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Influence of atopy on the clinical manifestations of coronavirus infection in adult volunteers

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Summary

In an attempt to understand the relationship between viral upper respiratory tract infection and the underlying virological and immunological mechanisms, thirty-four volunteers were inoculated intranasally with coronavirus 229E; subsequent virus shedding and/or antibody rises, indicating active infection, were observed in twenty-nine. There was a greater increase in independently measured scores of clinical severity, e.g. cold symptoms, in those with detectable IgE in nasal secretions ($P < 0{\cdot}01$). A similar association was found between clinical scores and serum IgE concentrations $\geqslant 150$ IU/ml, but the relationship with systemic atopy, as assessed by skin-prick tests to common allergens, was less marked. A more detailed study of twelve of the infected volunteers failed to explain these findings on the basis of mast cell mediator release, as concentrations of leukotriene B_4, the sulphidopeptide leukotriene C_4, and histamine, were not appreciably elevated in the nasal secretions following virus inoculation. Similarly, there was no evidence that circulating coronavirus specific IgE was produced. Thus, this study suggests that atopy may be related to the severity of cold symptoms produced by coronavirus 229E, although the exact connection has yet to be determined.

Introduction

In allergic subjects, particularly asthmatics, exacerbations commonly occur during respiratory virus infections suggesting the possible involvement of IgE [1]. There is little experimental evidence to confirm the relationship between symptoms of viral infection and atopy, despite the ready availability of measures of atopy, such as

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reactions to skin-prick testing and circulating IgE concentration [2–5]. Local IgE in secretions may also be important, as it is also associated with atopy [6, 7] and may be elevated even when serum concentrations are normal [8].

In this study all these measures of atopy have been made in volunteers infected with coronavirus 229E and compared with the severity of the cold, assessed subjectively and objectively by an independent medical observer.

In order to elucidate any direct role of mast cells or other mediator releasing cells, measurement was made of recognized mediators of nasal allergy in twelve of these infected volunteers. Both the sulphidopeptide leukotriene C_4 (LTC_4) and leukotriene B_4 (LTB_4), as well as histamine, have been shown to be released into nasal secretions after nasal allergen challenge [9,10]. Histamine also appears to be released into nasopharyngeal secretions during parainfluenza and respiratory syncytial virus (RSV) infections [11, 12]. In parainfluenza, RSV and rubella infections, specific serum and nasal IgE concentrations have been shown to be raised [11–13] and it has been suggested that it contributes to the wheezing in respiratory infections [11, 12].

Finally, evidence of increased infiltration of the nasal mucosa by leucocytes during infection was also sought.

Subjects and methods

Volunteers

Normal adult volunteers, between the ages of 18 years and 50 years, were recruited by a procedure that excludes those with recognized allergic disease, e.g. allergic rhinitis. Isolation, inoculation and assessment procedures have been described elsewhere [14, 15]. Thirty-four volunteers, twenty-five females and nine males, were inoculated intranasally with coronavirus 229E, contained in a filtered nasal wash, as previously described [16]. The trial was approved by the Northwick Park Ethical Committee.

Clinical scores

A daily clinical score was calculated from the record of nasal blockage, rhinorrhoea, sneezing and systemic symptoms. The numbers of used tissues and pyrexia are given heavy weighting [17]. The clinical score of all volunteers before virus challenge was zero.

Nasal washings

These were performed daily on 3 consecutive days before, and for 6 days after, virus inoculation. After volunteers refrained from nasal blowing for 1 hr, 1-ml portions of normal saline were instilled into each nostril with the head tilted backwards 30°. The individual expelled the mixture of mucus and saline into a plastic beaker [10]. This was repeated until 8 ml of wash fluid were recovered, followed by vortex mixing with glass beads to disperse the mucus. Aliquots for immunoglobulin measurement were centrifuged to sediment the mucus, and supernatants were stored at −20°C. Other aliquots for histamine and leukotriene assay, and virus culture were stored at −70°C. The presence of virus was detected using the C-16 line of MRC-C cells [18].

Enzyme-linked immunosorbent assay (ELISA) for total IgE in nasal washings

Washing methods, substrate and optical density (OD) measurements have been described elsewhere [16]. Nunc round-bottomed immunoplates (Gibco Ltd, Uxbridge,

U.K.) were coated with rabbit anti-human IgE antiserum (Hoechst UK Ltd, Hounslow, U.K.) at 1:3000 overnight at 4°C. After washing, plates were incubated with 0·1% bovine serum albumin (BSA) in phosphate buffered saline (PBS) for 2 hr at room temperature (RT). Nasal washings were added, undiluted, and incubated for 6 hr at 4°C. Goat anti-human IgE conjugated to alkaline phosphatase (Sigma Chemical Co., Ltd, Poole, U.K.) at 1:1000 was then added and left overnight at 4°C. After addition of the substrate, plates were read at 2 hr with a Titertek Multiskan ELISA reader (Flow Laboratories Ltd, Irvine, U.K.). Washings were considered positive for IgE if the OD was $\geqslant$OD + 2 standard deviations (s.d.) of that of the controls without nasal washing. This represented a minimum detectable limit of about 0·2 IU/ml.

ELISA for total circulating IgE

IgE in sera collected prior to, 6 days (intermediate), and 21 days (convalescent) after virus challenge, was measured as described above, omitting the blocking step with BSA. A standard curve on each plate used a standard human serum IgE (provided by Dr E.B. Mitchell, formerly of the Clinical Research Centre, Harrow, U.K.), and serum samples were diluted at $\frac{1}{5}$ and $\frac{1}{20}$ in 0·1% BSA and incubated for 4 hr at RT. The OD of the dilution that fell on the linear part of the standard curve was used to calculate the concentration. Concentrations of IgE $\geqslant$ 150 IU/ml were considered to be abnormally high [4,19,20].

Extraction of leukotrienes from nasal washings

Nasal wash fluid was mixed with an equal volume of HPLC grade methanol (Rathburn, Walkerburn, U.K.) using a Silverson grinder (Silverson Ltd, Chesham, U.K.) and stored at −20°C. After acidification to pH 4·0 with acetic acid the precipitate was removed by centrifugation (12 000 × ***g***, 10 min, 4°C) and the supernatant was diluted to give a 10% methanol/H_2O solution. The leukotrienes were then extracted using C18 Sep-paks (Water Associates, Northwick, U.K.) [10] and resuspended in PBS buffer.

Leukotriene measurement

The measurement of LTC_4 and LTB_4 by a double antibody radioimmunoassay (using antisera supplied by Dr E. Hayes, Merck Sharp & Dohme, Rahway, NJ, U.S.A.) has been described in detail elsewhere [21–23].

Briefly, rabbit anti-LTC_4 or anti-LTB_4 were incubated with unknown or dilutions of synthetic LTC_4 or LTB_4 (a gift from Dr J. Rockach, Merck Forest Laboratories, Quebec, Canada) together with 14,15^{-3}H-LTC_4 or 14,15^{-3}H-LTB_4 (New England Nuclear (NEN), Southampton, U.K.) together with normal rabbit immunoglobulins in PBS containing p-methyl sulphonyl fluoride (1×10^{-4} M) and 0·25% gelatin. After 2-hr incubation at 4°C, goat anti-rabbit immunoglobulin (Biorad, Watford, U.K.) diluted in 5% polyethylene glycol (PEG) 6000 was added. Two hours later the tubes were centrifuged (15 000 × ***g***, 15 min, 4°C). The radioactivity in the supernatant was measured with an LKB beta-1217 counter using Aquasol II (NEN) as scintillant. All assays were performed in duplicate and the leukotriene concentration determined using a smoothed spline computed analysis. Recovery experiments using synthetic leukotrienes confirmed a recovery of 82 + 9% for LTC_4 and 100% for LTB_4. The baseline pre-challenge nasal washing leukotriene concentration was obtained from the mean of 3 consecutive days prior to virus inoculation.

Recovery and measurement of histamine from nasal washings

After collection of the nasal washing, an aliquot of 200 μl wash fluid was mixed with 20 μl of 0·1 M EDTA pH 7·4 and stored at −80°C. Histamine measurement used a specific isotope enzymatic assay modified from that of Guilloux *et al.* [24], which had a sensitivity of 0·5 ng/ml. In brief, the sample was incubated with methyl transferase obtained from rat kidney in the presence of the tritium-labelled methyl donor s-adenylmethionine. The labelled methyl histamine so formed was extracted in chloroform and the activity of the sample compared with a standard curve obtained using histamine dihydrochloride (Sigma Ltd). On one set of samples a fluorimetric assay for histamine was performed and the results agreed closely with those obtained using the above methods.

Specific IgE in sera

Indirect ELISA. Plates were coated with coronavirus 229E or control antigen as described elsewhere [16]. Sera diluted $\frac{1}{5}$ in BSA diluent were incubated for 5 hr at RT, and IgE was detected as described for total IgE. The peroxidase-conjugated goat anti-human IgE (Miles Laboratories Ltd, U.K.), used by Welliver *et al.* [11, 12, 25], was not used as it was found to have a low-level cross-reactivity with IgG.

IgE capture ELISA. Plates were coated with anti-human IgE produced in goats (Hoechst) overnight as described for measurement of total IgE. Sera were diluted $\frac{1}{5}$ or $\frac{1}{20}$ in BSA diluent and incubated for 3 hr at RT. Virus or control antigen at 5% was added and left for 3 hr at RT. Rabbit antiserum to coronavirus 229E diluted 1:1000 was added and left overnight at 4°C, followed by alkaline phosphatase conjugated to anti-rabbit IgG (Miles Laboratories) at 1:4000 for 4 hr at RT. Substrate was added and ODs read at 405 nm as described.

Using both methods for measuring specific IgE, optimal dilutions of reagents were determined by chequerboard titrations. Optical densities obtained with control antigens were subtracted from those obtained with virus. Sera were considered positive if these adjusted ODs were $\geqslant$OD+2 s.d. of the controls without serum.

Allergen skin tests

Skin-prick tests were performed using standard allergens to grass pollen, trees, shrubs, *Dermatophagoides* sp., house dust, cat, dog, negative and positive histamine control (Pharmacia, Uppsala, Sweden). None of the volunteers developed a weal $\geqslant$3 mm to the negative control.

Nasal cytology

Nasal cytology smears were collected before and 3 and 6 days after virus challenge, by gently scraping the interior turbinate of each nostril with a specially designed nasal probe. The material was smeared onto a glass microscope slide, allowed to air-dry, fixed in methanol and stained with May-Grunwald/Giemsa. The slides were coded and counted blind by two independent investigators. A differential count was performed counting 200 cells.

Statistical methods

The statistical analyses were performed on a Sirius I microcomputer using the program, Statistical Package for Personal Computers (SPP, Patrick Royston, Clinical

Table 1. Pre-inoculation measures of atopy of volunteers subsequently infected with coronavirus and their clinical scores

Subject	No. positive skin tests with common allergens*	Nasal washing IgE†	Total serum IgE (IU/ml)	Total clinical score
M.D.	0	+	50	71·5
B.W.	4	+	850	51·5
E.M.	0	+	60	39
P.W.	1	+	340	31·5
M.B.	0	+	425	30·5
B.W.	0	−	157	29
L.M.	0	+	50	24·5
S.H.	3	+	74	24
B.B.	2	+	37	23
K.S.	0	+	12	23
V.D.	0	+	22	22·5
S.C.	1	+	5	22·5
M.G.	0	+	69	20
M.T.	0	−	15	20
A.B.	0	+	78	19
T.S.	0	+	43	19
S.R.	4	−	25	17·5
H.G.	1	−	13	17·5
R.B.	0	+	1050	12
H.G.	0	−	5	9·5
R.D.	0	−	5	8·5
S.W.	0	+	1	2
R.D.	3	−	145	0·5
S.M.	5	+	225	0
L.C.	0	−	108	0
K.G.	0	−	75	0
P.C.	0	−	59	0
J.H.	0	−	15	0
H.S.	0	−	13	0

* A positive skin test indicates a $\geqslant$3 mm weal.
† (+) Indicates ELISA ODs$\geqslant$control OD+2 s.d. on 1 or more days before virus inoculation.

Research Centre, Northwick Park Hospital, Harrow, U.K.). For correlating scores with IgE or leukotriene concentrations, Spearman's coefficient of rank correlation was calculated. The other non-parametric test used was a rank analysis of variance.

Results

Infection, illness and atopic status of volunteers

Of thirty-four volunteers inoculated with coronavirus 229E, twenty-nine became infected as indicated by virus shedding and twenty-three had some respiratory symptoms, with clinical scores ranging from 0·5 to 71·5 (Table 1). Nine of the infected volunteers had a positive skin test to one or more allergens and six, not necessarily the

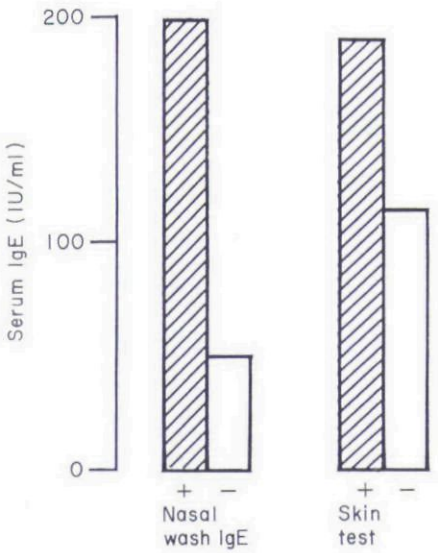

Fig. 1. Mean serum IgE (International Units/ml) in volunteers with detectable nasal wash IgE (optical density ⩾ control optical density + 2 s.d.) (+, $n = 17$) or without detectable nasal wash IgE (−, $n = 12$), and in volunteers with one or more positive skin prick tests (weal ⩾ 3 mm) to common allergens (+, $n = 9$) or negative skin prick tests (−, $n = 20$).

same individuals, had high (⩾ 150 IU/ml) serum IgE concentrations before the trial (Table 1). Seventeen volunteers had detectable amounts of nasal IgE and only eight were negative for all three measures of atopy.

Agreement between the measures of atopy

Those volunteers who had detectable nasal IgE had almost four times as much serum IgE compared with those who had no detectable IgE (Fig. 1). However, eleven volunteers who had negative skin tests had detectable nasal IgE and three had high concentrations of IgE in their sera (Table 1). None of these relationships was significant at $P < 0{\cdot}05$.

Relationship of measures of atopy to clinical scores

The presence or amount of local and serum IgE and the result of skin tests were all related to clinical scores (Fig. 2). The mean clinical score of those with detectable amounts of nasal IgE was over three times higher than the mean score of those with undetectable IgE ($P < 0{\cdot}01$). The mean clinical score of those with higher than normal serum IgE was about one and a half times higher than that of those volunteers whose serum IgE was within the normal range, although the difference was not statistically significant (in a two-tailed test). The mean clinical score of volunteers with positive skin tests was slightly higher than those with a negative test, although again this did not reach statistical significance. Groups that were positive for all three measures of atopy, or at least two of them, had a considerably higher mean score than the rest, although the differences were not statistically significant. The group that was positive for at least one of the criteria had a mean score of 23·8 compared with 4·7 for those who were negative for all three ($P = 0{\cdot}001$). None of the indicators of atopy appeared to be

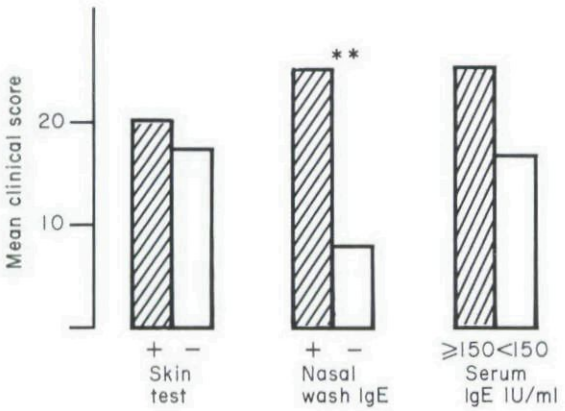

Fig. 2. Mean clinical score (i.e. severity of cold symptoms) of volunteers with (+, $n=9$) and without (−, $n=20$) at least one positive skin-prick test; with (+, $n=17$) and without (−, $n=12$) detectable nasal wash IgE (OD ≥ control OD + 2 s.d.); and in those with (+, $n=6$) and without (−, $n=23$) serum IgE ≥ 150 IU/ml. ** $P<0{\cdot}01$.

related to the amount of duration of virus shedding or to the size of specific IgG antibody rises (data not shown).

The following studies were carried out in twelve of the infected volunteers to try to elucidate the role of possible mediators.

Leukotrienes and histamine in nasal washings and relationship with measures of atopy and clinical scores

The concentration of the leukotrienes LTB_4 and LTC_4 in nasal washings was very low throughout the trial, compared with concentrations observed following provocation [10] (Fig. 3a). The mean concentrations showed fluctuations on different days, which did not appear to correlate with the changes in mean clinical scores (Fig. 3b). However, on most days after infection, LTC_4 concentrations in individual volunteers correlated positively with the clinical score, while with LTB_4 the reverse was the case; these correlations were not statistically significant however (data not shown).

Those volunteers classified as atopic by detectable IgE levels in nasal washing tended to have more LTC_4 ($P=0{\cdot}028$) and less LTB_4 (not significant) in their pre-inoculation washings than the non-atopics (Table 2). These differences persisted throughout the trial but values were so low that they may not be biologically important.

Histamine concentrations were also very low or undetectable. Only four volunteers had detectable amounts in their pre-trial washings; all four also had detectable nasal IgE and higher than average LTC_4 concentrations (Table 2). There was no indication that histamine concentrations increased after infection (data not shown) while one volunteer with high serum IgE (B.W.) and three with nasal IgE had no detectable histamine at any stage.

Coronavirus-specific IgE in serum

Optical densities significantly higher than the control were detected in only two pre-challenge sera (M.B. and L.M.) using the indirect ELISA. Because of these largely

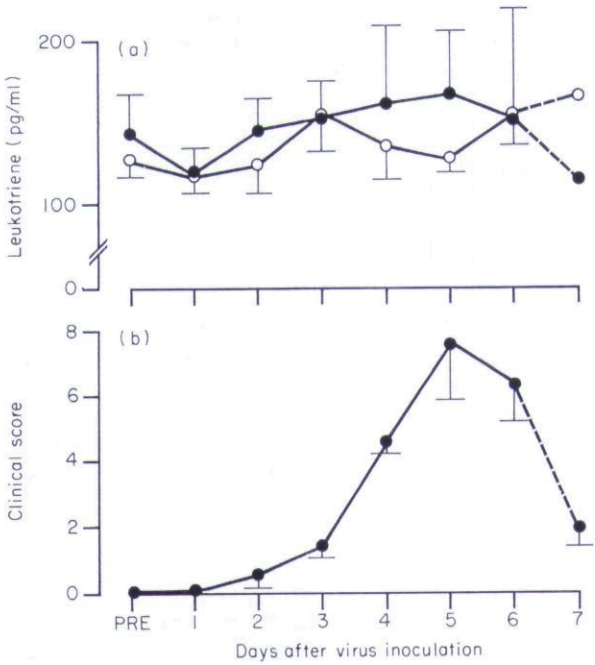

Fig. 3. (a) Mean LTC_4 (○) and LTB_4 (●) concentrations in volunteer nasal washings collected prior to (PRE: mean of 3 successive days before inoculation), and daily after intranasal virus inoculation (mean ± s.e.m., $n = 12$). (b) Mean clinical score of volunteer's cold symptoms prior to (PRE), and daily after intranasal virus inoculation (mean ± s.e.m., $n = 12$). Dotted lines indicates mean change in six volunteers only.

Table 2. Clinical scores and pre-inoculation measures of mediators of allergy in volunteers infected with coronavirus

		Pre-inoculation nasal washing concentrations			
Subject	Clinical score	Total IgE*	LTC_4 (ng/ml)	LTB_4 (ng/ml)	Histamine†
B.W.	51·5	+	0·102	0·075	−
E.M.	39	+	0·144	0·090	+
P.W.	31·5	+	0·146	0·106	+
M.B.	30·5	+	0·158	0·079	+
L.M.	24·5	+	0·142	0·173	−
S.C.	22·5	+	0·138	0·281	−
M.T.	20	−	0·141	0·280	−
R.B.	12	+	0·167	0·115	+
H.G.	9·5	−	0·134	0·078	−
R.D.	8·5	−	0·062	0·124	−
K.G.	0	−	0·137	0·262	−
J.H.	0	−	0·065	0·063	−

* (+) Indicates ELISA ODs ≥ control OD + 2 s.d. on 1 or more days before challenge.
† (+) Indicates ≥ 1 ng/ml on 1 or more days before challenge.

negative results an IgE capture ELISA was then tried. With this method, none of the pre-challenge sera appeared to contain specific IgE, although the convalescent sera of two volunteers (L.M. and S.C.) appeared to contain trace amounts, as did one intermediate serum (K.G.). No other samples from any volunteer contained detectable specific IgE. There was also no evidence that total IgE concentrations in the sera increased after infection (data not shown).

Nasal cytology

Most of the cells in the scrapes were epithelial or goblet cells. Neutrophils were occasionally seen but there were no differences between the pre- and post-infective cytology, except in one volunteer (H.G.) where neutrophils were present after infection, and another (S.C.) where neutrophils were seen before infection but not after. No eosinophils were seen in any subject.

Discussion

The variation in the indices of atopy used in this study is well recognized [20, 26, 27] although, as in other studies, the best agreement was between serum and nasal IgE concentrations [28, 29]. Detectable nasal IgE concentrations were most closely associated with a more severe cold, as assessed by a combination of subjective and objective clinical scores ($P < 0{\cdot}01$), followed by elevated serum IgE and positive skin-test results.

This interrelationship between the clinical severity of rhinitis and the presence of allergic processes is in accordance with the known capacity of virus infection to trigger asthma attacks in susceptible individuals [1, 30, 31].

Rhinovirus infections have been associated with asthma and bronchitis in both children and adults [1, 30, 31], but exacerbations only occurred in a minority of asthmatic adults during experimental rhinovirus infection [32]. No consistent association between the severity of colds and circulating or local IgE concentrations has yet been found in volunteers infected with rhinovirus (Callow, unpublished observation).

A number of possible mechanisms underlying the relationship were explored in this study. There was no evidence of coronavirus-specific circulating IgE prior to, or following, viral inoculation, and it may be that atopic individuals do not become sensitized to viral antigens [33]. In this case, direct triggering of cells, such as nasal mast cells or basophils, via specific surface-bound IgE directed against the virus would be unlikely.

The lack of involvement of mast cells or basophils was further suggested by the failure of this study to identify any rise in the concentration of histamine, sulphidopeptide leukotriene C_4 or leukotriene B_4 following virus inoculation. The small but statistically significant differences in baseline leukotriene concentrations in atopic and non-atopic volunteers were thought to be too small to be biologically important.

Similarly, the cytological findings did not identify any influx of inflammatory cells, in particular eosinophils, characteristic of the allergic response.

Although a significant increase of total protein is found in nasal secretions, following virus infection [34, 35; Callow, unpublished observations], other studies have, like ours, failed to detect a similar increase in histamine or leukotriene concentrations [36]. The observed increase in concentrations of these mediators

following allergen provocation may be due to the fact that inflammation and release of mediators is due to a large amount of allergen given in one dose whereas in virus infections, such effects are due to small amounts of infectious virus that are released over a much longer period. Furthermore, studies with allergen provocation are usually done with overtly atopic subjects [10], whereas the atopy detected in our volunteers was mild and not accompanied by allergic symptoms.

This study has raised the possibility of a relationship between the symptoms of a cold and the presence of atopy, in particular as detected by intranasal IgE concentrations. Studies attempting to dissect the mechanism of this relationship failed to identify a common pathway for viral and allergic rhinitis. Possibly an immunological imbalance, either humoral or cell-mediated, which has been shown to be associated with atopy [33, 37, 38] may independently lead to both atopy and increased severity of symptoms of virus infection.

Nevertheless, the results of this study show that increasing our understanding of any common pathways between virus- and allergen-induced rhinitis may provide insights which would have therapeutic implications for both conditions.

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