

Rhinovirus-specific antibody responses in preschool children with acute wheeze reflect severity of respiratory symptoms

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Abstract

Background: Some children with rhinovirus (RV) infections wheeze, but it is unknown whether this is due to more virulent strains of virus or differences in host immune responses. The aim of this study was to investigate the RV species-specific antibody responses measured at a follow-up visit in preschool children in relation to reported time with respiratory symptoms and the presence of different RV species during an acute episode of wheeze.

Method: Nasopharyngeal swabs and blood samples were taken among 120 preschool children (<4 years of age) at an acute episode of wheeze and at a follow-up visit (median 11 weeks later). Nested PCR was used to detect different RV strains, and serum levels of IgG₁ against purified recombinant VP1 proteins from representatives of the three RV species (RV-A, RV-B, and RV-C) were measured by ELISA.

Results: Rhinovirus was detected in 74% ($n = 80/108$) of the children at the acute visit, and RV-C was the most common subtype ($n = 59/80$, 74%). An increase in RV-specific IgG₁ was seen in 61% ($n = 73$) of the children at follow-up, most frequently against RV-A ($n = 61/73$, 86%) irrespective of the RV strains detected by PCR. Increases in RV-specific IgG₁ against RV-A or against RV-A and RV-C were significantly associated with more respiratory symptoms ($p = 0.03$, $p = 0.007$).

Conclusion: Antibody response to recombinant RV VP1 proteins was associated with longer time with respiratory symptoms.

Rhinovirus (RV) infections are common and usually only cause a mild cold, but some individuals will develop severe

respiratory infections. Wheezing caused by RV in children before 3 years of age is associated with an increased risk of asthma at 6 years (1–3). Rhinovirus can be divided into three species, RV-A, RV-B, and RV-C, with over 100 viral serotypes, and RV-C has been proposed to cause more severe attacks than other human rhinovirus groups (4).

It has been suggested that the innate immunity and T-cell responses are of most importance for the resolution of virus infection, while humoral responses with production of neutralizing IgG antibodies in serum are important for

Abbreviations

HRV, human rhinovirus; HSA, human serum albumin; ICS, inhaled corticosteroids; LRI, lower respiratory tract infections; MEGA, Molecular Evolutionary Genetics Analysis; RT-PCR, reverse transcription-polymerase chain reaction; SE, specific identification code; URI, upper respiratory tract infection; WBC, white blood cells.

protection against short-term reinfections (5). IgG in serum can be detected after 1–2 weeks after infection and can stay elevated for years (5, 6). An increase in RV-specific antibodies has been associated with severity of respiratory symptoms in adults (7). However, it is still not clear whether the clinical manifestations of RV infections are related to more virulent RV strains, or whether different strains evoke distinct host immune responses and airway inflammation.

The aim of this study was to characterize a group of preschool children (<4 years of age) recruited on presentation at the Astrid Lindgren Children's Hospital Emergency Department with an acute episode of wheeze and to evaluate different RV species detected at the acute visit, the changes in levels of serotype-specific serum IgG₁, and time with clinical symptoms measured at a follow-up visit.

Materials and methods

Study population and sample collection

During a time period from October 2008 to September 2012, children were recruited when visiting the Paediatric Emergency Department or after admission to the Paediatric Emergency Ward as a result of acute wheeze, at Astrid Lindgren Children's Hospital, Stockholm, Sweden, as previously described (8). Inclusion criteria were age 6 months to <4 years and presenting at the emergency ward with acute symptoms of wheeze. Exclusion criteria were prematurity (birth before 36 gestational weeks), any chronic disease, or

any simultaneous complications such as sepsis or bacterial pneumonia at the time point of inclusion. For study design and work flow, see Fig. 1, and for the basic characteristics of the children, see Table 1. All children included in the study were diagnosed by the treating physician at the Paediatric Emergency Department and treated with inhalations of salbutamol.

Blood samples were collected at the emergency ward at the acute visit and at a follow-up visit a few months later (median 11 weeks) and analyzed for total blood cell count as well as levels of species-specific IgG₁ antibodies against recombinant VP1 proteins of RV-A (RV89, 16, 2), RV-B (RV14), and RV-C (RV-YP) (Table S1). In addition, allergen-specific IgE antibodies were analyzed using Phadiatop and fx5 ImmunoCAP System (Thermo Fisher Scientific, Uppsala, Sweden) at the follow-up visit.

Nasopharyngeal swab samples were obtained at the acute visit and again at the follow-up visit by the research nurse and stored in the biobank at the Department of Clinical Microbiology, Karolinska University Hospital until subsequent nested PCR analyses and sequencing (see Tables S1 and S2 and Fig. S1) (8).

At the follow-up visit, all children were seen by the same study physician. The children underwent a clinical examination, and the legal guardian filled out a standardized questionnaire regarding history of earlier respiratory infections and wheeze, medication, and hereditary factors. The study physician interviewed the guardians and filled out a standardized questionnaire concerning the number of days the child had suffered from respiratory symptoms at home ('cough

Children with acute wheeze

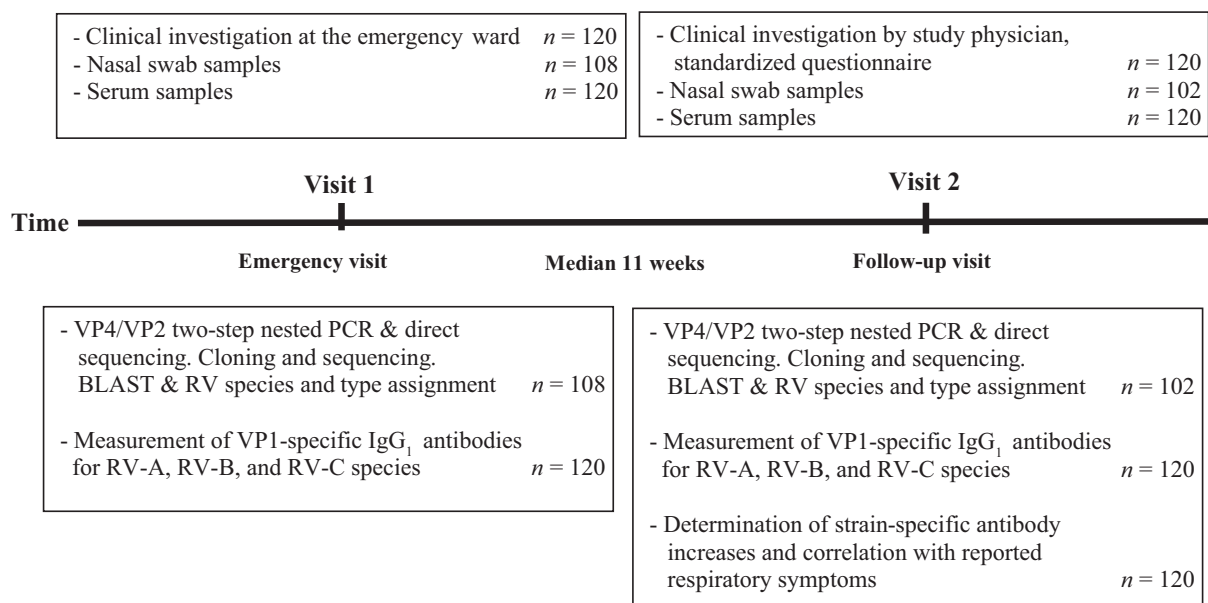


Figure 1 Study design and workflow for the detection of rhinovirus (RV) infection and species-specific antibody responses by

means of nested PCR and ELISA, respectively, in preschool children seeking emergency care due to an acute episode of wheeze.

and/or wheeze'), use of medication (β_2 -agonist and/or steroid inhalation), as well as about any emergency visits between the acute and follow-up visits. The reported number of emergency visits was checked in medical journals, and the guardians' personal diaries of days spent away from work when the child had respiratory symptoms.

RNA extraction and reverse transcription

Viral RNA was extracted from 280 μ l of virus-containing transport medium (Sigma-Virocult, Medical Wire & Equipment Co Ltd, Corsham, Wiltshire, UK) using a QIAmp Viral RNA Kit according to manufacturer's instructions (Qiagen, Hilden, Germany). Six microliters of the extracted RNA was reversely transcribed into cDNA using SuperScript[®] III First-Strand Synthesis System SuperMix with random hexamer primers according to manufacturers' instructions (Invitrogen, Carlsbad, CA, USA).

PCR and sequencing

For the amplification of the DNA coding for VP4 and a part of the VP2 region (i.e. VP4/VP2) of the RV genome, a nested PCR strategy with two primer pairs was used. (9) The first PCR amplification was performed using 2 μ l of cDNA and 22.5 μ l of AccuPrime[™] Pfx SuperMix (Invitrogen)

and 100 pM of each the outer primer pair (OS, outer sense: 5'-CCGGCCCCCTGAATGYGGCTAA-3'; OAS, outer antisense: 5'-ACATRTTYTSNCCAAANAYDCCCAT-3') (MWG Eurofins, Ebersberg, Germany). Then, 2 μ l of the first PCR product was re-amplified in a second round of PCR using the same amount of AccuPrime[™] Pfx SuperMix and the inner primer pair (IS, inner sense: 5'-ACCRAC-TACTTTGGGTGTCCGTG-3'; IAS, inner antisense: 5'-TCWGGHARYTTCCAMCACCANCC-3', where R = A, G; Y = C, T; M = A, C; H = A, C, T; N = A, G, C, T) (9). Reactions were run in a thermocycler (MWG-Biotech AG, Ebersberg, Germany) under the following conditions: 96°C for 2 min, 40 cycles at 96°C for 25 s, 55°C for 1 min, and 72°C for 2 min, and final elongation step at 72°C for 5 min. The amplification products of approximately 500 bp were visualized after electrophoresis on an ethidium bromide-stained 1% agarose gel. Positive PCR products were purified using Wizard[®] SV Gel and PCR Clean – Up System (Promega, Madison, WI, USA) and stored at –20°C or directly used for cycle sequencing on an ABI 3730XL sequencer with the same IS and IAS primers (MWG Eurofins). If the quality of sequences obtained by direct sequencing of the PCR product was not sufficient for type assignment (e.g. due to the presence of multiple RV types in one sample), 4 μ l of the amplicons were cloned into pCR[™]II-Blunt-TOPO vector using Zero Blunt[®] TOPO[®] PCR Cloning Kit (Invitrogen). One Shot[®] chemically competent *Escherichia coli* cells were then transformed with 1 μ l of TOPO cloning reaction, and LB plates containing kanamycin (50 μ g/ml) were used for the selection of positive clones. The nucleotide sequences of 8–10 positive clones for each isolate were determined using IS and IAS primers.

Table 1 Basic characteristics and clinical history of 120 children with acute episode of wheeze and reported days in hospital, exacerbations and time with wheeze/cough $\geq 25\%$ until follow-up visit after median 11 weeks

	Value
Characteristic	
Male, <i>n</i> (%)	77 (64)
Age in months at acute visit, median (min–max)	18 (6–42)
First time wheeze, <i>n</i> (%)	27 (22)
Atopic*, <i>n</i> (%)	33 (28)
Phadiatop ≥ 0.35 kU/l†	11 (10)
Fx5 ≥ 0.35 kU/l‡	28 (24)
Clinical history	
Weeks until follow-up, median (min–max)	11 (6–32)
Hospitalized acute, <i>n</i> (%)	97 (81)
Number of days in hospital	
PCR§ negative for RV (<i>n</i> = 28), median (min–max)	1 (0–6)
PCR§ positive for RV (<i>n</i> = 80) median (min–max)	1 (0–12)
Increase in anti-RV IgG ₁ (<i>n</i> = 73)	1 (0–8)
No increase in anti-RV IgG ₁ (<i>n</i> = 47)	1 (0–12)
Exacerbation until follow-up, <i>n</i> (%)	91 (76)
Wheeze/cough $\geq 25\%$ of the time until follow-up, <i>n</i> (%)	29 (24)

RV, rhinovirus.

*Allergen-specific IgE to food (fx5) and/or airborne allergens (Phadiatop) ≥ 0.35 kU/l.

†Phadiatop, *n* = 115 children analyzed.

‡Fx5, *n* = 118 children analyzed.

§PCR for RV, *n* = 108 children analyzed.

Nucleotide sequence analysis

Rhinovirus reference strains were retrieved from the NCBI database (Table S1) to allow a matching of the sequences determined from the samples obtained from the children according to sequence comparison. Each sample's name consists of two letters standing for Sweden (i.e. SE), followed by the individual patient number and the time of sampling (Table S2). Nucleotide sequences of the VP4/P2 regions for certain RV strains were identified by searching the nucleotide BLAST database available on the National Center for Biotechnology Information page (<http://www.ncbi.nlm.nih.gov/BLAST>). Multiple sequence alignment was performed by Clustal W method integrated in the Molecular Evolutionary Genetics Analysis (MEGA) software version 6 (www.megasoftware.net). GenBank accession numbers for 41 RV and 1 HEV sequences of the reference strains are listed in Table S1. The phylogenetic tree was constructed by the neighbor-joining (N-J) method using MEGA6 (Fig. S1) (10). The evolutionary distances between sequences were computed using the Kimura two-parameter model (11) with bootstrap values calculated from 1000 replicates (12). Scale bar indicates number of nucleotide substitutions per site.

ELISA experiments

ELISA plates were coated with 2 µg/ml of each of the recombinant VP1 proteins from RV2, 16, 89 (RV group A), RV14 (RV group B), and RV-YP (RV group C), respectively, and human serum albumin (HSA) for control purposes (7). Human sera were diluted 1 : 100, and bound human IgG₁ antibodies were detected with monoclonal mouse IgG₁ (BD Pharmingen, San Diego, CA, USA) diluted 1 : 1000 followed by detection with horseradish peroxidase-conjugated sheep anti-mouse IgG antibodies (Amersham Bioscience, Freiburg, Germany) diluted 1 : 2000. The optical density (OD) values corresponding to the levels of bound antigen-specific antibodies were measured at 405 and 490 nm in an ELISA reader (Dynatech, Denkendorf, Germany). All measurements were performed as duplicates with a variation of <5%. Plate-to-plate normalization was carried out by including a control serum (Ref) with established VP1-specific antibody levels on each of the plates (7). The reactivity to HSA, as determined for each patient's serum, was used as a negative control. Optical density values greater than double HSA value were considered positive (cut-off). ΔOD values were calculated as the OD value at the acute visit subtracted from the OD value at the follow-up. An increase of OD (ΔOD) ≥ 0.1 in antibody levels between the acute visit and follow-up was considered to show an immunological response to RV. The ELISA has been shown to differentiate well between antibody responses specific for RV group A, B, and C and was able to detect increases of RV-specific antibodies already 42 days after experimental infection (7).

Ethics

The study protocol was approved by the Regional Ethics Committee of Karolinska Institutet, Stockholm (2008/378-31/4, 2014/399-31/3), and written informed consent was obtained from the legal guardians. Anonymized serological analyses of serum samples were performed with the approval of the Ethics Committee of the Medical University of Vienna (EK1721/2014).

Statistical analysis

The chi-squared test was used to examine proportional differences between two groups of children. Independent samples Mann-Whitney *U*-test was used to compare medians and distribution between two groups, and Kruskal-Wallis median test was used to compare distribution between several groups. Initial serum levels of anti-RV IgG₁ were log-transformed for normality and analyzed in a linear regression model including age for correlation with times with symptom. Change in serum levels of anti-RV IgG₁ displayed a normal distribution and was analyzed in a linear regression model with time with symptoms as dependent variable and controlled for age. SPSS software (version 22; IBM Corporation, Armonk, NY, USA) was used for all statistical analyses.

Results

RV species and reported symptoms

The presence of RV was tested in 108 of 120 children with acute wheeze, and RV was detected in 74% ($n = 80$). RV-C was the most frequent species (74%, $n = 59/80$) (Tables 2 and S2). The median time spent at the hospital ward or reported time with respiratory symptoms until follow-up was not significantly different between the children with positive or negative PCR for RV (Table 1), or between different subtypes of RV (Table 2).

At the follow-up visit, nasopharyngeal samples were obtained from 102 of the 120 children, of these four were positive for RV. No relevant variations of RV species were observed during the observation period (Fig. S2).

Table 2 PCR findings and RV-specific IgG₁ antibody increase in children with acute wheeze and the relation to reported time with respiratory symptoms and use of β_2 -agonist in % of days until a follow-up visit

			Time with symptoms (%)		Time with β ₂ -agonist medication (%)	
	<i>n</i>	%	Median	IQR	Median	IQR
PCR findings*						
RV-A, single	16	15	15	9–48	36	15–91
RV-B, single	5	5	7	4–17	41	19–75
RV-C, single	49	45	10	4–22	22	6–35
RV-A + RV-C	9	8	10	4–30	20	13–47
RV-B + RV-C	1	1	6	6	6	6
PCR-negative	28	26	11	6–19	16	7–60
Total	108	100				
IgG ₁ † increase against						
RV-A‡	28	23	16§	7–32	29	7–71
RV-B¶	5	4	9	6–10	9	7–13
RV-C**	6	5	10	8–13	31	13–50
RV-A‡ + RV-B¶	6	5	7	1–18	18	3–83
RV-A‡ + RV-C**	18	15	28††	9–71	55‡‡	27–100
RV-B¶ + RV-C**	1	1	1	1	0	0
RV-A‡ + RV-B¶ + RV-C**	9	8	5	4–12	13	8–25
No IgG ₁ response	47	39	8	4–19	17	7–34
Total	120	100				

Numbers are reported in medians and interquartile range (IQR).

*Nasopharyngeal tests on 108 children.

†Levels of serum anti-RV IgG₁ measured on 120 children acute and at a follow-up visit.

‡RV-A = IgG₁ increase against VP1 protein of RV-89, RV-16 and/or RV-2.

§ $p = 0.03$.

¶RV-B = IgG₁ increase against VP1 protein of RV-14.

**RV-C = IgG₁ increase against VP1 protein of RV-YP.

†† $p = 0.007$.

‡‡ $p = 0.003$.

RV-specific IgG₁ antibodies

Of the 120 children included in the study, 92% ($n = 111$) had IgG₁ antibodies against RV at the acute visit. An increase in RV-specific IgG₁ antibodies was seen in 61% ($n = 73$) at the follow-up visit. The response to different subtypes is illustrated in Table 3. When comparing two age groups (6–18 vs 19–42 months), the older children had significantly higher levels of RV-specific IgG₁ at the acute visit, but the change in IgG₁ levels at the follow-up visit was comparable in both age groups (Fig. S3A,B). The reported time with respiratory symptoms and the initial levels of RV-IgG₁ at the acute visit or the change in IgG₁ levels values between acute visit and follow-up visit were not correlated in a linear regression model including age (data not shown).

Association of detected RV species, species-specific IgG₁ increase, and reported symptoms

Of the 80 children with RV detected at the acute visit, 59% ($n = 47/80$) displayed an IgG₁ increase against one or several of the RV-specific VP1 regions tested (Table S3). Children with RV detected and a RV-specific IgG₁ increase did not report significantly longer time periods with respiratory symptoms than children without anti-RV IgG₁ increase (Fig. 2).

In children with no detectable RV at the acute visit ($n = 28$), there was no significant difference in reported time with symptoms between children with ($n = 17$) or without ($n = 11$) increase in RV IgG₁ antibodies (Fig. 2).

In children with detectable RV-A ($n = 25$), species-specific IgG₁ increase was found in 68%. In children with RV-C ($n = 59$), 32% showed an increase in anti-RV IgG₁ against RV-C [of which only 4 (8%) were directed against RV-C only and had no cross-reaction against other RV species at the same time] (Table S3). Comparing children in two age groups (6–18 vs 19–42 months), no difference in detection of RV by PCR or anti-RV IgG₁ increase was found (Table S4).

Table 3 Presence of RV-specific IgG₁ antibodies found in 120 children with episode of acute wheeze and number of children with an increase of RV-specific IgG₁ antibodies at a follow-up visit median 11 week later

RV subtypes	IgG ₁ at acute visit $n = 111$ (92%)		IgG ₁ increase at follow-up visit $n = 73$ (61%)	
	n	%	n	%
RV-A89	102	85	52	43
RV-A16	103	86	49	41
RV-A2	106	88	54	45
RV-A89, 16 and 2	100	83	42	35
RV-B14	73	61	21	18
RV-C	93	78	33	28

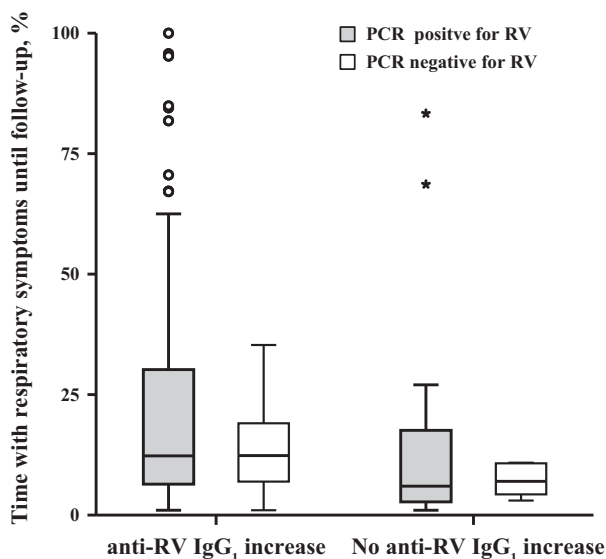


Figure 2 Presence of rhinovirus (RV) in nasal swabs by PCR typing and increase of species-specific IgG₁ in relation to time with respiratory symptoms in % of the time between the acute and follow-up visit.

Irrespective of the RV species detected at the acute visit, children with IgG₁ increase against RV-A (RV89, 16 and/or 2) or against both RV-A (RV89, 16, 2) and RV-C (RV-YP) had significantly longer time with respiratory symptoms compared with children without anti-RV IgG₁ increase ($P = 0.03$ for children with RV-A (RV89, 16 and/or 2) and $P = 0.007$ for children with both RV-A (RV89, 16 and/or 2) and RV-C (RV-YP) (Fig. 3).

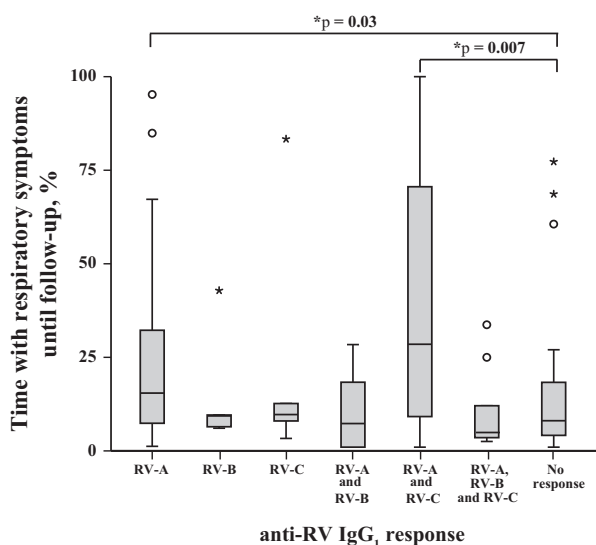


Figure 3 Rhinovirus species-specific IgG₁ response and time with reported respiratory symptoms between the acute and follow-up visits.

Children with most reported respiratory symptoms until follow-up visit

Among the 120 children with acute wheeze, 29 children reported respiratory symptoms (cough and/or wheeze) $\geq 25\%$ of the time until follow-up visit [median 40 days (IQR 28–60 days)] as compared to 91 children with $<25\%$ time with reported symptoms [median 7 days (IQR 3–10 days)]. The first subgroup tended to be younger than the larger group with symptoms $<25\%$ of the time (median age 15 vs 20 months) and showed increased anti-RV IgG₁ levels at the follow-up visit ($P = 0.005$, Table S5).

Asthma Predictive Index score, RV species, and reported symptoms

Asthma Predictive Index (API) is considered positive if one major criteria [mother and/or father with asthma and/or atopic eczema (with sensitization > 0.35 kU_A/l against Fx5 or Phadiatop)], or two minor criteria (Phadiatop ≥ 0.35 kU_A/l and eosinophils $\geq 4\%$ at revisit). (2) When API was assessed in the group of children with wheeze, 47% ($n = 55$) of total 117 children had a positive score. There was however no significant difference between children with a positive or negative API in relation to number of children with PCR positive for RV or increase in RV-specific IgG₁ levels, nor in time with respiratory symptoms or use of inhaled β_2 -agonists between the acute visit and the follow-up visit (Table S6).

Discussion

In this study, we followed the RV-specific IgG₁ response in a group of 120 preschool children aged between 6 months and <4 years seeking emergency care during an acute episode of wheeze and at a follow-up visit. The majority of the children reported a history of frequent episodes with respiratory infections. We show that RV was detected in as many as 74% of children at the acute visit and RV-C was the most frequent species. Importantly, we also show for the first time that children with RV-specific IgG₁ antibodies against RV-A and RV-C had more days with reported symptoms and used more β_2 -agonist medication until follow-up, irrespective of the RV species detected by PCR at the acute visit.

The finding that RV-C was the dominating RV species in this group of young children at the time of acute wheeze is consistent with earlier studies on older children with asthma (13, 14). The time spent with respiratory symptoms was, however, similar in children with nasopharyngeal RV-A and RV-C findings, or a combination of the two.

Rhinovirus species-specific serum IgG₁ antibodies against VP1 proteins from five selected representative strains of RV-A, RV-B, and RV-C were measured at the acute visit and at a follow-up visit and evaluated in relation to reported respiratory symptoms. More than 90% of these young children had species-specific antibodies against RV species at the acute visit. The increase in RV-specific IgG₁ antibodies seen in 61% of the children at the follow-up visit involved mainly IgG₁ antibodies against RV-A (RV2, 16, 89), irrespective of

the initial RV species detected by PCR. Interestingly, children with an increase in IgG₁ against both RV-A and RV-C had significantly more days with reported symptoms, and more days with β_2 -agonist medication until follow-up visit, again, irrespective of the RV species found at the acute visit. Children reporting most respiratory symptoms tended to be younger, and significantly, most of them had increased levels of anti-RV IgG₁ compared to the group of children with less reported symptoms, showing that although age is an important factor affecting the duration of symptoms, the antibody response might also play an important role or the combination.

The virulence of RV-C has been debated. Some studies support an equal association of RV-A and RV-C with serious illness (15, 16), whereas others suggest that RV-C is more pathogenic (4, 17) or that the low levels of species-specific antibody titers found against RV-C possibly reflect an inefficient immune response against this species (13). In our study, a species-specific increase in IgG₁ levels against RV-A was seen in 62% of children positive for RV-A by PCR, and in 36% of children positive for RV-C. The majority of the IgG₁ response against RV-C showed cross-reactivity with other RV species, and only four of the 59 children with RV-C in PCR displayed IgG₁ increases against only the VP1 protein of RV-C at the follow-up visit. These results are therefore similar to the findings on older children presented by Iwasaki et al. (13) where children with asthma had higher antibody responses to RV compared to nonasthmatic children, and where a very low increase in species-specific antibodies against RV-C was found. However, the interpretation of findings regarding the relevance of RV-C must be made with caution because the results were obtained with VP1 proteins from only one RV-C strain in our study and two strains in the study by Iwasaki.

Several studies have investigated the innate immune response in children with RV-induced respiratory symptoms, but the antibody response toward specific RV proteins is an emerging field. For example, children with nonatopic wheeze have been found to have lower interferon (IFN)-gamma which is important for the initiation of innate immunity (18). Resistance to RV has been shown to be a result of rapid induction of apoptosis and of IFN-beta in normal cells, whereas these responses seemed to be deficient in asthmatic cells (19). Vitamin D is known to be important for the induction of human antimicrobial peptide LL-37 production by macrophages, and we have shown that children with wheeze have lower levels of vitamin D than a healthy control group (8).

It has been speculated that in the absence of neutralizing antibodies or an effective innate immune response, RV-specific T cells may become central in virus eradication (20). It has recently been shown that some RV can induce T-cell activation directly without the involvement of antigen-presenting cells (21). This might explain why certain children have such an abnormal inflammatory response, displaying clinical respiratory symptoms despite a low species-specific antibody response against RV. One may speculate that RV-C has an ability to activate both cytokine production and local inflammation, as it is frequently identified in children with wheeze,

but induce only low levels of species-specific antibody titers. This might also lead to an inability for the immune system to quickly clear a reinfection, leading to recurrent respiratory infections in these young children. In addition, it has been shown that RV causes mucociliary impairment for 3 weeks after infection (22, 23), which might be a part of why children with difficulty to clear virus also suffer from secret stagnation and coughing for a long time. In our study, we could not find that initial levels of anti-RV IgG₁ titers at the acute visit predicted the duration of respiratory symptoms during the follow-up period of almost 3 months.

The combination of IgE sensitization and RV infection has been reported to be associated with inception of asthma and increased severity of wheeze (24, 25). We have previously reported that these children with severe wheeze constitute a group of children with frequent airway infections (8), but they are not more atopic than a healthy age-matched control group (8). Furthermore, we did not find that children with a positive API score or sensitization against food or airborne allergens had either increased levels of RV-specific IgG₁ or increased time with symptoms between the acute visit and the follow-up.

Seventeen of children showed an increase in RV-specific IgG₁ at the follow-up visit despite undetectable RV at the acute visit. In experimental RV inoculation, it has been shown that RV infection has a rapid onset within 24 h, but viral titers begin to decline already after 72 h and serum IgG can be detected within 2 weeks after infection and then remain for years (5). It is possible that these children with negative PCR had had their infections for more than 4 days when they came to the emergency care, which could affect viral load and the collection of representative nasopharyngeal samples.

One limitation of the study is that the number of children is not sufficient to make detailed subanalyses of the correlation between RV detection by PCR and the RV-specific immune response. The findings could also have been put into perspective by examining the presence of different RV species in a healthy control group. However, data from a cross-sectional study from Greece show a prevalence of 1–14% of RV colonization in children 2–5 years of age (26).

In conclusion, RV was detected in 74% of children with wheeze at the acute visit, where RV-C was the most frequent species (4, 27). Intriguingly, over 90% of this young group of children had species-specific antibodies against RV species at the acute visit, and 61% responded with an increase in RV-specific antibodies after the acute episode of wheeze. The time with reported respiratory symptoms seemed to relate more to the antibody response than the RV species found at the acute visit. The combined increase in IgG₁ levels against both RV-A and RV-C was associated with a longer time with reported symptoms. This group of young children, mainly toddlers, had either a less effective early innate immune response or a more symptomatic infection resulting in higher antibody responses. We propose that their antibody response could identify them as a particularly vulnerable group at greater risk of developing asthma. Further long-term studies are needed to investigate how RV-specific antibody response may be associated with the development of chronic asthma.

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Author contributions

Katarina M. Stenberg-Hammar participated in the study design, collected all clinical data, performed all statistical analysis and interpretation of data, made the first draft of the manuscript and critically revised the manuscript and gave final approval of the version to be published. Katarzyna Niespodziana participated in the study design, made all PCR and nucleotide sequence analyses and ELISA, participated in the interpretation of data, made the first draft of the manuscript and critically revised the manuscript and gave final approval of the version to be published. Clarissa R. Cabauatan participated in the PCR and nucleotide sequence analyses and ELISA, interpretation of data and critical revision and final approval of the version to be published. Rudolf Valenta, Gunilla Hedlin, Cilla Söderhäll, Anna James, Jon R. Konradsen, Erik Melén, Marianne van Hage participated in the study design, analysis and interpretation of data and critical revision and gave final approval of the version to be published.

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Conflicts of interest

The authors declare that they have no conflicts of interest.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Phylogenetic tree based on nucleotide sequences of RV strains detected from nasopharyngeal swab samples at acute visit.

Figure S2. Seasonal distribution of rhinovirus (RV) groups detected by PCR in nasopharyngeal swab samples of children hospitalized during acute wheezing episodes.

Figure S3. (A) Comparison of initial median levels of anti-RV IgG₁ in two age groups (6–18 and 19–42 months) show significantly higher levels in the older age group. (B) Median change of anti-RV IgG₁ levels in serum in 120 children at an acute episode with wheeze and at a later follow-up visit 11 weeks later.

Table S1. Characterization of RV reference strains used in this study.

Table S2. Attribution of PCR sequences from the samples of the children to RV species according to sequence similarity and clustering (left) and increases of VP1-specific IgG₁ levels between acute and follow up visit.

Table S3. Type of rhinovirus detected by PCR in 108

preschool children with acute episode of wheeze and increase in species-specific IgG₁ against RV at a follow-up visit median 11 weeks later.

Table S4. Comparison of two age groups of children at the time of acute wheeze with regard to gender, time with wheeze and the increase in anti-RV IgG₁ at a follow-up visit after median 11 weeks.

Table S5. Comparison of children with reported cough/wheeze more or less than 25% of the time until the follow-up visit, median 11 weeks later.

Table S6. Comparison of children with acute wheeze and positive or negative Asthma Predictive Index (API) in relation to PCR for RV and increase in RV species-specific IgG₁.

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