

Relationship between viral antibodies and bronchial hyperresponsiveness in 495 unselected children and adolescents

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The purpose of this study was to investigate whether recent and previous subclinical viral respiratory infection can explain the presence of increased bronchial responsiveness to histamine. We studied a randomly selected population of 495 children and adolescents, aged 7-16 years, from Copenhagen. If the subjects had had symptoms of respiratory infection recently, the examination was postponed for at least 6 weeks. Bronchial hyperresponsiveness (BHR) to inhaled histamine was found in 79 (16%) of the subjects, of whom 28 had asthma. Forty-eight subjects (10%) had increased levels of serum IgM antibodies against either parainfluenza, influenza, adenovirus, or respiratory syncytial virus (RSV), reflecting a recently acquired infection. No association between BHR and antibodies against respiratory viruses was found, as 7 (8.9%) of the 79 subjects with BHR and 41 (9.9%) of the 416 subjects without BHR had viral antibodies. Furthermore, no association between degree of bronchial responsiveness and viral antibodies was found. Moreover, 251 individuals (51%) had signs of earlier RSV infection, i.e. IgG antibodies against RSV. No relationship was found between age of the subjects and the presence of antibodies against either respiratory viruses in general or IgG-RSV. No relationship was found between the presence of antibodies against RSV and BHR; furthermore, evidence of earlier RSV infection was unrelated to the level of lung function and degree of bronchial responsiveness. We conclude that increased bronchial responsiveness in asymptomatic, unselected schoolchildren and adolescents is not likely to be caused by recent or previous viral respiratory infections.

Clinical aspects

Acute viral respiratory infection is associated with increased degree of bronchial responsiveness, wheezing, and reduced lung function. Sixteen percent of an unselected group of children and adolescents were found to have bronchial hyperresponsiveness and, to some degree, reduced lung function. The present study showed, however, that increased degree of bronchial responsiveness in population samples could not be explained by recent or subclinical viral respiratory infection. Furthermore, reduced lung function and asymptomatic bronchial hyperresponsiveness were found to be unrelated to previous RSV infection, a finding which contradicts findings in selected children with previous bronchiolitis. Our findings support the presumption that RSV infection might not in all cases induce chronic pulmonary disease.

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Viral respiratory infections (VRI) have been shown to trigger wheezing as well as an increase in bronchial responsiveness in asthmatic subjects (6, 17, 36). The relationship between respiratory infections

and bronchial hyperresponsiveness (BHR) poses an interesting problem, and increased knowledge may provide insight into the pathogenesis of BHR in non-asthmatic individuals.

Factors such as exposure to allergens (8), age (19), and a family background of asthma (26, 32) are known to influence the degree of bronchial responsiveness; furthermore, spontaneous changes in bronchial responsiveness to inhaled stimuli, such as histamine, are well known in both asthmatic and nonasthmatic subjects (27, 28). However, none of these factors can fully explain, either alone or in combination, the entire number of subjects with asymptomatic BHR. Furthermore, no complete explanation of the spontaneous variation of bronchial responsiveness in nonasthmatic subjects has been found. Acute symptomatic viral respiratory infection, in nonasthmatic subjects, leads to temporarily increased bronchial responsiveness (1, 12), and the degree of bronchial responsiveness has been shown to normalize within 6 weeks (1, 12). It is unknown, however, whether asymptomatic BHR can be caused by less severe viral infections, or whether asymptomatic BHR can be explained by subclinical, undiagnosed, viral respiratory infections.

Thus, the purpose of the present study was to investigate whether increased bronchial responsiveness to inhaled histamine in asymptomatic children and adolescents can be explained by recent or previous, subclinical viral respiratory infections.

Material

Subjects

A random sample of 983 children and adolescents living in the area surrounding the University Hospital in Copenhagen was drawn from the civil registration list and invited to participate in the study in 1986. All invited subjects were born in the first week of each month, were aged 7–16 years, and were Caucasian. A total of 527 (54%) accepted the invitation and were examined, of whom 262 were boys and 265 were girls. A sample of 100 nonresponders (families who did not respond when contacted by letter) was contacted by telephone; the description of the nonresponders has been presented in a previous paper (2).

All participants and their parents were interviewed by one of the authors (VB) and completed a questionnaire about respiratory and allergic (atopic) symptoms, i.e. symptoms of asthma (18), rhinitis (sneezing, running or blocked nose, not associated with colds), atopic dermatitis (an itchy dry rash on face, arms, or legs), and urticaria (itchy, generalized, irregular, wheal-like lesions) in themselves, their siblings, and their parents (2). Twenty-six subjects (5%) refused to continue the histamine challenge test after inhalation of saline or were unable to perform technically satisfactory FEV₁ maneuvers; furthermore, six subjects were excluded because of systemic diseases ($n = 5$) or FEV₁ below 1.0 l ($n = 1$). In cases of

known seasonal allergy, the examination was performed out of season. The definition of clinical asthma was based upon seven questions suggested by Hopp et al. (18). We found that 20 subjects had positive responses to questions 1–6, and eight subjects had positive responses to questions 1–3, plus any one of questions 4–7, whereas 77 subjects replied positively to only one question (Table 1). The participating subjects ($n = 495$) were divided into two groups.

Group 1. Children and adolescents with BHR ($n = 79$). Twenty-eight (35%) subjects fulfilled the criteria for having clinical bronchial asthma. All of these subjects had current symptoms of asthma at the time of this study. The median age of the subjects was 12 years (range 7–16), the median serum IgE was 95 kU/l, and six of the 28 subjects had a negative skin prick test. Fifty-one subjects with a PC₂₀ lower than 8 mg/ml histamine had no symptoms which could be classified as definite asthmatic symptoms, but 13 subjects had symptoms such as rhinitis or eczema. The median age of the nonasthmatic subjects was 10 years (range 7–16), 22 had a positive skin prick test, and the median serum IgE was 99 kU/l. Eleven (22%) of the 51 nonasthmatic subjects claimed to have respiratory symptoms, whereas 40 individuals had no respiratory symptoms. Seven of the 51 subjects had occasional cough, three had experienced shortness of breath, and one subject had experienced wheezing on one occasion.

Group 2. Children and adolescents without BHR (control subjects) ($n = 416$). A total of 416 subjects had a normal degree of bronchial responsiveness, i.e. a PC₂₀ histamine > 8 mg/ml, and no earlier or present respiratory symptoms suggesting a diagnosis of asthma. The median age was 12 years (7–16), 65 subjects had allergic disease, and the median serum IgE was 90 kU/l. Sixty-six subjects (16%) claimed

Table 1. Number of subjects with respiratory symptoms (1–7, Hopp et al. (18)) among 495 children and adolescents

	Asthmatic $n=28$	Nonasthmatic $n=416$	Total $n=495$
* Respiratory symptoms (Hopp et al. [18])			
1) Doctor's diagnoses of asthma	20	0	20
2) Wheezy or dry cough	28	71	99
Occasional dry cough	25	70	95
3) Shortness of breath with wheezing	28	6*	34
4) Doctor's treatment of asthma	20	0	20
5) Received antiasthmatic medication	20	0	20
6) Did the medication help	20	0	20
7) Different factors and pulmonary symptoms	28	0	28
Total respiratory symptoms	28	77	105

* All claimed to have unexplained attacks of dyspnea without wheezing.

to have respiratory symptoms, whereas 350 individuals had no respiratory symptoms. Sixty-three subjects had occasional cough, three had experienced shortness of breath, but none had experienced wheezing.

The protocol was evaluated and approved by the local ethical committee, and informed consent was obtained from all participants. The Helsinki II Declaration was adhered to throughout the study.

Methods

In subjects with recent or present symptoms of chest infection, i.e. cough, dyspnea, and malaise in combination with fever ($>38^{\circ}\text{C}$), the examination was postponed for 6 weeks from the day of initiation.

Lung function and bronchial challenge test

All subjects were asked to avoid antiasthmatic medication in accordance with the recommendations by Eiser et al. (11). The forced expiratory volume in 1 s (FEV_1) (Vitalograph[®]), the forced vital capacity (FVC), and the FEV_1/FVC ratio were measured in all participants. Predicted values (% pred.) based on the sex and height of the subjects were calculated according to the method of Zapletal et al. (38). Bronchial responsiveness to inhaled histamine was measured according to the method described by Cockcroft et al. (7). Histamine aerosol was generated by a Wright nebulizer[®] with an output of $0.14 \pm 0.015 \text{ l/min}^{-1}$. Nine concentrations of histamine were used, ranging from 0 (saline) to 8 mg/ml. Testing was terminated when the maximum concentration had been reached, or when a decrease of at least 20% of FEV_1 was observed. The percent reduction in FEV_1 per μmol ($\Delta\% \text{FEV}_1/\mu\text{mol}$) of the last inhaled concentration of histamine, i.e. the dose-response slope (DRS), was calculated and used in the analysis (25). The inspiration time was calculated as 40% of the inhalation period of 2 min. Before expression in log scale, 1.2% decline in FEV_1 per μmol was added to each value to eliminate negative and zero values. The DRS was found to increase with increasing degree of bronchial responsiveness; thus, a logDRS above 0.73 $\Delta\% \text{FEV}_1/\mu\text{mol}$ histamine was suggestive of BHR, i.e. a $\text{PC}_{20} \leq 8 \text{ mg/ml}$.

Skin prick test and total serum IgE

A skin prick test with nine common aeroallergens was performed in 524 (99%) of the participating subjects. The allergens used were birch, grass, mugwort, horse, dog, cat, house-dust mite (*Dermatophagoides pteronyssinus*), and two molds (*Alternaria iridis* and *Cladosporium herbarum*). The strength of

the allergens was 100 000 BU/ml (Pharmacia), and histamine HCl 10 mg/ml in 50% glycerol was used as positive reference; a negative reference was also included. We used a 1-mm lancet, which was pressed at an angle of 90% to the skin surface through a drop of test solution. The urticaria reactions were read after 15–20 min. The allergen wheal area was compared with the histamine wheal and classified as positive if the allergen wheal area was at least the same size as the histamine wheal (+++), moderate if the allergen wheal area was larger than the negative control but smaller than the histamine wheal (++ and +), and negative if the allergen wheal area was similar to the negative control (–). Three subjects, of whom one had symptoms, were not willing to have an allergen skin prick test performed.

Blood samples were drawn and stored at -20°C , without thawing, for approximately 30 d, after which the total serum IgE was determined by paper radio-immunosorbent test (PRIST, Pharmacia, Copenhagen, Denmark). The IgE analysis was performed in groups on 100 samples.

Serum immunoglobulin class-specific antibodies

Blood samples were analyzed as follows, by the enzyme-linked immunosorbent assay (ELISA) technique.

IgM-ELISA. Detection of IgM virus antibodies was performed as an immunoglobulin M antibody capture enzyme immunoassay, based on the principle originally described by Duermeier et al. (10) and Flehmig et al. (13). Rabbit antihuman IgM (μ -chain specific, CODE A-425, Dako a/s, Glostrup) was used as antibody capture in all six IgM-ELISAs (100 μl /well corresponding to 1 antibody μg /well). IgM immunoglobulin present in test sera was then added to duplicate wells in 100 μl volumes diluted 1/100. Optimal dilution of crude viral antigen preparations and corresponding control antigen from noninfected cells (14, 15) were added in duplicate to wells which had previously reacted with the serum to be tested. Binding of viral antigen to IgM antibodies was detected by the following indicator systems: 1) influenza A and B ELISA was performed with monoclonal antibodies against A and B virions, respectively (kindly supplied by Dr Nancy Cox, Influenza Branch, Centers for Disease Control, Atlanta, GA, USA), in combination with peroxidase-labeled rabbit antimouse immunoglobulin conjugate (Dakopatts, Code P-161); 2) adenovirus ELISA was performed with peroxidase-labeled goat polyclonal antibodies directed to Hexon protein, which is common to all adenovirus types (Biogenesis, UK, Code N. 0151-9204); 3) parainfluenza types 1 and 2 and RSV-ELISA were performed with biotin-labeled

polyclonal rabbit viral-specific antibodies (3) and commercially available avidinperoxidase conjugates (Dakopatts, Code P 360). Biotin-labeled rabbit antibodies against RSV were commercially available (Dako a/s, CODE E-346). Antibodies against parainfluenza virus were obtained in rabbits by three subcutaneous injections of highly purified virus antigens (33) in the presence of Freund incomplete adjuvant. Antibodies were made virus-specific by solid-phase absorption by means of a noninfected cell antigen preparation covalently bound to CL-4B Sepharose (Pharmacia, Uppsala), according to the manufacturer's instructions.

Purity of viral antibodies was assessed by SDS-PAGE and immunoblotting assays, before preparation of biotin conjugates. Biotinilation of antibodies was performed according to the procedure kindly provided by P. Grauballe, MD (Dako a/s). The optical density (OD) was measured at a wavelength of 492 nm (OD 492) in an Immuno Reader NJ-2000, Inter Med, TekNunc, Denmark. The ELISA E-value was obtained automatically from the reader as the OD 492 difference between the viral and control antigen readings.

ELISA cutoff values were calculated as two standard deviations above the mean obtained from a control group consisting of 100 individual serum samples taken from children (0–17 years old) submitted to the State Serum Institute for various diagnostic purposes except diagnostic testing of respiratory disease. Performance of each IgM-ELISA was evaluated in terms of specificity, by means of blocking experiments with sera from patients having acute RSV, parainfluenza (1 and 2), and influenza (A and B) viral infections, respectively. These sera were taken from patients with positive virus isolations from tracheal aspirates and/or nasopharyngeal swabs. Accordingly, blocking experiments were performed with serum samples from patients exhibiting high IgM antibodies titers against other paramyxoviruses such as mumps and measles, corroborated by clinical symptoms, and with sera from patients with high levels of IgM-rheumatoid factor. Sera from this category of patients were not able to block any of the IgM-ELISAs even when tested at rather low dilutions (1/10). On the other hand, complete blocking of patients' IgM antibody was obtained when acute serum samples from patients with positive virus isolation were tested at different dilutions in their corresponding ELISA (results not shown).

RSV-IgG-ELISA. RSV-IgG antibodies were detected by ELISA technique with the classic indirect approach in which viral and control antigen, at appropriate dilutions, are directly adsorbed to the microplates. The ELISA E-value was obtained as described for the IgM-ELISA.

Statistical analysis

Data in contingency tables were analyzed by chi-square (BHR vs. viral antibodies) or Fisher's exact test (respiratory symptoms vs. viral antibodies). The relationships between age and viral antibodies, and month of examination and viral antibodies were analyzed by one-way analysis. Differences in logDRS, FEV₁, FEV₁/FVC, age, and height among the three groups were analyzed by the Kruskal-Wallis test. When appropriate, the Mann-Whitney test was used to analyze differences among the three groups.

Results

We found that 79 (16%) of the subjects had BHR, i.e. PC-20 ≤ 8 mg/ml; 40 claimed to have no respiratory symptoms; 11 had monosymptomatic respiratory symptoms; and 28 were classified as having asthma. The subjects with asthma had increased bronchial response to inhaled histamine (logDRS) and reduced FEV₁ %pred., as compared with the control subjects ($P < 0.01$; Table 2).

Forty-eight of the 495 subjects (10%) had immunologic evidence of recent viral infection, as they had detectable levels of serum IgM antibodies and/or high levels of IgG antibodies against at least one of six common respiratory viruses. Thirty-eight of the 48 subjects had IgM antibodies, whereas 10 had signs of recent RSV reinfection (reactivated IgG-RSV), and 3 were found to have signs of double

Table 2. Lung function, FEV₁, % FEV₁/vital capacity (VC), FEV₁ % pred, and histamine challenge test, PC₂₀ and log dose-response slope (DRS) in 495 children and adolescents. Median values (range)

	Bronchial hyperresponsiveness			Total population n=495
	Yes		No	
	Asthma n=28 1	Nonasthmatic n=51 2	Control n=416 3	
FEV ₁ l	2.3 (1.1–4.1)	1.9 (0.9–1.9)	2.4 (1.0–6.1)	2.4 (0.9–6.1)
%FEV ₁ /VC	90.5 (73–99)	89.0 (71–98)	91.0 (61–99)	90.0 (61–99)
FEV ₁ (%pred)	88.7 (63–119)	93.8 (55–123)	96.9** (61–143)	96.1 (55–143)
PC ₂₀	2.0** (0.05–8.0)	5.6* (0.05–8.0)	> 8.0 (> 8.0)	> 8.0 (> 8.0)
DRS Δ%FEV ₁ /μmol	1.21*** (0.7–3.0)	0.94*** (0.7–3.0)	0.36 (–2.0–0.7)	0.41 (–2.0–3.0)

Mann-Whitney test:

* $P < 0.05$ (1 vs 2).

** $P < 0.01$ (1 vs. 3 and 2 vs. 3).

*** $P < 0.001$ (1 vs. 3 and 2 vs. 3).

Table 3. Test for specific viral antibodies (IgM and IgG ELISA) against six common respiratory viruses in a population sample of 495 children and adolescents

	Frequency of antibodies against respiratory viruses	
	Absolute no.	% positive tests <i>n</i> =495
IgM-ELISA		
Parainfluenza 1	4	0.8
Parainfluenza 2	3	0.6
Influenza A	6	1.2
Influenza B	20	4.0
Adenovirus	8	1.6
RSV	0	0.0
Total	41	8.3
IgG-ELISA		
RSV (high level)	10	2.0
RSV	251	50.7

infection (Table 3). No association between BHR and viral antibodies against respiratory viruses was found, as 7 (8.9%) of the 79 subjects with BHR and 41 (9.9%) of the 416 subjects without BHR had viral antibodies. Furthermore, no association between severe bronchial responsiveness, i.e. a PC₂₀ less than 4 mg/ml, and presence of viral antibodies was found. No relationship was found between age of subjects and presence of viral antibodies, whereas a significant relationship was found between month of examination and presence of viral antibodies ($P < 0.01$). Seventeen (35%) of the 48 children with viral antibodies had their sera samples taken during February and March, of whom 2 (12%) had BHR and 15 (88%) did not. Furthermore, a significant relationship between BHR and month of examination was found ($P < 0.01$): the highest frequency of BHR was found during April/May and September/November, whereas no increased frequency of viral antibodies was observed during these periods. No differences were found in the frequency of viral antibodies among asthmatic ($n = 2$, 7%), nonasthmatic ($n = 5$, 10%), and control ($n = 41$, 10%) subjects. Furthermore, no significant differences were found in the frequency of the specific viral antibodies among the three different groups (Table 4).

The degree of bronchial response to inhaled histamine (Table 5) was found to be unrelated to evidence of recent viral respiratory infection. Likewise, no differences in FEV₁ %pred. were found between those who had no evidence of respiratory viral infections and those who had had recent respiratory viral infection (Table 5).

Eleven (22%) nonasthmatic subjects with BHR and 66 (16%) control subjects without BHR claimed to have respiratory symptoms ($P = 0.4$); however, no significant relationship was found between antibodies against respiratory virus and respiratory symptoms among nonasthmatic subjects, as compared with controls; only 1 out of 11 subjects with BHR (Group 1) and 6 out of 66 healthy controls without BHR (Group 2) had both respiratory symptoms and virus antibodies.

A total of 251 subjects (51%) had evidence of past infection with RSV, i.e. a detectable level of IgG antibodies, of whom 225 had RSV IgG as the only positive finding (Tables 3 and 5). No relationship was found between presence of IgG-RSV and BHR (Table 4). The level of bronchial responsiveness and lung function was also found to be unrelated to the presence of earlier RSV (Table 5).

Furthermore, detectable levels of IgM and IgG antibodies against respiratory virus were unrelated to sex and a history of wheezy bronchitis before the age of 2 years.

Discussion

BHR and evidence of recent VRI

BHR is a characteristic feature of asthma (27) although not entirely restricted to asthmatic children (18, 27). Various factors are known to be of importance in the distribution of bronchial responsiveness among children. Acute symptomatic respiratory infections may lead to an increased degree of bronchial responsiveness, decreased lung function, and increased respiratory symptoms such as wheezing (12, 22, 23, 36, 37). Several studies have shown that acute viral, but not bacterial, respiratory infections can trigger symptoms of bronchial asthma (5) and

Table 4. Incidence of specific viral antibodies in 495 children and adolescents against parainfluenza viruses 1 and 2 (PARA1, PARA2), influenza A and B (INFLA, INFLB), adenovirus (ADENO), and respiratory syncytial virus (RSV)

	Frequency of antibodies against respiratory viruses						
	Immunoglobulin M					Immunoglobulin G	
	PARA1	PARA2	INFLA	INFLB	ADENO	RSV*	RSV
Asthmatic subjects ($n=28$)	0.0%	0.0%	0.0%	3.6%	3.6%	0.0%	50.0%
Nonasthmatic subjects with BHR ($n=51$)	0.0%	2.0%	2.0%	3.9%	2.0%	0.0%	47.1%
Nonasthmatic subjects without BHR ($n=416$)	1.0%	0.5%	1.2%	4.1%	1.4%	2.4%	50.5%

* Present reactivation of IgG antibodies.

Table 5. Bronchial challenge test, log dose-response slope (DRS), and lung function, FEV₁ %pred, in relation to specific respiratory virus antibodies. Median values (range). Kruskal-Wallis (KW) and *P* values

	Respiratory viral antibodies present in serum			
	none 1	IgM and IgG-RSV* 2	IgG-RSV 3	KW (<i>P</i> =)
Subjects with BHR (<i>n</i> =79)				
Number	39	7	33	
DRS Δ%FEV ₁ /μmol	0.97 (0.7–2.7)	0.93 (0.8–1.6)	1.03 (0.7–3.0)	2.4 (0.3)
FEV ₁ %pred	90 (55–123)	89 (84–114)	96 (70–119)	1.9 (0.4)
Subjects without BHR (<i>n</i> =416)				
Number	183	41	192	
DRS Δ%FEV ₁ /μmol	0.38 (–1.2–0.7)	0.42 (–0.5–0.7)	0.35 (–2.0–0.7)	4.1 (0.1)
FEV ₁ %pred	97 (71–143)	99 (61–128)	95 (65–128)	5.1 (0.08)
All subjects (<i>n</i> =495)				
Number	222	48	225	
DRS Δ%FEV ₁ /μmol	0.42 (–1.2–2.7)	0.44 (–0.5–1.6)	0.40 (–2.0–3.0)	3.0 (0.23)
FEV ₁ %pred	96 (55–143)	99 (61–128)	96 (65–128)	2.9 (0.2)

* IgG-RSV reactivation.

increased bronchial responsiveness among healthy subjects without provoking respiratory symptoms (21). Furthermore, a well-known association between allergy (atopy) and increased bronchial responsiveness exists (27, 29). We found a significant relationship between month of examination (April/May and September/November) and increased degree of bronchial responsiveness, a finding which supports the thesis that a close relationship between allergy (atopy) and bronchial responsiveness to inhaled histamine may exist.

On the other hand, BHR in asymptomatic children and adolescents cannot be entirely explained by allergic background (27, 32), allergic symptoms (8, 29, 37), or atopic disease (27), as a multivariate regression analysis of our data showed that these factors could explain only 23% of the variation in level of bronchial responsiveness (unpublished data). Therefore causal factors other than allergy and atopy seem to be involved in the development of BHR, and recent viral infection might be a potential provocative factor. We performed a serologic investigation of antibodies against six respiratory viruses in unselected children and adolescents with and without BHR. We found that 10% of the examined population had serologic signs of recent viral respiratory

infection. Since only a few serum samples from children and adolescents with positive virus isolation were available, it was not possible to provide data on the sensitivity and specificity of the serologic tests. In any case, clinical data of respiratory illness are of uncertain value, since many of the respiratory viruses examined in the present study were accompanied by similar symptoms. Vikerfors et al. (35) have found that the sensitivity of the ELISA to influenza A was 86%, and Meurman et al. (24) have found similar sensitivity of the ELISA-RSV (79%), suggesting that a substantial number of children and adolescents developed specific viral antibodies during a viral respiratory illness. Nevertheless, the diagnostic value of serum antibodies against all respiratory viruses are at present not available. However, these findings may still lead to an underestimation of children and adolescents with recent viral infection.

In spite of the fact that none of the subjects had had symptoms of respiratory infection within the preceding 6 weeks, we found a relatively high number (43%) of the subjects with antibodies against viruses had IgM antibodies against influenza B virus – probably caused by a known epidemic of influenza B in Denmark just before the study period. We therefore assume that children and adolescents with mild or subclinical respiratory infection might have developed specific antibodies. We found, however, no increased frequency of BHR among children and adolescents, aged 7–16 years, with antibodies against any of the six respiratory viruses. Evidence of recent respiratory virus infection was found in seven subjects with BHR ($PC_{20} \leq 8$) and 41 control subjects. Furthermore, no correlation was found between severe BHR ($PC_{20} \leq 4$) or a generally increased degree of bronchial responsiveness and serologic evidence of recent respiratory viral infection, indicating that viral infections could not explain the BHR among asymptomatic schoolchildren and adolescents.

Our findings are different from findings in other studies (5, 12, 21). Yet, these earlier studies were performed either in selected hospitalized younger children (5) or in individuals with acute viral respiratory illness (12, 21, 31). Previous studies of acute respiratory infection have shown that the degree of bronchial responsiveness increases on the third day after the infection (20), reaching its peak in the second week (4), after which it decreases to preinfection levels within 6 weeks (1, 12). Therefore, if viral respiratory infection is the cause of BHR in asymptomatic subjects, it seems reasonable to believe that measurement of serum antibodies, as performed in the present study, can prove or refute the hypothesis that asymptomatic BHR is caused by recent viral respiratory infection. On the other hand, Vikerfors et al. (35) found that serum antibodies against influ-

enza A remain increased for at least 60 d, suggesting that the subjects in the present study with viral antibodies might have had respiratory illness within the last 60 d. These findings indicate that the subjects with antibodies might earlier have had BHR, but then, more than 6 weeks later, had returned to bronchial responsiveness within normal range, indicating that we overestimated recent or subclinical respiratory infection inducing BHR in the present study. Thus, only two subjects (12%) had BHR and viral antibodies against influenza B, whereas 15 subjects examined in the same period of 4 weeks, in which the epidemic was going on, had bronchial responsiveness within normal range. Furthermore, a criticism is that an early VRI without the presence of viral antibodies at the time of examination might have been present; another is that no convalescent sera were examined. No tendency, however, exists towards correlation between bronchial response to histamine and viral antibodies, and misclassification of some subjects as having or not having viral antibodies would not change the correlation; this suggests that no major errors have been made.

BHR and previous VRI

Various respiratory viruses have been found to induce an increase in bronchial responsiveness (5). The virus identified, RSV, was the most frequent and the most likely virus to provoke asthma (5). It is unknown, however, whether RSV is the virus that most frequently provokes asymptomatic BHR. It has been shown that RSV infection is a frequent cause of acute bronchiolitis, and that bronchiolitis may lead to persistently increased bronchial responsiveness and decreased lung function (9, 30). Half of the study population showed evidence of previous RSV infection, which is in accordance with other studies from Scandinavian countries (34). The subjects in the present study with RSV antibodies had both the same degree of bronchial responsiveness and level of lung function as the subjects without signs of RSV infection. The relationship between bronchiolitis and reduction in lung function has been demonstrated in children hospitalized because of VRI (16). These former studies included younger children, and the difference between our findings and these former findings might be related to the range of age groups in this population sample of schoolchildren. However, in the present study, we found no correlation between young children, aged 7–10 years, and increased bronchial responsiveness in the presence of RSV antibodies. The differences between our findings and the findings by others are, therefore, most likely to have been caused by the unselected, cross-sectional nature of our population. Another possible explanation is that these children and adolescents

had had their RSV infection late in their childhood period, because it is known that RSV infections in young children are associated with severe respiratory distress, whereas RSV in older children causes only mild respiratory illness. It therefore seems likely that only the more severe episodes of viral bronchiolitis cause persistent abnormalities in the airways, whereas the risk in less severe respiratory infections is negligible.

In conclusion, this study demonstrates that increased bronchial responsiveness in otherwise healthy children and adolescents is not likely to be caused by recent viral respiratory infection, and, furthermore, that common respiratory infections in early childhood do not seem to be followed by persistent BHR in later childhood and early adulthood.

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References

1. AQUILINA AT, HALL WJ, DOUGLAS RG, UTELL MJ. Airway reactivity in subjects with viral upper respiratory tract infections: the effects of exercise and cold air. *Am Rev Respir Dis* 1980; 122: 3–10.
2. BACKER V, BACH-MORTENSEN N, DIRKSEN A. Prevalence and predictors of bronchial hyperresponsiveness in children aged 7–16 years. *Allergy* 1989; 44: 214–19.
3. BAYER E, SKUTELSKY E, WILCHEK M. The avidin-biotin complex in affinity cytochemistry. *Methods Enzymol* 1979; 62: 308.
4. BRIGHAM K, SPRAGG R. The 3rd international conference on the adult respiratory distress syndrome. *Intensive Care Med* 1989; 15: 332–4.
5. BUSSE WW. Respiratory infections: their role in airway responsiveness and the pathogenesis of asthma. *J Allergy Clin Immunol* 1990; 85: 671–83.
6. CLARKE CW. Relationship of bacterial and viral infections to exacerbation of asthma. *Thorax* 1979; 34: 344–7.
7. COCKCROFT DW, KILLIAN DN, MELLON JJA, HARGREAVE FE. Bronchial reactivity to inhaled histamine: a method and clinical survey. *Clin Allergy* 1977; 7: 235–43.
8. COCKCROFT DW, MURDOCK KY. Changes in bronchial responsiveness to histamine at intervals after allergen challenge. *Thorax* 1987; 42: 302–8.
9. COLLIER AM, PIMMEL RL, HASSELBLAD V, CLYDE WA Jr, KNELSON JH, BROOKS JG. Spirometric changes in normal children with upper respiratory infections. *Am Rev Respir Dis* 1978; 117: 47–53.
10. DUERMAYER W, WIELAND F, VAN DER VEEN JC. A new principle for the detection of specific IgM antibodies applied in an ELISA for hepatitis A. *J Med Virology* 1979; 4: 25–32.
11. EISER NM, KERREBIJN KF, QUANJER PH. Guidelines for standardization of bronchial challenges with (nonspecific) bronchoconstricting agents. *Bull Eur Physiopathol Respir* 1983; 19: 495–514.

12. EMPEY DW, LAITINEN LA, JACOBS L, GOLD WM, NADEL JA. Mechanisms of bronchial hyperreactivity in normal subjects after upper respiratory tract infection. *Am Rev Respir Dis* 1976; 113: 131-9.
13. FLEHMIG B, RANEKE M, BERTHOLD H, GERTH HJ. A solid phase radioimmunoassay for detection of IgM antibodies to hepatitis A virus. *J Infect Dis* 1979; 140: 169-75.
14. GLIKMANN G, PEDERSEN M, MORDHORST C-H. Detection of specific immunoglobulin M to mumps virus in serum and in cerebrospinal fluid samples from patients with acute mumps, using an antibody capture enzyme immunoassay. *Acta Pathol Microbiol Immunol Scand* 1986; 94: 145-56.
15. GLIKMANN G, MORDHORST C-H. Serological diagnosis of mumps and parainfluenza 1 virus infections by enzyme-immunoassays, with comparison of two different approaches for detection of mumps IgG antibodies. *Acta Pathol Microbiol Immunol Scand* 1986; 94: 157-66.
16. GURWITZ D, MINDORFF C, LEVISON H. Increased incidence of bronchial reactivity in children with a history of bronchiolitis. *J Pediatr* 1981; 98: 551-5.
17. HALPERIN SA, EGGELSTON PA, BEASLEY P, et al. Exacerbations of asthma in adults during experimental rhinovirus infection. *Am Rev Respir Dis* 1985; 132: 976-80.
18. HOPP RJ, BEWTRA AK, NAIR NM, TOWNLEY RG. Specificity and sensitivity of methacholine inhalation challenge in normal and asthmatic children. *J Allergy Clin Immunol* 1984; 74: 154-8.
19. HOPP RJ, BEWTRA A, NAIR NM, TOWNLEY RG. The effect of age on methacholine response. *J Allergy Clin Immunol* 1985; 76: 609-13.
20. INOUE H, HORIO S, ICHINOSE M, et al. Changes in bronchial reactivity to acetylcholine with type C influenza virus infection in dogs. *Am Rev Respir Dis* 1986; 133: 367-71.
21. LAITINEN LA, ELKIN RB, EMPYE DW, JACOBS L, MILLS J, NADEL JA. Bronchial hyperresponsiveness in normal subjects during attenuated influenza virus infection. *Am Rev Respir Dis* 1991; 143: 358-61.
22. LITTLE JW, HALL WJ, DOUGLAS RG Jr, MUDHOLKAR GS, SPEERS DM, PATEL K. Airway hyperreactivity and peripheral airway dysfunction in influenza A infection. *Am Rev Respir Dis* 1978; 118: 295-303.
23. MCINTOSH K, ELLIS EF, HOFFMAN LS, LYBASS TG, ELLER JJ, FULGINITI VA. The association of viral and bacterial respiratory infections with exacerbations of wheezing in young asthmatic children. *J Pediatr* 1973; 82: 578-90.
24. MEURMAN O, SARKKINEN H, RUUSKANEN O, HÄNNINEN P, HALONEN P. Diagnosis of respiratory syncytial virus infection in children: comparison of viral antigen detection and serology. *J Med Virol* 1984; 14: 61-5.
25. O'CONNOR GT, SPARROW D, TAYLOR D, SEGAL M, WEISS S. Analysis of dose-response curves to methacholine. *Am Rev Respir Dis* 1987; 136: 1412-17.
26. PATTEMORE PK, ASHER MI, HARRISON AC, MITCHELL EA, REA HH, STEWART AW. The interrelationship among bronchial hyperresponsiveness, the diagnosis of asthma, and asthma symptoms. *Am Rev Respir Dis* 1990; 142: 549-54.
27. PEAT JK, SALOME CM, SEDGWICK CS, KERREBUN J, WOOLCOCK AJ. A prospective study of bronchial hyperresponsiveness and respiratory symptoms in a population of Australian schoolchildren. *Clin Exp Allergy* 1989; 19: 299-306.
28. REDLINE S, TAGER IB, SPEIZER FE, ROSNER B, WEISS ST. Longitudinal variability in airway responsiveness in a population-based sample of children and young adults. *Am Rev Respir Dis* 1989; 140: 172-8.
29. SALOME CM, PEAT JK, BRITTON WJ, WOOLCOCK AJ. Bronchial hyperresponsiveness in two populations of Australian schoolchildren. I. Relation to respiratory symptoms and diagnosed asthma. *Clin Allergy* 1987; 17: 271-81.
30. SAMET JM, TAGER IB, SPEIZER FE. The relationship between respiratory illness in childhood and chronic air-flow obstruction in adulthood. *Am Rev Respir Dis* 1983; 127: 508-23.
31. TELLEZ A, PEREZ BP, FERNANDEZ PMV, LEON P, ANDA P, NAJERA R. Acute respiratory disease in Spain: seven years of experience. *Rev Infect Dis* 1990; 12: 745-53.
32. TOWNLEY RG, BEWTRA A, WILSON AF, et al. Segregation analysis of bronchial response to methacholine inhalation challenge in families with and without asthma. *J Allergy Clin Immunol* 1986; 77: 101-7.
33. UDEM SA. Measles virus: conditions for the propagation and purification of infectious virus in high yield. *J Virol Methods* 1984; 8: 123-6.
34. UKKONEN P, HOVI T, VON BONSDORFF C-H, SAIKKU P, PENTTINEN K. Age-specific prevalence of complement-fixing antibodies to sixteen viral antigens: a computer analysis of 58 500 patients covering a period of eight years. *J Med Virol* 1984; 13: 131-48.
35. VIKERFORS T, LINDEGREN G, GRANDIEN M, VAN DER LOGT J. Diagnosis of influenza A virus by detection of specific immunoglobulin M, A, and G in serum. *J Clin Microbiol* 1989; 27: 453-8.
36. WELLIVER RC, WONG DT, SUN M, MIDDLETON E, VAUGHAN RS, OGRA PL. The development of respiratory syncytial virus-specific IgE and the release of histamine in nasopharyngeal secretions after infection. *N Engl J Med* 1981; 305: 841-6.
37. WELLIVER RC, WONG DT, MIDDLETON E, SUN M, MCCARTHY N, OGRA PL. Role of parainfluenza virus-specific IgE in pathogenesis of croup and wheezing subsequent to infection. *J Pediatr* 1982; 101: 889-96.
38. ZAPLETAL A, SAMANEK M, PAUL T. Lung function in children and adolescents. Methods, reference values. In: HERZOG H, ed. Basel: Karger, 1987: 1-220.

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