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Serology of Respiratory Viruses in Relation to Asthma and Bronchial Hyperresponsiveness

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ABSTRACT

Do individuals with serological evidence of infections with respiratory viruses have an increased prevalence of asthma or bronchial hyperresponsiveness (BHR)? Antibodies to four common respiratory viruses were measured in the sera of 81 subjects without a history of a recent respiratory infection. The subjects underwent an interview, spirometry, a methacholine provocation test and skin prick tests. In sera where virus antibodies were found, IgG titer values of ≥1:40 were regarded as evidence of a recent infection. A significant relationship was found between serological markers of a recent respiratory syncytial (RS) virus infection and physician diagnosed asthma (odds ratios (OR) 14.5, 95% confidence intervals (CI) 1.4-151) or BHR (OR 12.7, 95% CI 1.2-132)($p<0.05$). Furthermore, the total blood eosinophil count was significantly higher in subjects with serological signs of a current or recent RS virus infection than in those without such signs (364±198 $x10^6$/L and 175±118$x10^6$/L respectively, $p<0.05$)

In conclusion, a recent RS viral infection may be an important factor in the induction of symptoms and signs suggestive of asthma.

INTRODUCTION

Clinical observations have shown that viral respiratory infections intimately correlate to attacks of wheezing in patients with asthma and may temporarily induce bronchial hyperresponsiveness (BHR) in previously healthy individuals (4,8). Despite difficulties in virus isolation, respiratory viruses have been identified in up to 50% of wheezing illnesses and asthma

exacerbations occurring in childhood, and in up to 20% of those in adults (15). Both in children and adults the predominant organisms identified have been rhinoviruses, respiratory syncytial (RS) virus and parainfluenza viruses.

The actual mechanisms by which viral infections induce bronchial obstruction may include the release of preformed mediators, the induction of IgE synthesis, potentiation of neural and epithelial damage (3, 17) and since this worsening can partly be overcome by simultaneous steroid inhalation (9) inflammatory cells are likely to be involved.

We report the findings from a population survey in which serological evidence of respiratory virus infections were related to airway symptoms, objective findings suggestive of airway inflammation and lung function.

MATERIAL AND METHODS

Study population (Fig 1)

The European Community Respiratory Health Survey is an extensive study into the prevalence of allergies and asthma that has been conducted at 48 centres in 23 countries throughout Europe and in other parts of the world in the last five years (7). Sweden contributes with data from three such centres, one of which is Uppsala.

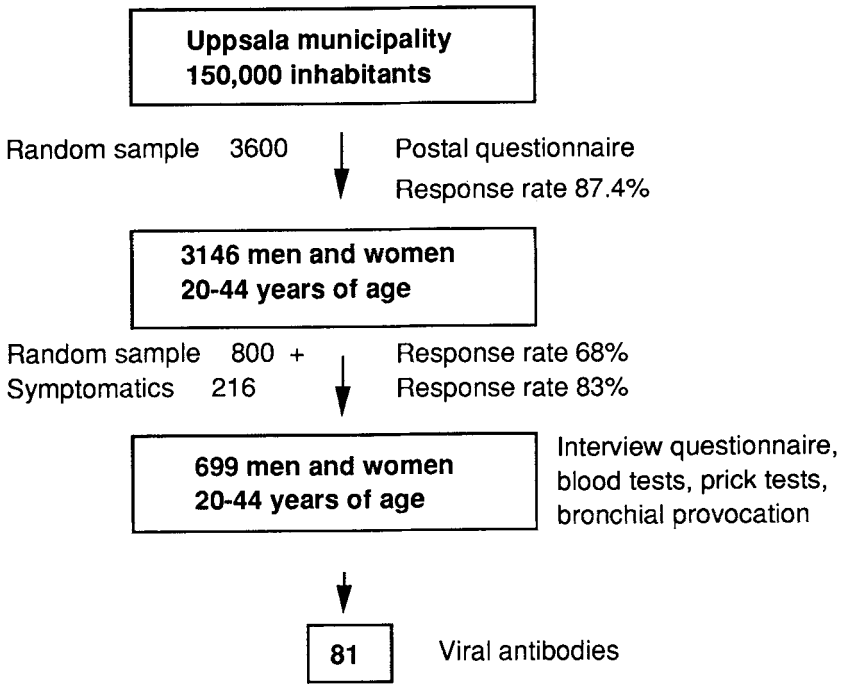
In December 1990, a questionnaire comprising 7 questions on asthma-related symptoms was mailed to 3,600 men and women, aged 20-44 years, who had been randomly selected from the population register in the municipality of Uppsala.

From this cohort, a random sample of 800 was selected for further investigation including an interview, skin prick tests for 10 common allergens and a bronchial provocation test with methacholine.In addition, all persons from the original sample, who reported the use of asthma medication, attacks of asthma or awakening because of shortness of breath, were invited to participate. A total of 1,016 persons were thus invited but the full protocol was carried out on 699 as previously reported (5).

Immunoglobulin G (IgG) levels specific for 4 common respiratory viruses (adenovirus, influenza A and B- and RS virus) were measured in 81 subjects with and without symptoms of asthma (Figure 1).

All persons gave their informed consent. The protocol of the study was approved by the Ethics Committee of the Medical Faculty of Uppsala University.

Figure 1. Selection of population and response rates



Lung function and bronchial hyperresponsiveness

The forced expiratory volume in one second (FEV_1) was measured using the Spiro Medics computerized dry-rolling seal spirometer system 2130 (Sensor Medics, Anaheim, California, USA). The predicted values were calculated for each patient (11).

Methacholine challenge was performed using a Mefar dosimeter (Mefar, Brescia, Italy) (13) The subjects were asked about respiratory infections during 6 weeks prior to the challenge. If a history of infections was obtained, the procedure was postponed until a 6 week period without a respiratory infection had surpassed.

The peak expiratory flow (PEF) (best of three measurements) was recorded twice daily during one week with a Mini-Wright Peak Flow Meter (Clement Clarke, London, UK). Peak flow variability was calculated by dividing the difference between the highest and lowest daily PEF reading by the daily mean PEF value (12).

Blood samples.

The following venous blood samples were obtained:

a. Five mL supplemented with EDTA (0.34 mol/L), which was used for the analysis of the blood eosinophil count (B-Eos) on a Hemalog 2R (Technicon Chemicals Company, Tournai, Belgium).

b. Ten mL from which serum was collected and analysed for total serum levels of immunoglobulin E (S-IgE) (Pharmacia CAP system® ,Pharmacia Diagnostics, Uppsala, Sweden).

c. Ten mL the serum of which was analysed for viral antibodies.

The same investigation sequence was applied to all persons. The blood was drawn immediately prior to the methacholine provocation test. The serum samples were kept frozen at -20°C until analysis.

Detection of specific antibodies

Antigens used for the complement fixation test were type-specific antigens for influenza virus types A and B produced in the chorioallantois membrane, an adenovirus group-specific antigen produced in Hela cells and a RS virus antigen produced in Vero cells/MA 104 cells (rhesus kidney cell line) (16).

Questionnaire

The screening questionnaire and the questionnaire used in the structured interview are based on the International Union Against Tuberculosis and Lung Disease questionnaire (6). In the interview, the patients answered questions relating to respiratory symptoms, respiratory

disorders, medication and environmental factors.

Definitions

The following definitions were used:

1. Bronchial hyperresponsiveness (BHR) was defined as a positive methacholine test, i.e. a reduction in FEV_1 by at least 20% after the inhalation of 2 mg or less of methacholine (2).
2. Atopy was defined as:

   a. a prick test reaction to at least one of the above-mentioned allergens with a mean diameter of ≥3mm, and

   b. no dermatographism (10)
3. Serological evidence of a recent virus infection was defined as an IgG level ≥1:40 in all the viruses studied.

Statistical methods

The Chi-square-test and Mann-Whitney U test were used for analysing differences between subjects with and without serological evidence of infections. Odds ratios (OR) with 95% confidence intervals (CI) were determined for binary independent variables. Logistic regression analysis was performed in order to estimate adjusted OR when taking several independent variables into account. The statistical analysis was performed on a Macintosh IIci computer with the software package Statistica 4.0 (StatSoft Inc, Tulsa, USA). A p value of <0.05 (two-tailed test) or an OR with the lower limit of the 95% CI ≥ 1.0 were regarded as statistically significant.

RESULTS

Serological measurements of respiratory virus antibodies were performed in 81 subjects.The characteristics of the study population are presented in Table 1.

Table 1 Characteristics of the study population (B-Eos=blood eosinophil count, S-IgE=total serum concentration of immunoglobulin E, FEV_1 =forced expiratory flow rate in one second, PEF=peak expiratory flow).

	n	(%)
Total number	81	(100)
Male	41	(51)
Physician diagnosed asthma	16	(19)
Atopy	33	(42)
Bronchial hyperresponsiveness	16	(22)
Current smoker	26	(32)
Ex-smoker	18	(22)
	Mean±SD	range
Age (years)	34±8	21-45
B-Eos ($x10^6$/L)	185±128	22-710
S-IgE (kU/L)	80±146	2-788
FEV_1 (% predicted)	107±15	56-138
PEF variability (%)	4±3	0-13

Serologic signs of a respiratory virus infection were found in 29 (36) subjects. Evidence of a recent infection with adenovirus was found in 14 (17%), with influenza A virus in 14 (17%), with influenza B virus in 6 (7%) and with RS virus in 4 (5%).

Significant relationships between evidence of a recent RS-virus infection and physician diagnosed asthma ($p<0.001$), and measured BHR were found ($p <0.01$) (Table 2).

Table 2. Number of patients with serological evidence of current or recent virus infections in relation to reports of ever having had asthma and bronchial hyperresponsiveness (RS= respiratory syncytial, BHR= bronchial hyperresponsiveness)(OR = odds ratio, CI = 95% confidence interval).

	Physician diagnosed asthma			BHR		
	Yes	No	OR (CI)	Yes	No	OR (CI)
Total	17	64		16	56	
Virus infection	6	23	1.1(0.3-3.3)	7	20	1.4(0.5-4.3)
Adeno virus	4	10	1.5 (0.4-5.4)	2	11	0.6 (0.1-3.2)
Influenza A	5	9	2.1 (0.6-7.1)	5	9	1.9 (0.6-6.6)
Influenza B	0	6		-0	5	-
RS-virus3	1	13.5 (1.3-139)*		3	1	12.7 (1.2-132)*

(* = p<0.05)

B-Eos and total S-IgE were significantly higher in subjects with evidence of a recent RS-virus infection (Table 3).

Table 3. Relationship between objective asthma related variables and serological evidence of respiratory syncytial (RS)-virus infection (mean±SD). (B-Eos = total blood eosinophil count, S-IgE = total serum immunoglobulin E, FEV1= forced expiratory volume in one second, PEF = peak expiratory flow)

	RS-virus infection	
	Yes (n=4)	No (n=77)
B-Eos (x106/L)	364±198	175±118*
S-IgE (kU/L)	346±284	68±129*
FEV1 (% predicted)	104±16	107±15
PEF-variability (%)	5±4	4±2

(* = p<0.05)

No other significant relationships between serological signs of recent virus infections and asthma or asthma-related variables were found.

DISCUSSION

This study was performed to establish whether any connections existed between serologic signs of respiratory virus infections and symptoms or signs of asthma in subjects without a clinical infection. Significant correlations between RS-virus specific antibodies and asthma-like symptoms and BHR were found.

This study has some limitations, in particular the small size of the sample which may have caused us to miss subtle connections. Furthermore the questionnaire inquired about asthma-like symptoms during the previous 12 months and did not include questions on recent symptoms of respiratory infections. We thus had no means of establishing the temporal relationship of these variables.

The viruses studied are those that have earlier been implicated in episodes of wheezing (14) except for rhinovirus, as the large number of serotypes precludes serology for screening for this virus. The only significant relationship found, however, was between evidence of a recent RS-virus infection and BHR and physician diagnosed asthma.In another serological study, Backer et al (4) found no connection between IgM antibodies to parainfluenza, influenza, adenovirus or RS-virus and BHR, whereas studies applying virus isolation have estimated RS-virus to account for 1-5% of virus induced asthma exacerbations in adults (3).

In conclusion, this study provides some evidence that RS virus infections may be relevant to the development of symptoms and objective signs of asthma in adults.

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