Rhinovirus-induced wheezing in infancy—the first sign of childhood asthma?

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Background: Although known as common causes of upper respiratory infections, rhinoviruses, enteroviruses, and coronaviruses are poorly studied as inducers of wheezing in infants, and their possible role in the development of childhood asthma has not been investigated.

Objective: The purposes of this study were to assess the occurrence of RV, enterovirus, and coronavirus infections in wheezing infants and to evaluate the association of these viral findings with early school-age asthma.

Methods: In 1999, outcome in relation to asthma was studied in 82 of 100 initially recruited children who had been hospitalized for wheezing in infancy during the period 1992-1993. In 2000, etiologic viral studies regarding the index episode of wheezing were supplemented by rhinovirus, enterovirus, and coronavirus detection by RT-PCR from frozen nasopharyngeal aspirates in 81 of the children for whom adequate samples were available. Of these children, 66 had participated in the follow-up in 1999.

Results: Rhinoviruses were identified in 27 (33%) of the 81 children, enteroviruses in 10 (12%), and coronaviruses in none. Rhinoviruses were present as single viral findings in 22 (81%) of the 27 rhinovirus-positive cases, and rhinovirus infections were associated with the presence of atopic dermatitis in infancy. Enteroviruses were commonly encountered in mixed infections and had no association with atopy. As single viral findings, rhinoviruses were associated with the development of asthma ($P = .047$; odds ratio, 4.14; 95% CI, 1.02-16.77 versus rhinovirus-negative cases [by logistic regression adjusted for age, sex, and atopic dermatitis on entry)].

Conclusion: Our results present rhinoviruses as important inducers of wheezing even in infancy. The association with atopy and subsequent asthma calls for reevaluation of the role of rhinoviruses in the development of asthma. (J Allergy Clin Immunol 2003;111:66-71.)

Abbreviations used
NPA: Nasopharyngeal aspirate
OR: Odds ratio
RSV: Respiratory syncytial virus
RV: Rhinovirus

Respiratory syncytial virus (RSV) has been considered to be the most common virus inducing wheezing in infants. Rhinoviruses (RVs) and coronaviruses, in contrast, are well-known causes of upper respiratory infections at all ages. There is increasing evidence that RVs also cause lower respiratory infections in young children,[1,2] and they might precipitate wheezing symptoms.[3-5] In older children, RVs appear to be the most important viruses in producing exacerbations of asthma.[6] Likewise, there is recent evidence that enteroviruses might be more common than expected in respiratory infections.[7]

Viral isolation, antibody assays, and antigen detection tests are available for common respiratory viruses such as RSV.[8] For RVs, in contrast, isolation has thus far been the only available diagnostic method. There are more than 100 currently known serotypes, a fact that has hampered the development of antigen and antibody assays.[9] The recent development of PCR for RVs and enteroviruses[10] and for coronaviruses[11] has allowed reevaluation of the role of these viruses in respiratory infections of young children.

We have prospectively followed a group of children hospitalized for wheezing in infancy. In this selected cohort, earlier viral studies revealed a negative association of RSV with asthma, RSV having been an uncommon viral finding in infants developing asthma later in childhood.[12,13] To supply the earlier antibody and antigen findings on respiratory viruses, RT-PCR was performed to detect RVs, enteroviruses, and coronaviruses in frozen nasopharyngeal aspirate (NPA) specimens obtained during the index episode of wheezing.

The aims of the present study were to assess the occurrence of RV, enterovirus, and coronavirus infections in wheezing infants and to evaluate the association of these viral findings with early school-age asthma.

METHODS

As described in detail earlier,[14] 100 children aged 1 to 23 months were admitted to the hospital because of respiratory infection-related wheezing during a period of 18 months in 1992 and 1993. Each of 82 of these children—61 boys and 21 girls—attended a study
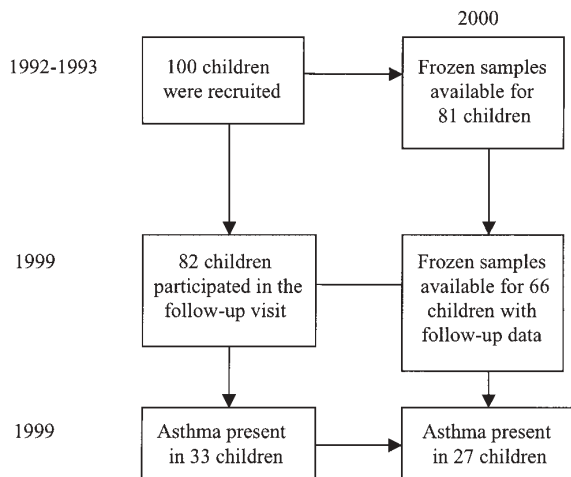
FIG 1. Subgroups of the children in the present study. The term *frozen samples* refers to frozen NPA specimens obtained during the index episode of wheezing in infancy. PCR tests were performed to identify RVs, enteroviruses, and coronaviruses in these NPAs.

FIG 2. RV and RSV findings in 81 infants hospitalized for wheezing, presented in 4 age groups. The viral findings have been classified into 3 categories: RSV-positive, RV-positive, and other/no viral findings. In 1 case (in the 1-to-5-months age group), both RSV and RV were identified; in the bar graph, that case is included in the RV-positive group.

visit approximately 6 years later (median, 6.3 years; range, 5.3-7.2 years), at a median age of 7.2 years (range, 5.6-8.8 years).

On admission, NPAs were taken by suctioning a mucus specimen through the nostrils with a disposable extractor.[14] Direct detection of viral antigens by time-resolved fluoroimmunoassay was available for all of the following: RSV; parainfluenza viruses type 1, 2, and 3; influenza A and B viruses; and adenoviruses.[14] A part of each NPA specimen was frozen for use in later studies.[14] Complement fixation serology was studied in paired sera taken 6 weeks apart for the same 7 respiratory viruses.[14]

In addition, blood eosinophil counts and total serum IgE were measured.[14] The medical histories of the children, including information on the presence of atopic dermatitis and the data on earlier episodes of wheezing, were obtained in parental interviews.[14]

In 1999, each of the children was studied clinically, the exercise challenge test was performed,[15] and the parents were interviewed for the child's medical history through use of a structured questionnaire. The medical records of our university hospital were available, and the cards of regional hospitals and public health care centers were checked in case the child had been treated in one of them. The presence of asthma was defined according to the following criteria: the child (1) had ongoing continuous maintenance medication for asthma at the study visit or (2) had had asthma-suggestive symptoms (repeated parent-reported episodes of wheezing [≥2 episodes] and/or prolonged cough [≥4 weeks] apart from infection during the preceding 12 months), and the exercise challenge test was positive.[15,16] Eighty-two children attended the follow-up visit in 1999, as seen in Fig 1. Frozen NPA specimens, taken in 1992-1993 and stored at –40°C, were available for 81 children for RV, enterovirus, and coronavirus studies in 2000. In all, there were 66 children for whom both adequate follow-up data and frozen NPA specimens were available.

An RT-PCR assay was used for direct detection of RVs and enteroviruses in NPA samples, as described recently in more detail.[10] In brief: Before the PCR test, the nucleic acids from the samples were isolated by means of a High Pure Viral Nucleic Acid Kit (catalog no. 1 858 874, Roche), used according to the manufacturer's instructions. The primers were from the conserved region of the RV and enterovirus genomes (a biotinylated positive-sense primer with map-position 454-473; a negative-sense primer with map-position 548-568). 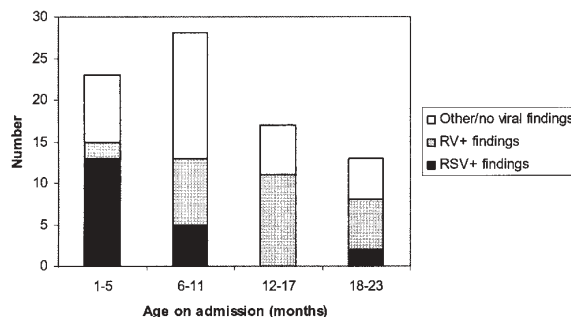The PCR products were detected by agarose gel electrophoresis and ethidium bromide staining. The results were confirmed in a liquid-phase hybridization assay through use of the oligonucleotide probes, a maximum difference between RVs (a samarium-labeled probe with map-position 528-544) and enteroviruses (an europium-labeled probe with map position 534-549) being used to differentiate the RV and enterovirus amplicons. The probes could be detected simultaneously by time-resolved fluorometry.[17,18] The sensitivity and specificity of the RT-PCR and hybridization methods have been validated against prototype (American Type Culture Collection) RVs and enteroviruses. In a study by Lönnrot et al,[10] the 30 most common enterovirus serotypes (obtained from American Type Culture Collection) among 64 known human serotypes were tested; each gave a positive signal by RT-PCR. Nine known RV serotypes (obtained from American Type Culture Collection), among 101 known serotypes, and 20 clinical isolates of unknown serotypes were tested, and the RT-PCR assay amplified all tested RVs. All but 1 amplicon gave a positive signal in the subsequent hybridization assay.

The NPA samples were also tested for sequences of human coronavirus strains 229E and OC43 by an RT-PCR assay similar to that for RVs and enteroviruses.[7] The primers and probes were modified either from the primers and nucleotide sequences described earlier[11,19] or from those available in GenBank.

The data were analyzed through use of SPSS/PC+ 9.0 software (SPSS Inc). Statistical significance of the differences between the groups was assessed with the χ^2 test for proportions supplemented by odds ratios (ORs) for positive test results. The Fisher exact test was used when the expected frequency for any cell was <5. Logistic regression analysis was used to calculate the adjusted ORs and related 95% CIs. Two-tailed tests were used in all analyses. P values less than .05 were considered statistically significant.

The study was approved by the Research Ethics Committee of Kuopio University Hospital. Informed written consent was obtained from the parents of the children.

RESULTS

RVs were identified in 27 (33%) of the 81 specimens analyzed (Table I). Twenty-five (93%) of the RV-positive children were 6 months of age or older (P = .003 versus children <6 months of age; Fig 2). RV infections were most common in the 12- to 17-months age group, being present in 65% of the children at that age. Enterovirus infection was found in 10 children, with no significant

TABLE I. Viral identifications in 81 children under the age of 2 years who were hospitalized for wheezing

Viral identifications*†	No. of subjects (%) (n = 81)
Single identification	47 (58)
RV	22 (27)
Enterovirus	5 (6)
Coronavirus	0 (0)
Other respiratory viruses	20‡ (25)
Multiple identifications	12 (15)
RV and enterovirus	1 (1)
RV and other respiratory viruses	4§ (5)
Enterovirus and other respiratory viruses	4‖ (5)
Other combination of respiratory viruses	3¶ (4)
No viral identifications	22 (27)

RV, Rhinovirus; *NPA,* nasopharyngeal aspirate; *RSV,* respiratory syncytial virus.

*PCR was used to detect RV, enterovirus, and coronavirus in frozen NPA specimens.

†Antigen and antibody assays were performed to detect other respiratory viruses, including RSV, parainfluenza, and adenoviruses.[14]

‡RSV in 13 cases, parainfluenza 3 virus in 5 cases, and parainfluenza 2 virus in 2 cases.

§RSV in 1 case, adenovirus in 2 cases, and adenovirus and parainfluenza 3 virus in 1 case.

‖RSV in all 4 cases.

¶RSV and parainfluenza 3 virus in 2 cases; RSV and parainfluenza 3 virus and adenovirus in 1 case.

age dependence; coronavirus infection was found in none of the children. Of the 81 children, RSV infection had been diagnosed in 21, most (67%) of whom were less than 6 months of age (Fig 2). Parainfluenza viruses had been identified in 11 children (type 3 in 9 cases) and adenoviruses in 4 children.

In most (81%) of the 27 cases with RV identified there were no other viral findings. In 5 cases there were concomitant viral identifications: adenovirus in 3 children (1 had parainfluenza virus also), RSV in 1 child, and enterovirus in 1 child. Enterovirus infections were mixed in 5 cases (50%), 4 with RSV and 1 with RV. In all, 1 or more viruses were detected in 59 (73%) of the 81 children with adequate frozen NPA samples available.

The 1992-1993 recruitment period occurred between 2 RSV infection epidemics: the peak incidence of RSV infections appeared at the beginning of 1992, representing an ongoing winter epidemic started at the end of 1991, and this was followed by a minor spring epidemic in 1993. In contrast, the peak incidence of RV infections occurred at the end of 1992, between the major and minor RSV epidemics.

Among the baseline characteristics in the 82 children followed to school age, an earlier episode of wheezing (1 separate physician-diagnosed wheezing episode that had occurred before the index hospitalization; OR, 4.29; 95% CI, 1.02-17.99), atopic dermatitis (OR, 4.11; 95% CI, 1.42-11.84), a total serum IgE level of ≥60 kU/L (OR, 3.41; 95% CI, 1.03-11.33), and a blood eosinophil count of ≥0.45 × 10⁹/L (OR, 7.02; 95% CI, 2.26-21.79) were in age- and sex-adjusted analyses significantly predictive of asthma. In this selected group of wheezing children, RSV identification was negatively associated with later

childhood asthma (OR, 0.23; 95% CI, 0.06-0.93), again independently of age and sex. The same baseline characteristics were predictive of asthma in the 66 children with NPA samples available for RV and enterovirus studies (data not shown). Therefore, these characteristics are evaluated in further analyses on RVs and enteroviruses (Table II).

As seen in Table II, RV findings were significantly associated with the presence of atopic dermatitis in infancy, independently of age and sex. In contrast, no associations were found between RV infections and elevated total serum IgE, blood eosinophilia, or history of an earlier episode of wheezing. Enterovirus identifications had no dependence on age, sex, or any of the other aforementioned factors (Table II).

Of the 66 children with frozen NPAs and follow-up data available, RVs were identified in 25 (38%) and enteroviruses in 9 (14%). Asthma was considered to be present in 27 (41%) of the 66 children at early school age (Table III). Asthma was present in 15 (60%) of the RV-positive cases and in 2 (22%) of the enterovirus-positive cases (Table IV). As single viral identifications (n = 20), RVs were associated with early school-age asthma, independently of age, sex, and atopic dermatitis on entry into the study. When the mixed RV infections (n = 5) were included in the analyses, the risk for asthma remained increased (OR, 2.29) but the statistical significance was lost. In contrast, enteroviruses had no association with later asthma. In all, RVs were identified in frozen NPAs in more than one half (56%) of the later asthmatics. The figure varied from 25% to 75% in the 4 different age groups, from the youngest to the oldest group, respectively.

DISCUSSION

This study shows that RVs are commonly encountered in young children needing hospitalization for wheezing. From the age of 6 months onward, RVs were the most prevalent of the respiratory viruses identified, whereas under the age of 6 months the most common viral finding—as was to be expected—was RSV. We also found that the infants with atopic dermatitis were especially likely to wheeze during RV infection. Interestingly, hospitalization for RV-induced wheezing in infancy was significantly associated with school-age asthma: the risk for asthma was more than 4-fold that for other wheezing children with no RVs identified, even when age, sex, and the presence of atopic dermatitis in infancy were included as confounding factors in the analyses.

Hospitalization for wheezing in infancy is a well-known risk factor for later asthma.[13] In accord with earlier findings considering the same study cohort,[12,13] RSV infection was found to be associated with a relatively low risk of later childhood asthma among young children hospitalized for wheezing. However, when the later occurrence of asthma among RSV-positive children (15%) is compared with the prevalence of asthma (4%) among nonselected school-aged children of our area,[16] the risk for asthma is increased among RSV-positive children as

TABLE II. Baseline characteristics on entry to the study in the 81 children

Baseline characteristics*	No. of subjects (%)		
	All children (n = 81)	RV-positive† (n = 27)	Enterovirus-positive‡ (n = 10)
Earlier episode of wheezing	11 (14)	5 (19)	0 (0)
Atopic dermatitis	25 (31)	14 (52)§	3 (30)
Total serum IgE of ≥60 kU/L‖	16 (21)	8 (30)	3 (30)
Blood eosinophil count of ≥0.45 × 10^9/L¶	28 (36)	14 (54)	4 (40)

Analyses were performed by logistic regression, with adjustment for age and sex. *RV,* Rhinovirus.

*Only the characteristics significantly predicting asthma in the 82 children followed to school age are presented.

†Multiple viral findings in 5 cases (1 with enterovirus).

‡Multiple viral findings in 5 cases (1 with RV).

§*P* = .013 versus RV-negative children.

‖Sample obtained for 76 children; 27 of them were RV-positive, 10 of them enterovirus-positive.

¶Sample obtained for 78 children; 26 of them were RV-positive, 10 of them enterovirus-positive.

TABLE III. Criteria for the presence of asthma at early school age

	Asthma: no. of subjects (%)		
	Maintenance medication* (n = 24)	No maintenance medication (n = 3)	Asthma not present (n = 39)
Exercise challenge test +†	6 (25)	3 (100)	2‡ (5)
Wheezing§	17 (71)	1 (33)	2‖ (5)
Cough¶	3 (13)	2 (67)	8‖ (21)

*Continuous inhaled anti-inflammatory medication for asthma at the study visit.

†A 15% or greater fall in FEV$_1$ and/or auscultable wheezing after free running for 8 minutes.

‡Neither of the children reported wheezing or prolonged cough.

§At least 2 episodes of wheezing (with or without prolonged cough) during the preceding 12 months.

‖Exercise challenge test was positive in none of the symptomatic children.

¶Prolonged (≥4 weeks) cough apart from infection during the preceding 12 months; no wheezing reported.

well. However, the RSV-associated risk for later asthma was not as high as that in a controlled study by Sigurs et al,[20] in which the risk was increased up to 12-fold.

Enteroviruses were as common as parainfluenza viruses in the wheezing infants. Similar proportions—5% to 10%—have been suggested in earlier studies of respiratory infections in wheezing children.[3-5] No association was found, however, between enteroviruses and atopy or later asthma. There is 1 previous study stressing the role of coronaviruses in lower respiratory tract infections in children.[21] In accord with findings by Rakes et al,[5] we were not able to identify any coronavirus positive cases. RVs are considered the most common causative agents in upper respiratory infections at all ages.[22] There is evidence that RVs might also cause lower respiratory infections in young children.[1] The figure in the present study (33%) is close to that published by Juvén et al[2] for pneumonia in children. In asymptomatic children with no preceding or concurrent respiratory symptoms, RVs have been detected in 12% to 13% of the NPAs by RT-PCR.[23,24] Those detection rates are clearly lower than the RV detection rate in wheezing children in the present study.

Johnston et al[6] found that in 9- to 11-year old children RVs were the most common viruses to trigger exacerbations of asthma. However, previous reports on RV-induced wheezing in infancy are few.[3-5] Our findings stressing the role of RVs in young children hospitalized for wheezing and the association between RV-induced wheezing and atopy are in accord with findings in earlier studies.[4,5] The new, heretofore unpublished finding of clinical importance

TABLE IV. Viruses associated with wheezing in infancy and the presence of asthma at early school age

Viral identifications	Asthma at early school age: no. of subjects (%)	
	Present (n = 27)	Not present (n = 39)
Single identification	18 (67)	24 (62)
RV	14 (52)*	6 (15)
Enterovirus	0 (0)	5 (13)
RSV/other respiratory viruses	1 (4)/3 (11)	9 (23)/4 (10)
Multiple identifications	2† (7)	8‡ (21)
No viral identifications	7 (26)	7 (18)

RV, Rhinovirus; *RSV,* respiratory syncytial virus.

*P = .047; OR, 4.14; 95% CI, 1.02-16.77 versus RV-negative cases, as determined by logistic regression with adjustment for age, sex, and atopic dermatitis on entry into the study.

†RV and enterovirus in 1 case; enterovirus and RSV in 1 case.

‡Includes RV in 4 cases and enterovirus in 2 cases.

is our observation of substantially increased risk of asthma after RV-induced wheezing in early childhood.

It is not fully established how RVs trigger wheezing in susceptible people, but several mechanisms have been proposed.[25] Respiratory epithelial cells are the host cells for RV replication, and the ability of RVs to infect respiratory airway epithelium seems to depend on the density of viral receptors on the cell membrane.[25] Most (90%) of the RV serotypes use intercellular adhesion molecule 1 receptor,[26] which is highly expressed in bronchial epithe-

lial cells, especially during asthmatic airway inflammation.[27] Consequently, high expression of intercellular adhesion molecule 1 in bronchial epithelial cells would render asthmatic persons more susceptible to lower airway RV infections.[25] In addition, in vitro and in vivo studies indicate that RV infection promotes secretion of several cytokines, such as IL-6, IL-8, IL-11, and GM-CSF from epithelial cells.[28-30] IL-11 might have direct effects on bronchial hyperresponsiveness,[29] and the other cytokines have profound effects on inflammatory cells that can potentiate asthma.[25]

The present study has 2 main strengths. First, it was prospective, the follow-up time being long (6 years on average) and the attendance (despite the length of the follow-up time) good (>80%). At the follow-up visits at early school age the children were 7 years old, on average, and at that age, the continuing symptoms can be considered to be asthma rather than transient wheezing.[31] Second, the viral studies were extensive; 7 conventional respiratory viruses had been studied on each child's entry into the study in infancy,[14] and the findings were later supplemented by RV, enterovirus, and coronavirus studies. RT-PCR, used in the present study, has been shown to be more sensitive than conventional virus isolation for RVs and enteroviruses.[9,10] Liquid-phase hybridization assay, done after RT-PCR, further increases the sensitivity and specificity.[10] In comparison with virus isolation from clinical respiratory samples, the sensitivity of PCR has been excellent (98%).[9] Inasmuch as RVs were rarely involved in mixed viral infections, it was possible to study their specific effect on outcomes in the children.

The present study has 2 shortcomings. Viral PCR was studied approximately 6 years after the acute episode, and therefore good-quality frozen NPA specimens obtained on entry into the study were available for only 80% of the children. Thus the risk analyses for the later outcome at early school age had to be restricted to those 66 cases with both NPA specimens and adequate follow-up data available. In addition, we did not have a control group with no history of early childhood wheezing. Nevertheless, there was a recent Finnish study on RV, enterovirus, and coronavirus findings by RT-PCR in asymptomatic children[24] that showed the viral detection rate by RT-PCR to be low (5%) when no preceding, concurrent, or following respiratory symptoms were reported. In the present study, the viral detection rate by RT-PCR in wheezing children was greater than 8-fold (44% in all). In case of RVs, the detection rate in wheezing children was approximately 3-fold. Consequently, we consider RVs to be important infective agents that are able to induce wheezing in susceptible children. In addition, the prevalence of school-age asthma has been carefully studied in our area in recent years, the criteria for asthma being with minor modifications similar to the criteria used in the present study; the prevalence in those studies has been stable (4% to 4.5%).[16,32] Thus, RV-induced wheezing in infancy was associated with multiplied occurrence of asthma (up to 70%) in later childhood.

In conclusion, the development of PCR methods for RVs, enteroviruses, and coronaviruses has allowed us to reevaluate the role of these viruses in wheezing children. Our results present RVs as important inducers of wheezing even in children under the age of 2 years. RV-induced wheezing leading to hospitalization seems to predict the development of asthma. The association with atopy suggests that there is active asthmalike bronchial inflammation in children wheezing during RV infection. Thus, our results call for reevaluation of the role of RV in the development of asthma.

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Asthma, rhinitis, other respiratory diseases