	0	29	10	0–64
11	21	N	0	ND	0	13	10	0–32
12	47	A1	0	23	0	16	8	0–2
13	33	A1	0·4	57	0	36	4	8–8
14	32	A1	0	45	0	20	8	0–32
15	20	A1	0·5	125	0	25	2	8–8
16	22	A1	1·0	41	0	24	5	0–32
17	27	A2	0	232	0	24	12	4–16
18	26	A2	0	1627	0	18	6	8–8
19	22	A2	2	936	2	24	6	0–4
20	26	A2	0·4	133	0	18	12	4–64
21	23	A2	0·4	153	0	24	12	4–16
22	22	A2	0·5	334	2	14	11	ND

*N. Normal. A1. Atopic, non-asthmatic. A2. Asthmatic and atopic. §. Average number of drinks/day. †. Calculated using the method of Jackson [7]. ‡. Viral culture: total CPE for three daily nasal washes. Maximum score possible is 12. Serology titres: pre-inoculation—convalescence. ND. Not done. IgE. IU/ml. U. Unknown.

marked difference was noted between normal and atopic individuals in their symptomatic responses to infection. Normal volunteers with detectable antibody present ($\geqslant 1:2$ dilution) tended to have mild symptoms in keeping with lower culture scores and attenuated development of neutralizing antibody. This finding was in contrast to atopic individuals who had high cold symptom scores after correction in spite of the presence of preinoculation antibody, low culture scores and mild or absent serum antibody responses. The trend was for normals to have mild to severe symptoms whereas atopics tended to all have severe symptoms irrespective of preinoculation neutralizing antibody status (Table 2). Although the subgroups were small, the differences in symptom scores between normal and atopic preinoculation antibody positive individuals were highly significant ($P=0{\cdot}014$). It was noteworthy that normal volunteers with absence of antibody developed some of the most severe colds (cases 5, 6, 10—Table 1).

Levels of IgE were not correlated with symptom scores, viral CPE scores or serological responses.

Measurement of albumin in nasal washes

Nasal washes were obtained on day 2 (before inoculation) and on day 5 (third day of cold). Results of albumin measurements on the respective days are shown in Table 2. Normal subjects with neutralizing antibody present ($\geqslant 1:2$) had a median decrease in nasal wash albumin of 8·5 μg/ml. When pre-inoculation antibody was absent, normal subjects developed an increased nasal albumin (median increase 74·5 μg/ml) during the cold.

Contrasting results were obtained in the atopic group. In spite of neutralizing antibody being present, atopic individuals developed significant increases in nasal albumin levels (median increase 22·5 μg/ml—$P=0{\cdot}01$ vs normals). This difference was not present in the antibody negative group (atopic vs normals: $P=0{\cdot}9$), or in the normal vs atopic group as a whole ($P=0{\cdot}54$). There was a graded increase in nasal albumin changes associated with a cold, being lowest in normal antibody positive individuals and highest in atopic antibody negative

Table 2. Relationship of baseline neutralizing antibody to symptom scores and nasal wash albumin levels in normal and atopic subjects

Subject no.	Baseline titre	Group	Symptom score	Albumin D2 μg/ml	Albumin D5 μg/ml	Change albumin (D5–D2)
1	1/4	N	5	102	12	−90
2	1/2	N	6	21	18	−3
4	1/8	N	7	24	21	−3
8	1/2	N	10	54	40	−14
m			6·5	39	20	−8·5
13	1/8	A1	36	130	150	+20
15	1/8	A1	25	65	132	+67
17	1/4	A2	24	84	96	+12
18	1/8	A2	18	21·5	36	+14·5
20	1/4	A2	18	32	57	+25
21	1/4	A2	24	54	54	0
m			24*	59·5	76·5	+22·5†
5	0	N	28	18·5	90	+71·5
6	0	N	36	18	162·5	+144·5
7	0	N	15	15	14	−1
10	0	N	29	36	330	+294
11	0	N	13	23	97·5	+74·5
m			28	18·5	97·5	+74·5
12	0	A1	16	20	28	+8
14	0	A1	20	180	375	+195
16	0	A1	24	65	465	+400
19	0	A2	24	295	255	−40
m			22	122·5	315	+101·5

D2. Day 2 (before cold). D5. Day 5 (during cold). m. Median value. N. Normal volunteers. A1. Atopic non-asthmatic. A2 Atopic asthmatic. *$P=0·01$ vs normal antibody positive group. †$P=0·01$ vs normal antibody positive group.

subjects, again confirming increased sensitivity conferred by the atopic trait ($P=0·04$).

No significant correlations were found between IgE and nasal albumin (normals: $r=-0·2$, $P=0·57$; atopics: $r=0·1$, $P=0·8$). In pre-inoculation antibody positive subjects symptom scores were correlated with nasal albumin ($r=0·78$, $P=0·007$) but not in antibody negative individuals ($r=0·36$, $P=0·33$).

Discussion

We have produced experimental HRV infection in normal and atopic volunteers. Good clinical colds were induced as assessed by symptom scores and infection was confirmed by viral shedding with culture and serology. Atopic individuals, in contrast to normal volunteers, tended to have more severe cold symptoms as well as greater increases in nasal albumin levels and this was not prevented by pre-inoculation neutralizing antibody. High IgE levels did not correlate with more severe clinical disease.

Historically, induction of experimental infectious disease in volunteers was initiated to permit exploration of various infectious, epidemiological and pathogenetic aspects under controlled conditions. Such human models have been particularly useful in the investigation of viral diseases and have helped to clarify transmission and disease patterns of many respiratory viruses, including rhinoviruses [3,4,10]. Recently, attention has focused on the interaction of diseases such as asthma and bronchiec-

tasis with respiratory viruses, and studies have examined their role in either direct causation [11] or exacerbations of pre-existing disease [1]. Experimental HRV disease has in the past involved groups of normal volunteers in studies of viral transmission and antibody responses, and a few studies have reported data obtained in patient sub-groups at particular risk for respiratory complications, such as atopics and asthmatics [5,12]. Doyle *et al.* [6] found no evidence to support increased susceptibility of atopic individuals to HRV 39 in spite of a battery of sophisticated and sensitive investigations.

In the past, experimental HRV colds have often been induced by instillation of virus-containing drops by pipette into the nose [13]. Aerosolized viruses may induce colds more reliably [14], and recent studies have suggested that the use of an atomiser releasing a fine spray may mimic naturally occurring HRV transmission better [15]. We have employed both methods and, coupled to relatively high inoculation titres of virus, this method yielded high infection rates as assessed by viral shedding and seroconversion. A high percentage of subjects developed clinical colds as measured by symptom scores and atopic volunteers tended to have moderate to severe colds irrespective of preinoculation neutralizing antibody. This contrasted with normal subjects who had only mild symptoms when preinoculation antibody in excess of 1:2 dilution was demonstrated. This relatively subjective observation was supported by changes in nasal wash albumin levels found during the cold. Naclerio et al. [16] have found a good correlation between intensity of cold symptoms and albumin levels in the nose and we employed the same measurement to obtain an objective parameter of severity in our group of volunteers. Albumin influx represents a passive transudation of plasma likely reflecting degrees of nasal mucosal inflammation associated with clinical cold symptoms. Changes in albumin levels during the cold were significantly higher in atopic subjects who had neutralizing antibodies present compared with normal individuals (Table 2). The largest changes in albumin levels were noted in atopic antibody negative volunteers, but when total groups were compared irrespective of pre-inoculation antibody, no differences were noted for either symptom scores or albumin levels.

Our results suggest that the presence or absence of neutralizing antibody is the primary determinant of the host's clinical response to a HRV cold. This overrides the effects of atopy and conceals its influence in any comparisons because of the relative severity of disease in normal antibody negative individuals. However, when these antibody negative normal and atopic groups are excluded from analysis, the increased sensitivity of atopic subjects becomes obvious. We cannot exclude that we have identified pre-existing rhinitic symptoms; however, baseline symptoms, if present, were subtracted to adjust the final scores. Furthermore, only two atopic subjects in this sub-group had a history of atopic rhinitis and symptom scores were corroborated by changes in nasal albumin levels.

Our data do not correspond to the findings of Doyle *et al.* [6]. All subjects in their study were antibody negative at screening and for reasons outlined above, differences between atopic and normal individuals would not have been apparent unless stratification was possible for positive and negative preinoculation antibody levels. Although other studies [17] have also tried to compare severity and found no differences, their study design and methods were not comparable to our study. In support of our results a study of experimental coronavirus 229E infection suggested that atopy may be related to the severity of cold symptoms. Associations were also found between clinical cold scores and nasal and serum IgE concentrations [18].

The discrepancy between absent initial HRV 16 neutralizing antibody levels and low titres on the day prior to inoculation was unexpected. Our repeat measurements of neutralizing antibody titres on the same sample are within one doubling dilution and assays were always performed in duplicate. Because of consistent results in duplicate experiments and minor variability in repeat assays, technical factors are unlikely to explain changes. A possible reason may be the development of crossreactive neutralizing antibody as a response to HRV colds caused by other serotypes. This phenomenon has been reported and an antibody crossreactive relationship has been demonstrated between HRV 1A and 30 heterotypic serotypes [19]. Although we have no definite evidence, we surmise that the majority of these volunteers had developed HRV-associated colds in the interim period before inoculation and developed neutralizing antibody cross-reactive to HRV 16. Previous inoculation studies have reported a similar lack of serological response after a cold in the presence of neutralizing antibody levels equal to or exceeding 1:8 dilution [20] as demonstrated in this study. This low level antibody did not prevent the development of infection in both normal and atopic volunteers, probably the result of the relatively high doses of virus used for inoculation. No associations were found between age and sex and the various parameters of infection; a finding similar to earlier reports [4].

The role of high IgE serum levels as a marker of increased susceptibility to rhinoviral disease has not been evaluated. Although our data in a limited number of subjects suggests greater susceptibility of atopic individuals (as defined by skin testing), severity of symptoms and nasal albumin levels were not correlated with IgE

levels. Tissue events may not parallel serum markers and serum IgE may thus not reflect levels of IgE or other atopy-associated mediators in nasal and other tissues. Alternatively, although the symptom score that we employed is sensitive to detect colds, it may be relatively insensitive in the presence of severe symptoms and not suitable to discriminate between moderately severe and very severe colds. Lack of such distinction will naturally hamper correlations sought with IgE levels.

Atopic individuals may therefore exhibit an increased sensitivity to the consequences of a common cold, prompting the suggestion that allergic tissues were 'pre-primed' by the existing allergic disease [21]. Although the mediators involved in cold symptoms are not defined, it can be speculated that the quantities of various cytokines (for example IL-8, GM-CSF) as well as inflammatory mediators (leukotrienes, kinins) may be amplified in atopic tissues, causing more severe symptoms when released. Kinins are potent vasoactive peptides identified in nasal secretions during colds; they cause vasodilation and increased vascular permeability, and are probably generated in the nose during a cold [16]. Additionally, because intercellular adhesion molecule-1 (ICAM-1) is the cellular receptor for the major group of HRVs and may be upregulated in allergic inflammation, atopic individuals may be predisposed to higher rates of HRV infection and even further increases in ICAM-1 accompanied by the nasal recruitment of mediator cells able to amplify existing inflammation [22].

In summary, our studies employing an experimental HRV disease programme in normal and atopic volunteers yielded significant upper airway cold symptoms of greater severity in the atopic group. Further utilization of this or similar protocols will allow the clarification of some of the operative pathophysiological mechanisms and their relationship to atopy and asthma.

Acknowledgements

Supported by a grant from the British Lung Foundation. Philip G. Bardin is the recipient of a Post-doctoral Fellowship from the South African Medical Research Council and David J. Fraenkel is an Australian Commonwealth Scholar. The help and advice of Dr David Tyrrell (Director Common Cold Unit, Salisbury, UK) is gratefully acknowledged. We thank Sister Gill Smith and her staff for dedicated patient care and the Southampton General Hospital for the facilities provided. Expert statistical advice was given by Ms Fiona Lampe.

References

1 Johnston S, Pattemore P, Smith S, et al. The association of viral infections with longitudinal changes in respiratory symptoms and/or peak flow recordings in schoolchildren. Eur Resp J 1992; 5:109.
2 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.
3 Jackson GG, Dowling HF. Transmission of the common cold to volunteers under controlled conditions. IV. Specific immunity to the common cold. J Clin Invest 1959; 38:762–9.
4 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.
5 Calhoun WJ, Swenson CA, Dick EC et al. Experimental Rhinovirus 16 infection potentiates histamine release after antigen bronchoprovocation in allergic subjects. Am Rev Respir Dis 1991; 144:1267–73.
6 Doyle WJ, Skoner DP, Fireman P et al. Rhinovirus 39 infection in allergic and nonallergic subjects. J Allergy Clin Immunol 1992; 89:968–78.
7 Jackson GG, Dowling HF, Spiesman IG, Boand AV. Transmission of the common cold to volunteers under controlled conditions. Arch Int Med 1958; 101:267–78.
8 Cohen S, Tyrrell DA, Russell MAH, Jarvis MJ, Smith AP. Smoking, alcohol consumption, and susceptibility to the Common Cold. Am J Public Health 1993; 83:1277–83.
9 Weeke B. Rocket immunoelectrophoresis. Scand J Immunol 1973; 1:37–46.
10 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.
11 Matsuse T, Hayashi S, Kuwano K et al. Latent adenoviral infection in the pathogenesis of chronic airways obstruction. Am Rev Respir Dis 1992; 146:177–84.
12 Halperin SA, Eggleston PA, Beasley P et al. Exacerbations of asthma in adults during experimental rhinovirus infection. Am Rev Respir Dis 1985; 132:976–80.
13 Cohen S, Tyrrell DAJ, Smith AP. Psychological stress and susceptibility to the common cold. New Engl J Med 1991; 325, 9:606–12.
14 Couch RB, Cate TR, Douglas GR Jr, Gerone PJ, Knight V. Effect of route of inoculation on experimental respiratory viral disease in volunteers and evidence for airborne transmission. Bacteriol Rev 1966; 30:517–29.
15 D'Alessio DJ, Peterson JA, Dick CR, Dick EC. Transmission of experimental Rhinovirus colds in volunteer married couples. J Infect Dis 1976; 133:28–36.
16 Naclerio RM, Lichtenstein LM, Kagey-Sobotka A et al. Kinins are generated during experimental rhinovirus colds. J Infect Dis 1988; 157:133–42.
17 Wiselka MJ, Kent J, Cookson JB, Nicholson KG. Impact of respiratory virus infection in patients with chronic chest disease. Epidemiol Infect 1993; 111:337–46.
18 Callow KA, Tyrrell DAJ, Shaw RJ et al. Influence of atopy on the clinical manifestations of coronavirus infection in adult volunteers. Clin Allergy 1988; 18:119–29.

19 Cooney MK, Wise JA, Kenny GE, Fox JP. Broad antigenic relationships among Rhinovirus serotypes revealed by cross-immunization of rabbits with different serotypes. J Immunol 1975; 114:635–9.

20 Mufson MA, Ludwig WM, James HD Jr et al. Effect of neutralizing antibody on experimental Rhinovirus infection. JAMA 1963; 186:578–84.

21 Bardin PG, Johnston SL, Pattemore PK. Viruses as precipitants of asthma symptoms II. Physiology and mechanisms. Clin Exp Allergy 1992; 22:809–22.

22 Johnston SL, Bardin PG, Pattemore PK. Viruses as precipitants of asthma symptoms III. Rhinoviruses: molecular biology and prospects for future intervention. Clin Exp Allergy 1993; 23:237–46.