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Effect of experimental rhinovirus 16 colds on airway hyperresponsiveness to histamine and interleukin-8 in nasal lavage in asthmatic subjects *in vivo*

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Summary

Background Asthma exacerbations are closely associated with respiratory virus infections. However, the pathophysiological consequences of such infections in asthma are largely unclear.

Objective To examine the effect of rhinovirus 16 (RV16) infection on airway hypersensitivity to histamine, and on interleukin-8 (IL-8) in nasal lavage.

Methods Twenty-seven non-smoking atopic, mildly asthmatic subjects participated in a placebo-controlled, parallel study. A dose of $0.5–2.9 \times 10^4$ TCID50 RV16 or placebo was nasally administered. Cold symptoms were recorded by questionnaire throughout the study. Histamine challenges were performed at entry, and on days 4 and 11 after inoculation. Nasal lavages were obtained at entry, and on days 2 and 9. The response to histamine was measured by PC_{20} (changes expressed as doubling doses: DD). IL-8 levels were obtained by ELISA, and were expressed in ng/ml.

Results RV infection was confirmed by culture of nasal lavage and/or by antibody titre rise in each of the RV16-treated subjects. Among the 19 RV16-treated subjects, eight developed severe cold symptoms. Baseline FEV_1 did not change significantly during the study in either treatment group ($P = 0.99$). However, in the RV16-treated subjects there was a decrease in PC_{20} at day 4, which was most pronounced in those with a severe cold (mean change ± SEM: -1.14 ± 0.28 DD, $P = 0.01$). In addition, IL-8 levels increased in the RV16 group at days 2 and 9 ($P < 0.001$). The increase in nasal IL-8 at day 2 correlated significantly with the change in PC_{20} at day 4 ($r = -0.48$, $P = 0.04$).

Conclusion We conclude that the severity of cold, as induced by experimental RV16 infection, is a determinant of the increase in airway hypersensitivity to histamine in patients with asthma. Our results suggest that this may be mediated by an inflammatory mechanism, involving the release of chemokines such as IL-8.

Keywords: asthma, asthma exacerbation, common cold, rhinovirus, airway hyperresponsiveness, interleukin-8, nasal lavage, chemokines, lymphocytes, neutrophils

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Introduction

Asthma is a chronic disease of the airways, characterized by episodic chest tightness and wheezing, and by airway hyperresponsiveness to non-sensitizing stimuli, as measured by the sensitivity to inhaled histamine or methacholine [1]. Airway inflammation underlies both stable asthma and exacerbations of the disease, as appears from a predominantly eosinophilic and mononuclear cell infiltrate in bronchial biopsy specimens [2]. It is generally considered that the exacerbations are due to exposure to respiratory

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virus infections and/or to airborne allergens, potentially leading to a flare-up of airway inflammation [1,2].

Several clinical and epidemiological studies have described a close temporal association of respiratory virus infections with asthma exacerbations [3]. Respiratory viruses can be identified in 10 to 44% of the asthma exacerbations in adults [4,5], whilst in children identification rates vary from 26 to 83% [6–9]. The use of sensitive techniques to detect rhinovirus and coronavirus in the two most recent studies have resulted in the highest identification rates so far [5,9]. Among the various respiratory viruses identified, rhinovirus predominates in most of these studies [5,7–9]. Interestingly, rhinovirus shedding in the absence of cold symptoms does not seem to be associated with clinical worsening of asthma [7].

The effects of experimental rhinovirus infection on airway responsiveness to inhaled histamine are somewhat controversial. Lemanske *et al.* demonstrated an induction of hypersensitivity to histamine after experimental rhinovirus 16 (RV16) infection in non-asthmatic patients with atopic rhinitis [10], whereas others have not observed such an effect when using other rhinovirus serotypes [11,12]. In asthmatic subjects, Halperin *et al.* found increased hypersensitivity to histamine in only four out of 22 subjects after experimental rhinovirus (serotype 39 and HH strain) infection [13], whilst in the most recent study by Fraenkel *et al.* a rather small, but significant increase in sensitivity to histamine could be detected in six asthmatic subjects after infection with RV16 [14]. Since none of these studies was placebo-controlled, it seems mandatory to examine the effect of experimental rhinovirus infection on airway sensitivity to histamine in asthmatic subjects by using such a design. This has successfully been employed in our previous study, showing excessive airway narrowing to methacholine after RV16 infection in atopic asthmatic subjects [15].

Rhinovirus infection has been shown to lead to infiltration of inflammatory cells into nasal secretions and mucosa [16–18] as well as into the bronchial mucosa [14] in normal and/or atopic subjects. *In vitro*, there is evidence that bronchial epithelial cell lines, fibroblasts and mononuclear cells produce pro-inflammatory cytokines in response to infection with rhinovirus [19–21]. *In vivo*, the levels of chemokines such as interleukin-8, RANTES and MIP-1α were found to be elevated in nasal secretions of asthmatic children during naturally acquired colds [22,23]. Consequently, one can postulate that chemokines such as IL-8 drive recruitment of inflammatory cells [24], thus promoting airway inflammation, and thereby airway sensitivity to histamine.

In the present study, we hypothesized that experimental RV16 colds in atopic asthmatic patients increase airway sensitivity to histamine, particularly in those with severe cold symptoms. In addition, we postulated that this effect is associated with a rise of IL-8 in nasal secretion. To that end, we measured dose-response curves to inhaled histamine and levels of IL-8 in nasal washings before and after placebo-controlled nasal inhalation of wild type RV16 in atopic, mildly asthmatic patients.

Materials and methods

Subjects

Twenty-seven non-smoking, atopic asthmatic subjects participated in this study. The subjects had not used inhaled or oral corticosteroids for at least 3 months, nor had they used theophyllines, antihistamines, sodium cromoglycate, or nedocromyl sodium for at least 6 weeks preceding the study. Symptoms of asthma were stable and controlled by on demand usage of inhaled salbutamol alone, that was withheld for at least 8 h before the measurements. There was no history of relevant exposure to allergens from 2 weeks before until the end of the study. The patients were not selected on basis of a history of virus-induced exacerbations. Among the eight subjects who received placebo-inoculation, four did not have neutralizing antibodies in their undiluted sera against 20–25 tissue culture infective dose ($\leq$ 1:1), and four had titres between 1:2 and 1:128 serum dilution. Fourteen out of the 19 subjects who received virus did not have neutralizing antibodies, and five had titres in the range of 1:2 to 1:16 serum dilution. The study was conducted from July to December 1994. The study was approved by the Hospital's Medical Ethics Committee, and informed consent was obtained from all participants. The subjects' characteristics are listed in Table 1.

Design

The study had a double-blind, placebo-controlled parallel design. Prior to the study, each subject was screened for inclusion and exclusion criteria. Three days before the experimental inoculation of virus or placebo, a histamine inhalation test was carried out. Subsequently, virus or placebo (diluent) was administered on two successive days. Histamine challenges were repeated at days 4 and 11 after the first inoculation of virus or placebo. Nasal washing was performed and a blood sample was taken immediately before the first inoculation of virus or placebo, and then at days 2 and 9. Four weeks after inoculation all subjects returned to the laboratory for a final nasal washing and a blood sample to determine the convalescent antibody titre.

RV16 inoculation

The RV16 virus strain and stock was the same as used in previous experiments in humans *in vivo* by others [10] and

by ourselves [15]. The virus was cultured according to standards of good laboratory practice and the inoculum was tested to be safe for human *in vivo* usage [25]. Nasal inoculation of the rhinovirus was performed following a previously described method [10], that was slightly modified by adding nasal virus inhalation [15]. A total dose of 0.5–2.9×10^4 $TCID_{50}$ diluted in 3 ml Hanks' balanced salt solution (HBSS) with 0.5% (w/v) gelatin was administered to each subject. This dose was divided over 2 days. On each day the same procedure for virus inoculation was followed. First, 0.5 ml of the inoculum was inhaled through the nose by using a nebulizer (DeVilbiss 646: median mass aerosol diameter (MMAD) 2.4 μm) connected to a face mask. Second, 0.5 ml was sprayed by atomizer (DeVilbiss 286, powered by a compressor, MMAD: > 10 μm) into the nostrils. And finally, 0.5 ml was instilled into the nostrils by pipette.

Laboratory confirmation of infection

We considered a four-fold or greater increase in virus-specific neutralizing antibody in the serum and/or recovery of the virus from nasal washes as confirmation of RV16 infection [10,15]. Before and 28 days after virus or placebo inoculation, levels of neutralizing antibodies were determined by a neutralization assay using homologous virus [10,15]. Nasal lavages were obtained before the first virus or placebo administration, and subsequently on days 2, 9 and 28. Human embryonic lung fibroblast (HEL) cultures were inoculated with these lavages and incubated at 32°C for 14 days. If the culture showed the characteristic rhinovirus-induced cytopathic effects, RV16 was identified by a neutralization assay, using RV16 specific guinea pig immune serum (1126AS/GP-VR; American Type Culture Collection, Rockville, MD). All nasal washes were also inoculated into rhesus monkey kidney (LLC-MK2) cells, HEp-2 cell cultures and HEL cells, and cultured at 37°C, in order to exclude any intercurrent respiratory virus infection.

Diary cards

Throughout the study period, symptoms of common cold and asthma were evaluated with a questionnaire that was completed by the participants three times daily. Cold symptoms included sneezing, sore throat, nasal discharge, stuffy nose, headache, cough, malaise, chills or fever, which were graded: 0 = absent, 1 = mild, 2 = moderate, 3 = severe and added up to a total cold symptom score. In subjects in whom infection was confirmed, those who recorded a total cold symptom score ≥ 11 at any time-point after inoculation were defined as having a severe cold, whereas subjects with scores < 11 were considered to have a mild cold [10,15,25].

In the same manner, asthma symptoms including cough, breathlessness, wheeze, chest tightness, and nocturnal symptoms were recorded [15]. The daily consumption of inhaled salbutamol as rescue medication was also noted.

Histamine challenge

Standardized histamine challenge tests [26] were performed using histamine-di-phosphate in phosphate buffered saline. Histamine was stored at 4°C, and nebulized at room temperature in serial doubling concentrations ranging from 0.03 to 8 mg/ml. A DeVilbiss 646 nebulizer (DeVilbiss Co., Somerset, PA) (output 0.13 ml/min) was used, in connection to the central chamber of an in- and expiratory valve box with an expiratory aerosol filter (Pall Ultipor BB50T). The aerosol was inhaled by tidal breathing for 2 min at 5 min intervals with the nose clipped. The lung function response was measured as FEV_1, obtained by a dry rolling spirometer (Morgan spiroflow, Rainham, United Kingdom). First, baseline FEV_1 was determined as the mean of three reproducible values (within 5%). Subsequently, single measurements of FEV_1 were made 90 s after each histamine dose. The tests were discontinued if FEV_1 decreased by more than 20% from baseline. Afterwards the subjects inhaled 200 μg salbutamol to provide immediate bronchodilation.

Nasal lavage

A modified 'nasal pool device' was used for nasal lavage [27]. This consists of a syringe, connected to a rubber tube, equipped with an inflatable balloon, serving as a nasal adapter. The balloon was inserted into the *vestibulum nasi*, and gently inflated. While the patient was sitting in a writing position, 10 ml of pre-warmed Hanks' balanced salt solution (HBSS) (one nostril with and the other without 0.5% gelatin) was instilled by gently compressing the syringe. After 5 min the fluid was recovered by retracting the syringe. This procedure was carried out in each nostril. The lavage recovered from the first (with 0.5% gelatin) and second nostril (without gelatin) was used for confirmation of RV infection and to determine the IL-8 level, respectively.

IL-8 in nasal washing

Immediately after recovery, nasal washings were centrifuged at $250 \times g$ for 10 min. Supernatant was removed and stored at −70°C until further analysis. The IL-8 levels were determined by ELISA (CLB, Amsterdam, The Netherlands), according to the manufacturer's directions. The detection limit of this assay was 40 pg/ml.

Leucocyte counts in peripheral blood

Before, and on days 2 and 9 after placebo or virus administration, absolute and differential leucocyte counts

were assessed by automated blood count analysis (Technicon H1, Technicon, Tarrytown, NY).

Statistical analysis

The highest individual total cold symptom score (referred to as cold score), and the cumulative asthma score recorded from day 0 to 5 after inoculation minus the cumulative scores from 4 days to 1 day before inoculation (referred to as asthma score) were used for correlation testing [15]. The response of FEV_1 to histamine was expressed as percentage fall from baseline value [26], and was plotted against log nebulized concentration of histamine in mg/ml. The concentration-response curves were characterized by their position, expressed as the provocative concentration causing 20% fall in FEV_1 from baseline value (PC_{20}), which was calculated by log-linear interpolation between the last two adjacent data-points [26]. The logarithm of PC_{20} was used in the analysis, and changes in PC_{20} were expressed in doubling doses (DD). IL-8 levels were expressed in ng/ml, peripheral blood leucocyte numbers were expressed in cells/L.

Changes in the variables were analysed by repeated-measure analysis of variance (MANOVA), with placebo,

Table 1. Characteristics of study subjects

Subject number	Sex	Age (yr)	FEV_1 (% pred.)	$PC_{20}FEV_1$* (mg/ml)	Cold score	Asthma score	Antibody titre† (before/after)	Lavage culture day 2/day 9	Medication	Allergy‡
Placebo										
1	M	25	76	0.28	1	0	<1:1/ <1:1	Neg/neg	None	H
2	M	26	68	0.60	4	2	<1:1/ <1:1	Neg/neg	Salbutamol	H, C
3	M	18	71	0.64	2	1	1:32/ 1:32	Neg/neg	Salbutamol	H, C
4	F	26	75	0.78	2	0	1:8/ 1:8	Neg/neg	Salbutamol	H, C, P
5	M	25	73	0.80	1	0	1:16/ 1:8	Neg/neg	Salbutamol	H, C
6	M	24	80	1.37	2	2	1:128/ 1:128	Neg/neg	None	H, C, P
7	M	24	89	1.85	11	5	<1:1/ <1:1	Neg/neg	None	C
8	M	25	87	1.93	4	0	1:1/ <1:1	Neg/neg	Salbutamol	H, C, P
Mean ± SEM			77.4 ± 2.6	0.87 ± 0.4						
RV16										
9	F	25	71	0.12	15	32	<1:1/ 1:4	Pos/neg	Salbutamol	H, P
10	F	23	77	0.25	10	3	<1:1/ 1:4	Pos/neg	Salbutamol	H, P
11	M	21	103	0.32	4	0	<1:1/ 1:4	Pos/neg	None	H, P
12	M	18	80	0.34	6	6	<1:1/ 1:16	Pos/pos	Salbutamol	H, C
13	M	19	81	0.37	5	−10	1:1/ 1:128	Pos/pos	None	C
14	M	22	85	0.41	0	0	<1:1/ 1:32	Pos/neg	Salbutamol	H, C, P
15	M	20	87	0.71	16	23	<1:1/ 1:128	Pos/pos	Salbutamol	H, P
16	F	25	88	0.86	11	23	<1:1/ <1:1	Pos/pos	None	H, C, P
17	M	26	108	0.87	2	2	<1:1/ 1:4	Pos/neg	Salbutamol	H
18	F	21	92	1.19	12	23	1:2/ 1:32	Neg/neg	Salbutamol	C
19	M	20	101	1.33	12	15	1:16/ 1:256	Pos/pos	Salbutamol	H, C, P
20	M	21	80	1.39	16	28	<1:1/ 1:64	Pos/neg	None	H, C, P
21	F	24	89	1.76	22	49	1:8/ 1:512	Pos/pos	None	H, C, P
22	M	22	89	2.03	6	8	1:4/ 1:512	Pos/neg	Salbutamol	H, C
23	F	23	77	2.12	14	29	<1:1/ 1:128	Pos/pos	None	H, C, P
24	M	26	69	2.39	6	4	1:4/ 1:8	Pos/neg	Salbutamol	H, C
25	M	24	77	2.69	5	2	<1:1/ 1:32	Pos/neg	Salbutamol	H, C, P
26	M	21	90	2.98	3	0	<1:1/ 1:32	Pos/pos	None	C
27	F	22	105	4.17	6	0	<1:1/ 1:16	Pos/pos	None	H, C
Mean ± SEM			86.4 ± 2.6	0.90 ± 0.2						

* Airway hyperresponsiveness to histamine (provocative concentration of histamine causing a 20% fall in FEV_1) measured at entry of study.

† Titre of neutralizing antibodies against RV16 measured on screening day and at day 28 after inoculation.

‡ Allergy: C = cat; H = house dust mite; P = pollen (grasses and/or trees); determined by skin prick test (Vivodiagnost, ALK, Benelux).

RV16 treatment or severe cold and mild cold as between-group factors and time as a within-group factor. Significant MANOVA effects were explored with Student's *t*-tests. Differences in PC_{20}, IL-8 levels and leucocyte numbers within the groups between the study days were examined using two-tailed paired *t*-tests, and differences between the groups were analysed using unpaired *t*-tests. The summary statistics were expressed as means ± SEM. For evaluation of associations between the variables, the Pearson's correlation test was used. *P* values less than 0.05 were considered statistically significant.

Results

One of the RV16-treated subjects (subject 9) dropped out of the study at 7 days after the first inoculation because of a moderate exacerbation of asthma, requiring treatment with oral prednisone, to which she responded well. One nasal washing sample was excluded from the analysis (subject 7, day 9), because of a recent nose bleed.

Confirmation of infection

In the placebo group all cultures of nasal washes remained negative for RV16 during each visit. In the virus-treated group RV16 could not be detected in the nasal lavage before inoculation, whilst at day 2 RV16 was detected in the nasal lavage of all but one subject (subject 18). At day 9, RV16 was identified in 10 out of 19 subjects, whereas at day 28 all nasal washings were negative. No other respiratory viruses were identified in any of the nasal washings (Table 1).

In the placebo group none of the subjects showed an increase in RV16 neutralizing antibodies. In the RV16 group all subjects but two (subjects 16 and 24) showed at least a four-fold increase in neutralizing antibodies in the convalescent sera (range: 4-fold to 128-fold increase) (Table 1).

Cold and asthma scores

In the placebo group, there was no significant change in cold score or asthma score (MANOVA, $P \geq 0.52$). In the RV16 group, there was a significant increase in cold score (MANOVA, $P < 0.001$), that peaked 1 day after the first inoculation, gradually returning to baseline within 5 days. The highest cold scores were significantly different between the groups ($P < 0.001$). Eight of the RV16-infected subjects had a severe cold as shown by a symptom score ≥ 11 (Table 1).

In the RV16-treated subjects there was a significant increase in asthma symptoms (MANOVA, $P < 0.001$) that peaked on the second and third day after the first inoculation, and returned to baseline within 5 days. The asthma score in the subjects with a severe cold was significantly higher than the asthma score in those with a mild cold and the placebo-treated subjects ($P < 0.001$) (Table 1). Cold score and asthma score were significantly correlated in the RV16 group ($r = 0.92$, $P < 0.001$). The use of salbutamol did not change significantly within the groups at any time point (MANOVA, $P = 1.00$).

Baseline lung function

Before RV16 or placebo inoculation, FEV_1 % predicted was slightly higher in the RV16 group as compared to the placebo group ($P = 0.04$) (Table 1). During the course of the study, there were no significant effects on baseline FEV_1 within either the placebo group or in the RV16-treated subjects with a mild or severe cold (MANOVA, $P = 0.99$) (Figure 1). The maximal change in FEV_1 after infection did not correlate significantly with the asthma score ($P = 0.98$).

Sensitivity to histamine

Before inoculation of RV16 or placebo, the mean PC_{20} was not different between the two treatment groups ($P = 0.93$). In the placebo group, there was no significant change in PC_{20} during the study (MANOVA, $P = 0.67$) (Figure 2). In the RV16 group, there was a significant decrease in PC_{20} at day 4 (mean difference ± SEM: -0.65 ± 0.25 DD, $P = 0.02$), which was no longer significant at day 11 (mean difference ± SEM: -0.40 ± 0.30 DD, $P = 0.19$). These changes were not significantly different from placebo ($P = 0.10$ and $P = 0.27$, respectively).

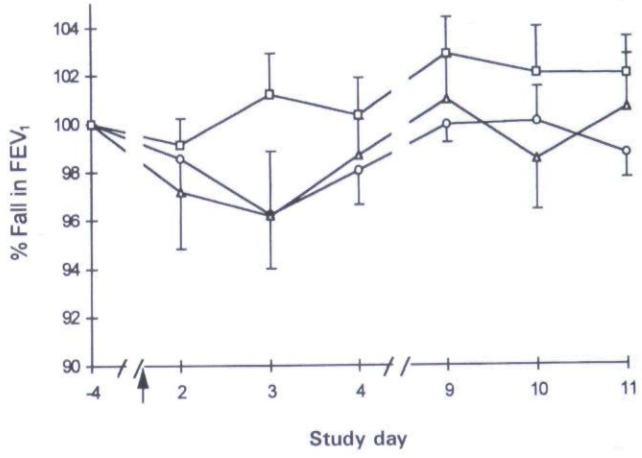

Fig. 1. Change in baseline FEV_1 as compared to the value at entry, expressed in percentage fall in FEV_1 from the individual mean baseline value (100%) in the placebo group (□), the mild cold group (△) and the severe cold group (○). There was no significant effect on FEV_1 during the study in any group (MANOVA: $P = 0.99$).

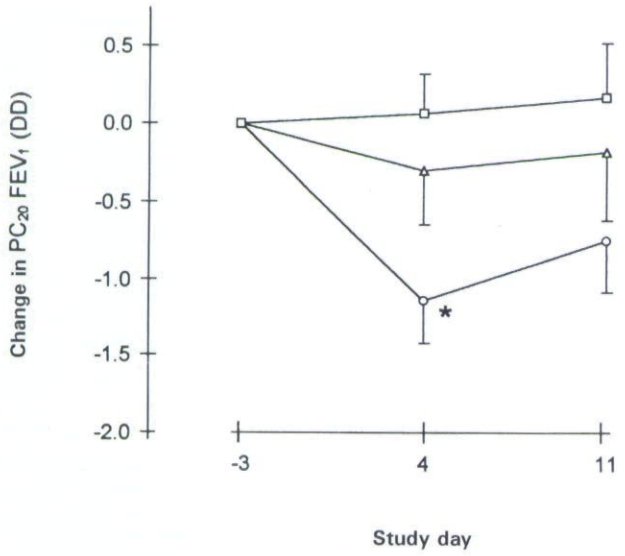

Fig. 2. Change in sensitivity to inhaled histamine (PC_{20}) as compared to the value at entry, expressed in doubling doses (DD, geometric mean ± SEM) in the placebo group (□), the mild cold group (△) and the severe cold group (○). In the severe cold group the changes were significantly different from placebo at day 4 ($P = 0.01$), but not at day 11 ($P = 0.09$).

However, in the subjects with a severe cold, this decrease was more pronounced: mean difference ± SEM: −1.14 ± 0.28 DD, $P = 0.005$ at day 4, with a trend towards a decrease at day 11 (mean difference ± SEM: −0.75 ± 0.34 DD, $P = 0.07$) (Figure 2). This change was significantly different from placebo at day four, but not at day 11 ($P = 0.01$ and $P = 0.09$, respectively). In the mild cold group there was neither a change in PC_{20} at day 4 (mean difference ± SEM: −0.30 ± 0.35 DD, $P = 0.42$), nor at day 11 (mean difference ± SEM: −0.18 ± 0.44 DD, $P = 0.68$) (Figure 2).

The changes in PC_{20} in the five subjects with pre-existing neutralizing antibodies against RV16 were not statistically different from the changes in those without such antibodies (MANOVA, $P = 0.33$) (day 4: mean change ± SEM: −0.53 ± 0.46 DD, $P = 0.31$, and −0.69 ± 0.31 DD, $P = 0.04$, respectively. Day 11: −1.10 ± 0.28 DD, $P = 0.02$, and −0.13 ± 0.38 DD, $P = 0.73$, respectively).

IL-8 in nasal washing

IL-8 in the nasal washings did not change significantly in the placebo group (MANOVA, $P = 0.06$) (Figure 3). In the RV16 group, IL-8 increased both at days 2 and 9 ($P < 0.001$) as compared to day −5. In subjects with a severe cold this increase was significant at both time-points (within group, $P \leq 0.04$), and also as compared to placebo ($P < 0.05$). In those with a mild cold the increase in IL-8 was only significant at day 9, both within the group ($P = 0.008$) and compared to placebo ($P < 0.01$) (Figure 3). The increase in IL-8 at day 2 in the RV16 group correlated significantly with the cold score ($r = 0.80$, $P < 0.001$), asthma score ($r = 0.68$, $P = 0.001$), and with the change in PC_{20} at day 4 ($r = -0.48$, $P = 0.04$) (Figure 4a, 4b).

Leucocyte counts in peripheral blood

In the RV16 treated subjects differential leucocyte counts showed a significant rise in the number of neutrophils ($P < 0.001$) and a concomitant decrease in lymphocyte number at day 2 after inoculation ($P < 0.001$) (Figure 5). Only in subjects with a severe cold were the cell numbers at day 2 significantly different from placebo ($P \leq 0.01$). Neutrophil and lymphocyte numbers had returned to baseline at day 9 ($P > 0.17$).

The number of neutrophils at day 2 correlated significantly with cold score ($r = 0.59$, $P = 0.008$), asthma score ($r = 0.65$, $P = 0.002$), the change in PC_{20} at day 4 ($r = -0.49$, $P = 0.03$), whilst there was a trend towards a significant correlation with the change in IL-8 levels at day 2 ($r = 0.39$, $P = 0.10$) (Figure 4c). Furthermore, the number of lymphocytes at day 2 was also significantly related to the cold scores ($r = -0.69$, $P = 0.001$), asthma score ($r = -0.62$, $P = 0.004$) and the change in IL-8 at day 2 ($r = -0.73$, $P < 0.001$) (Figure 4d), with a trend towards a

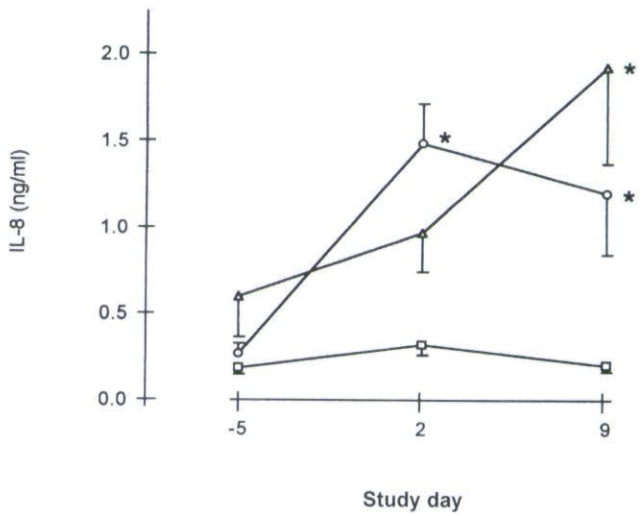

Fig. 3. Levels of IL-8 in nasal lavage fluid (ng/ml, mean ± SEM) in the placebo group (□), the mild cold group (△) and the severe cold group (○). In the severe cold group, the changes in the levels of IL-8 compared to the values at entry were significantly different from placebo, both at days 2 and 9 ($P = 0.002$ and $P = 0.04$, respectively), whilst in the mild cold group there was only a significant change at day 9 ($P = 0.01$).

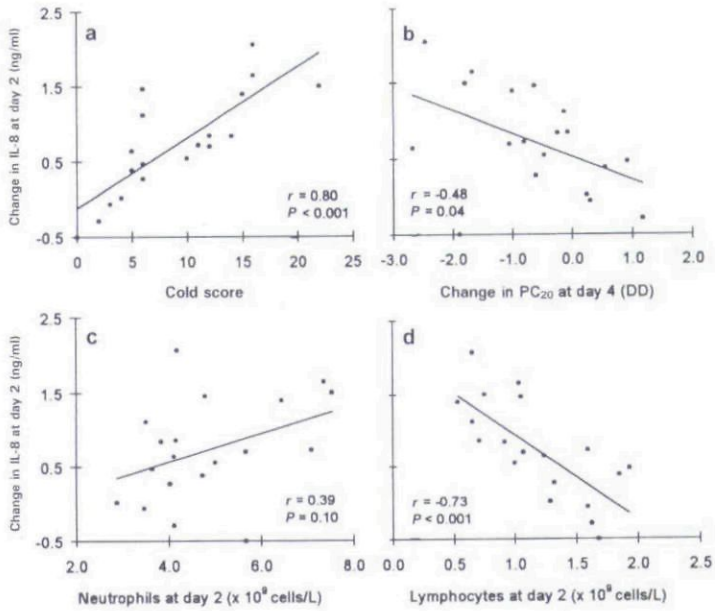

Fig. 4. Relationship (Pearson's correlation coefficient, r) between the changes in IL-8 levels in nasal washing at day 2 compared to the values at entry, and cold score (a), changes in PC_{20} at day 4 compared to the values at entry (b), and the number of neutrophils (c) and lymphocytes (d) in peripheral blood at day 2.

correlation with the change in PC_{20} at day 4 ($r = 0.42$, $P = 0.07$).

Discussion

This study shows that experimental rhinovirus 16 infection in atopic asthmatic subjects induces airway hypersensitivity to histamine, particularly in those patients who develop a severe cold. In addition, we have demonstrated that the levels of the pro-inflammatory chemokine IL-8 in nasal secretions rise after infection. This rise is associated with cold score, change in airway hyperresponsiveness, and numbers of neutrophils and lymphocytes in peripheral blood after infection. These findings suggest that the severity of the cold is a major determinant of rhinovirus-induced airway hyperresponsiveness in asthma. Our results fit in with the hypothesis that this is mediated through an inflammatory mechanism involving locally produced chemokines.

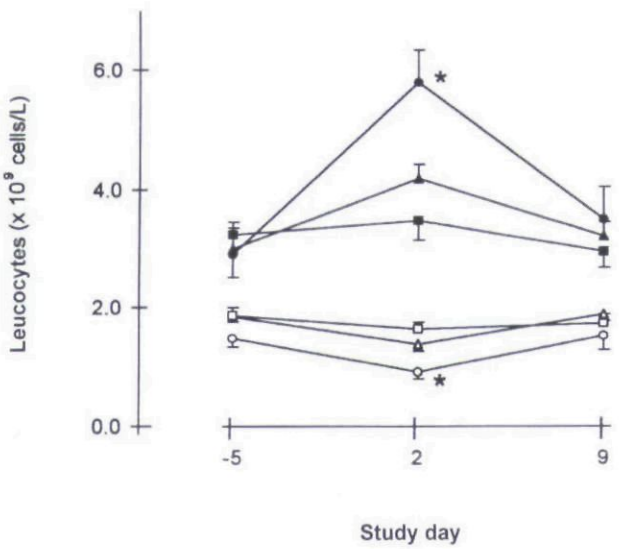

Fig. 5. Number of neutrophils (closed symbols) and lymphocytes (open symbols) in peripheral blood during the study in the placebo group (■ □), the mild cold group (▲ △), and the severe cold group (● ○). Only in the severe cold group was the number of neutrophils at day 2 significantly elevated ($P = 0.01$), whilst the number of lymphocytes was significantly reduced compared to placebo ($P = 0.003$).

This is the first placebo-controlled study showing the development of airway hypersensitivity to histamine after experimental infection with wild type rhinovirus. The change in airway hypersensitivity in the asthmatic subjects with severe colds was about 1 doubling dose, which is similar to what is usually observed after allergen challenge [28]. Rhinovirus-induced hypersensitivity of such magnitude has also been demonstrated in patients with atopic rhinitis by Lemanske *et al.* [10]. However, in the latter subjects the histamine hypersensitivity lasted up to 4 weeks after infection, whereas in our study the effect was no longer significant at day 11. One could speculate that the prolonged effect in atopic rhinitis may have been due to the additional allergen challenges during that study.

Two previous studies on experimental rhinovirus infection in asthma [13,14] showed small and variable changes in airway hypersensitivity. Our findings suggest that this might be explained by the severity of the colds that were induced. After taking this into account, it appears that experimental rhinovirus infection in asthma does lead to substantial worsening of PC_{20} to histamine. Interestingly, this also

occurred in the small number of subjects who had pre-existing RV16 neutralizing, but possibly cross-reactive circulating antibodies. The latter may not be surprising, since atopic subjects with low titres of neutralizing antibodies, as opposed to normal subjects with such titres, have been shown to develop severe cold symptoms after experimental RV16 inoculation [29].

The design of the present study allowed us to differentiate the responses to rhinovirus inoculation from normal fluctuations in symptoms and airway physiology that are characteristic to asthma. The study was performed in the months July to December, but no attempt was made to exclude coinciding allergen exposure (pollen, house dust mite), since this would have been hard to accomplish. However, the present circumstances can be considered as those encountered during naturally occurring infections. Despite the fact that the subjects were clinically stable as assessed by history, and by symptom control with p.r.n. β_2 adrenergic medication alone, a moderate exacerbation of asthma developed in the subject who had the lowest PC_{20} at entry into the study. This underlines the potential of exacerbations after rhinovirus infection in patients with asthma [3,9,30], despite the usually small accompanying changes in lung function [13–15].

In this study, we applied validated procedures for inoculation and measuring the responses to rhinovirus infection. First, by using a combination of three methods of virus administration, including nasal inhalation, the natural ways of transmission were mimicked [31]. In this way, the virus may even have reached the intrapulmonary airways [32]. Second, commonly used and well-standardized methods for lung function testing and histamine challenges were used [26]. Third, we applied a validated method for nasal lavage [27], which allowed IL-8 to diffuse into the lavage fluid during a 5-min exposure period of the nasal epithelium, resulting in IL-8 levels well above the detection limit of the IL-8 ELISA.

How can the present results be interpreted? The increase in airway hypersensitivity, in the absence of a significant decrease in lung function, during the acute phase of infection might be explained by physiological phenomena such as airway wall swelling, potentiating the airway narrowing effect of smooth muscle shortening [33]. Such an explanation would be in keeping with the observations by Cheung *et al.* [15], who showed that experimental RV16 infection leads to excessive airway narrowing in response to inhaled methacholine in subjects with asthma. Airway wall swelling in asthma is generally considered to be a consequence of inflammation [2]. Indeed, Fraenkel *et al.* [14] recently described the infiltration of inflammatory cells, particularly lymphocytes and eosinophils into the bronchial mucosa in patients with asthma after experimental RV16 infection. The presently observed correlation between the numbers of neutrophils and lymphocytes in peripheral blood after infection and the change in airway hypersensitivity indirectly supports an active role of these cells in the virus-induced airway inflammation.

We found a marked rise in IL-8 in nasal secretions after RV16 infection. In general, this confirms the ability of rhinovirus to increase the release of a number of pro-inflammatory mediators and/or cytokines within the airways, such as kinins [16,34] and interleukin-1 [35] in nasal secretions, and histamine in broncho-alveolar lavage fluid [36]. The present results obtained by experimental rhinovirus infection are in keeping with the preliminary data of Teran *et al.* [22], who showed that levels of IL-8 in nasal secretions were elevated in nasal secretions during a naturally acquired cold in children with asthma. Our results extend these previous observations by showing an association between the increase in IL-8 in nasal washings and cold or asthma symptoms, as well as the degree of worsening of airway hyperresponsiveness. IL-8 is a CXC-chemokine, produced by tissue cells (epithelial cells, fibroblast and endothelial cells), leucocytes, macrophages and mast cells [24,37] and displays various activities, such as chemotactic activity for neutrophils, lymphocytes and basophils [38]. In addition, IL-8 may be involved in the recruitment of primed eosinophils, implicating its involvement in allergic inflammation [39]. Since rhinovirus *in vitro* induces the production of IL-8 in epithelium, fibroblasts and peripheral blood mononuclear cells [20,21,40], our findings support the hypothesis that the release of mediators, such as the chemokine IL-8, can drive the airway inflammation, and thereby the hypersensitivity to histamine after rhinovirus infection in allergic asthma.

What are the clinical implications of this study? First, a common cold aggravates airway hypersensitivity in patients with asthma, fitting in with the close epidemiological association between rhinovirus infections and exacerbations of asthma [5,9]. And second, it appears that atopic asthmatic patients with low titres of neutralizing antibodies may not be fully protected against experimental RV16 infection, and its detrimental effects on their asthma. This observation in a small number of subjects in the present study first needs confirmation in larger series of experimental or naturally occurring rhinovirus infections in patients with allergic asthma.

In conclusion, experimental RV16 infection can be employed as a useful laboratory model for the development of airway hypersensitivity during an asthma exacerbation. One of the potential mechanisms for this might be the rhinovirus-induced release of pro-inflammatory chemokines. This hypothesis needs further testing in models of rhinovirus infection *in vitro* and *in vivo*, focusing on the pathological mechanisms in the intrapulmonary airways in patients with asthma.

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