A Mechanistic Role for Type III IFN-λ_1 in Asthma Exacerbations Mediated by Human Rhinoviruses

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Rationale: **Human rhinoviruses (HRV) are the leading cause of upper respiratory infections and have been postulated to trigger asthma exacerbations. However, whether HRV are detected during crises because upper respiratory infections often accompany asthma attacks, or because they specifically elicit exacerbations, is unclear. Moreover, although several hypotheses have been advanced to explain virus-induced exacerbations, their mechanism remains unclear.**

Objectives: **To determine the role of HRV in pediatric asthma exacerbations and the mechanisms mediating wheezing.**

Methods: **We prospectively studied 409 children with asthma presenting with upper respiratory infection in the presence or absence of wheezing. Candidate viral and immune mediators of illness were compared among children with asthma with different degrees of severity of acute asthma.**

Measurements and Main Results: **HRV infections specifically associated with asthma exacerbations, even after adjusting for relevant demographic and clinical variables defined *a priori* (odds ratio, 1.90; 95% confidence interval, 1.21–2.99; $P = 0.005$). No difference in virus titers, HRV species, and inflammatory or allergic molecules was observed between wheezing and nonwheezing children infected with HRV. Type III IFN-λ_1 levels were higher in wheezing children infected with HRV compared with nonwheezing ($P < 0.001$) and increased with worsening symptoms ($P < 0.001$). Moreover, after adjusting for IFN-λ_1, children with asthma infected with HRV were no longer more likely to wheeze than those who were HRV-negative (odds ratio, 1.18; 95% confidence interval, 0.57–2.46; $P = 0.66$).**

Conclusions: **Our findings suggest that HRV infections in children with asthma are specifically associated with acute wheezing, and that type III IFN-λ_1 responses mediate exacerbations caused by HRV. Modulation of IFN-λ_1 should be studied as a therapeutic target for exacerbations caused by HRV.**

Keywords: asthma; interferon-λ; rhinovirus; children; asthma exacerbation

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AT A GLANCE COMMENTARY

Scientific Knowledge on the Subject

Human rhinoviruses (HRV) are the leading cause of upper respiratory infections and are thought to trigger asthma exacerbations. However, whether HRV infections play a causal role in the development of asthma exacerbations is unclear.

What This Study Adds to the Field

Our findings suggest that HRV infections in children with asthma are specifically associated with acute wheezing, and that type III IFN-λ_1 responses mediate exacerbations caused by HRV. Modulation of IFN-λ_1 may provide a therapeutic target for exacerbations caused by HRV.

Asthma exacerbations are the main cause of hospitalization in children, and occur in association with respiratory viral infections (1, 2). Human rhinoviruses (HRV) are frequently isolated in the upper airways of children during respiratory infections and during asthma attacks, and have been postulated to trigger these crises (3, 4). However, whether HRV are detected during asthma crises because they are the most frequent cause of upper respiratory infections (URI; which accompany asthma attacks), or because they can specifically elicit asthma exacerbations is unclear. To our knowledge, no pediatric study has compared the viral etiology of URI in patients with asthma with and without wheezing to investigate the specific association between HRV URI and asthma attacks.

Several hypotheses have been advanced to explain the mechanisms that trigger asthma crises during respiratory infections (4–7). Focused mainly on HRV-associated episodes, two nonexclusive theories attribute asthma attacks to direct viral injury and immune-mediated exacerbations (7–10). Postulated direct effects of HRV associate wheezing episodes with high viral load (9, 11) or specific HRV species (10, 12). These theories are supported in part by studies where inoculation of an attenuated HRV in adults with asthma and healthy control subjects led to higher HRV titers in the respiratory tract of the former group (13, 14), and other observations suggesting a potential association between HRV species C (HRVC) and wheezing or asthma (12, 15). Meanwhile, other leading hypotheses have been postulated to explain immune-mediated pathways to exacerbations associated with HRV. The first

attributes exacerbations to respiratory tract innate immune inflammation (4) and is supported by experimental exposures and epidemiologic studies demonstrating that HRV enhance the production of inflammatory cytokines (e.g., IL-8 and IL-6) and elicit neutrophilia in the lungs of patients with asthma (16, 17). The second of these hypotheses suggests that HRV-mediated asthma crises are elicited by polarization of the adaptive immune response toward Th2 responses (8). A variety of respiratory viruses may recruit allergen-specific (not virus-specific) Th2 cells to the lungs of an atopic host, even with viruses (e.g., HRV) that do not routinely elicit virus-specific Th2 responses in healthy children (18). The third theory proposes that loss of regulatory cytokine expression (5) leads to asthma attacks. Finally, recent reports attributed asthma crises to altered modulation of type III IFN-λ after HRV infection in patients with asthma (14). A role for type III IFN-λ_1 in asthma exacerbations has been postulated, but the directionality of this modulation is not clear (14).

This study investigated the specific role of HRV in the inception of asthma attacks. Then, the role of HRV load and HRV species in eliciting wheezing in children with asthma was explored. Finally, the role of immune mediators, with a special emphasis on type III IFN-λ_1, in asthma crises and disease severity was analyzed.

METHODS

Study Population

Children between 5 and 18 years of age with a medical diagnosis of asthma presenting with URI symptoms in the presence or absence of wheezing and visiting the Asthma Clinics of Hospital Mi Pueblo, Florencia Varela; Hospital Evita Pueblo, Berazategui; Hospital Iriarte, Quilmes; and Hospital V. Lopez y Planes, General Rodriguez in Buenos Aires, Argentina, were invited to participate. The four Asthma Clinics serve a population of low socioeconomic status in the outskirts of Buenos Aires (*see* Table E1 in the online supplement for socioeconomic data). Children with asthma in these neighborhoods attend the Clinics routinely, receiving free specialized medical care and are therefore unlikely to seek medical attention elsewhere. Enrollment was conducted by pediatric pulmonologists staffing the Asthma Clinics. Children with asthma with a URI and an acute asthmatic exacerbation were considered cases, whereas children with a preexisting diagnosis of asthma and with signs and symptoms of URI but no wheezing were considered control subjects. Patients with oropharyngeal malformations, neuromuscular disorders, immune suppression, or chronic cardiopulmonary diseases other than asthma were excluded from participation. Children were enrolled between March 1st (fall) and November 1st (spring) in 2007 and 2008. Children were not enrolled during the summer, because of the low incidence of asthma exacerbations. The Institutional Review Boards of the participating hospitals approved the study. Written informed consent was obtained from parents or guardians; assent was obtained from invited children.

Demographic and Clinical Data

Demographic and clinical data were ascertained from parents by questionnaire and chart review and included such variables as age, sex, breastfeeding history, number of siblings, prior asthma hospitalizations, prior intensive care unit admissions for asthma, home tobacco smoke exposure, history of allergic diseases and asthma, use of corticosteroids, birth history, allergen exposure, and other treatments at home.

Score for baseline asthma status was determined by frequency of symptoms (days per week, nights per month) and FEV_1 (*see* Table E2) (19).

URI and asthma exacerbation severity was defined as none (URI with no wheezing), mild, moderate, or severe using a modified functionalized Global Initiative for Asthma (GINA) asthma exacerbation severity scheme (*see* Table E3) (20).

Viruses and Cytokines

Nasal washes were obtained by gently flushing the children's nostrils with 4 ml of sterile saline solution (AnalytiCals, Buenos Aires, Argentina). Secretions were aliquoted, immediately snap frozen using dry ice, and stored at −80°C. Aliquots of frozen samples were analyzed for viral RNA in our laboratories at Johns Hopkins University and Vanderbilt University and for cytokines (mRNA and protein) in our laboratories at Fundacion INFANT in Buenos Aires. Respiratory specimens from children were tested by MultiCode-Plx Assay (EraGen, Madison, WI) for HRV, respiratory syncytial virus, parainfluenza virus, influenza virus, human metanepneumovirus, adenovirus, and human coronaviruses. RNA was extracted from 200 μl of pooled nasal wash samples on a Roche (Indianapolis, IN) MagNApure LC automated nucleic acid extraction instrument and quantitative real-time reverse transcriptase polymerase chain reaction (RT-PCR) for HRV was performed with the Smart Cycler II (Cepheid, Sunnyvale, CA) using primers and probe sequences (CY+ AGCC+TGCGTGGC, GAAACACGGACACCCAAAGTA, TCCTC CGGCCCCTGAATGYGGC) directed at a highly conserved HRV 5′-noncoding region (21). HRV viral load was quantified using an HRV RNA runoff-transcript standard curve. Conventional RT-PCR was then performed on HRV-positive samples by using primers that amplified a fragment of approximately 548 nt, encompassing the VP4/VP2 region, and the hypervariable region in the 5′-NCR (22, 23). Amplified fragments were sequenced bidirectionally with the ABI PRISM BigDye Terminator Kit (Applied Biosystems, Carlsbad, CA) on a 3730xl DNA Analyzer (Applied Biosystems). Sequences were edited and aligned with MacVector version 11.1 (MacVector, Cary, NC). Seven published strains from GenBank and 152 field strains from this study were included. Phylogenetic analysis was performed using Molecular Evolutionary Genetics Analysis (MEGA) (Tempe, AZ) version 4 with three bootstrapped replicates and the neighbor-joining algorithm with HRV87 as outgroup (24). Cytokines were measured with a cytometric bead array (Becton Dickinson, Franklin Lakes, NJ). IFN-α, IFN-λ_1, and IFN-$\lambda_{2/3}$ were measured by ELISA and real-time RT-PCR. Specific blood IgE to common foods and aeroallergens (Table E4) was tested in 89 subjects by Immunetech (Foster City, CA) using MyAllergyTest System.

Statistical Analysis

Continuous and categorical variables were compared using Wilcoxon rank-sum, chi-square, and Fisher exact tests as appropriate. Risk factors for wheezing were assessed using logistic regression with covariates selected *a priori*. Multivariate models with and without type III IFN-λ_1 were compared using a log likelihood ratio test. A Bonferroni correction was used to account for multiple cytokine comparisons. Analyses were performed using R statistical software (www.r-project.org). Complete details are available at http://biostat.mc.vanderbilt.edu /ArchivedAnalyses.

RESULTS

Study Population

Four hundred and nine 5- to 18-year-old children with asthma with URI symptoms (200 presenting with wheezing and 209 not wheezing) were prospectively enrolled during the fall, winter, and spring of 2007 and 2008. Fourteen (3%) families declined to participate; these children had similar characteristics as those enrolled in the study (not shown).

Wheezing children had more frequent prior hospitalizations because of asthma and admissions to intensive care unit (Table 1). Conversely, greater baseline severity of illness (National Asthma Education and Prevention Program [NAEPP] II score does not consider prior hospitalizations [19]) and more frequent use of inhaled steroids were observed in nonwheezing control subjects (Table 1).

Rhinoviruses and Asthma Exacerbations

Respiratory viruses were detected in respiratory secretions of 333 (82%) children (Table 1). HRV were the agents detected most frequently, in 106 (56%) wheezing children and 74 (37%) nonwheezing patients (Figure 1A) ($P < 0.001$). Those who were

HRV-positive were more likely to wheeze than those who were HRV-negative (odds ratio [OR], 2.17; 95% confidence interval [CI], 1.44–3.25; $P < 0.001$). The relationship between HRV and wheezing persisted after adjustment for age, sex, baseline asthma severity, familial history of asthma, and prior hospitalizations (OR, 1.90; 95% CI, 1.21–2.99; $P = 0.005$ (Table 2)]. A similar association was not observed for any other assayed viral pathogen. RNA from other respiratory viruses was detected infrequently and at similar rates between cases and control subjects (19% vs. 14%, respectively; $P = 0.24$) (Table 1).

Moderate-to-severe baseline asthma was associated with less frequent acute wheezing. Conversely, more than one hospitalization because of asthma increased the risk of wheezing (Table 2).

Viral Load and Asthma Exacerbations

We first compared HRV titers in respiratory secretions obtained from cases (HRV URI and wheezing) and control subjects (HRV URI and not wheezing). Comparison revealed no differences. Median viral load for cases was 42,700 (interquartile range, 2,826–942,300) and for control subjects was 52,320 (interquartile range, 6 158–1,967,000) ($P = 0.68$) (Figure 1B).

Subsequently, we investigated whether HRV species associated with disease exacerbations (Figures 1C and 1D). HRVC has recently been recognized to cause severe respiratory disease in children (25–27) and has been postulated to be associated with asthma exacerbations (10, 12, 15, 25). Of 180 HRV-positive samples, 60 (33%) were HRVA, 18 (10%) HRVB, 47 (26%) HRVC, and 55 (31%) could not be sequenced. Thirty-two (68%) children with HRVC wheezed, compared with 8 (44%) with HRVB, 30 (50%) with HRVA, 36 (66%) with untypable HRV, and 74 (56%) all non-C HRV combined ($P = 0.19$, for C vs. all non-C combined) (Figure 1D). HRVC strains were more diverse than those among species A and B (Figure 1C). The findings cannot confirm a role for HRV titers or species in asthma exacerbations.

Inflammation and HRV-mediated Asthma Exacerbation

IL-6 is an inflammatory cytokine associated with production of acute-phase reactants (28), and IL-8 a potent proinflammatory cytokine with a key role in neutrophil recruitment and activation during inflammation (17, 28). Previous studies showed an association between IL-6 and IL-8 levels and production of other inflammatory molecules (17). Interestingly, no significant differences in IL-6 and IL-8 levels in respiratory secretions of cases and control subjects were observed ($P = 0.59$ and $P = 0.11$, respectively). Moreover, inflammatory cytokine

TABLE 1. COMPARISON OF DEMOGRAPHIC AND CLINICAL CHARACTERISTICS BY WHEEZING AND RHINOVIRUS STATUS IN THE STUDY POPULATION

	All Children			All Children			HRV Positive		
	Wheezing (*n = 200*)	No Wheezing (*n = 209*)	*P* Value	HRV Positive (*n = 180*)	HRV Negative (*n = 211*)	*P* Value	Wheezing (*n = 106*)	No Wheezing (*n = 74*)	*P* Value
Age	8.6 (6.8, 11.2)	8.8 (6.7, 11.2)	0.938	8.6 (6.9, 11.1)	8.8 (6.7, 11.6)	0.589	8.4 (6.6, 11)	8.9 (7.1, 11.2)	0.297
Female (%)	91 (46)	83 (38)	0.279	80 (44.4)	87 (41.2)	0.591	48 (45.3)	32 (43.2)	0.906
Baseline severity (%)									
Mild intermittent	70 (35)	29 (14)	<0.001	60 (33.3)	36 (17.1)	0.003	41 (38.7)	19 (25.7)	<0.001
Mild persistent	79 (40)	53 (25)		51 (28.3)	74 (35.1)		38 (35.8)	13 (17.6)	
Moderate	42 (21)	125 (60)		65 (36.1)	95 (45)		23 (21.7)	42 (56.8)	
Severe	9 (4)	2 (1)		4 (2.2)	6 (2.8)		4 (3.8)	0 (0)	
Prior asthma hospitalization (%)									
0	61 (31)	95 (46)	0.004	68 (37.8)	87 (41.4)	0.346	36 (34)	32 (43.2)	0.449
1	26 (13)	27 (13)		19 (10.6)	29 (13.8)		12 (11.3)	7 (9.5)	
⩾2	113 (57)	86 (41)		93 (51.7)	94 (44.8)		58 (54.7)	35 (47.3)	
Prior asthma ICU (%)									
0	190 (95)	198 (96)	0.058	171 (95.5)	200 (95.7)	0.971	101 (95.3)	70 (95.9)	0.096
1	8 (4)	2 (1)		4 (2.2)	5 (2.4)		4 (3.8)	0 (0)	
⩾2	2 (1)	6 (3)		4 (2.2)	4 (1.9)		1 (0.9)	3 (4.1)	
Asthma in family (%)									
Mother	35 (18)	34 (16)	0.841	33 (18.3)	34 (16.1)	0.656	22 (20.8)	11 (14.9)	0.418
Father	24 (12)	21 (10)	0.636	11 (6.1)	27 (12.8)	0.04	7 (6.6)	4 (5.4)	0.989
Siblings	39 (20)	57 (27)	0.082	33 (18.3)	56 (26.5)	0.071	17 (16)	16 (21.6)	0.449
Allergies in family (%)									
Mother	20 (10)	35 (17)	0.064	17 (9.4)	36 (17.1)	0.041	9 (8.5)	8 (10.8)	0.791
Father	7 (4)	20 (10)	0.023	4 (2.2)	23 (10.9)	0.002	2 (1.9)	2 (2.7)	0.882
Siblings	15 (8)	25 (12)	0.176	14 (7.8)	25 (11.8)	0.242	7 (6.6)	7 (9.5)	0.674
Inhaled steroid use (%)	80 (40)	128 (61)	<0.001	94 (52.2)	107 (50.7)	0.844	46 (43.4)	48 (64.9)	0.007
HRV positive (%)	106 (56)	74 (37)	<0.001	180 (100)	—	<0.001	—	—	—
Positive for other viruses (%)									
Missing	34 (17)	42 (20.1)	—	18 (10)	40 (19)	—	12 (11.3)	6 (8.1)	—
Coinfected	16 (8)	13 (6.2)	0.685	26 (14.4)	3 (1.4)	<0.001	16 (15.1)	10 (13.5)	0.858
Any other virus	32 (19.3)	24 (14.4)	0.244	26 (16)	30 (17.5)	0.77	16 (17)	10 (14.7)	0.829
RSV	12 (7.2)	9 (5.4)	0.509	11 (6.8)	10 (5.8)	0.823	7 (7.4)	4 (5.9)	0.762
Influenza	8 (4.8)	2 (1.2)	0.061	3 (1.9)	7 (4.1)	0.338	3 (3.2)	0 (0)	0.265
PIV	6 (3.6)	1 (0.6)	0.067	4 (2.5)	3 (1.8)	0.717	4 (4.3)	0 (0)	0.14
Adenovirus	5 (3)	13 (7.8)	0.087	9 (5.6)	9 (5.3)	1	3 (3.2)	6 (8.8)	0.167
HMPV	0 (0)	0 (0)	—	0 (0)	0 (0)	—	—	—	—
Coronaviruses	2 (1.2)	4 (2.4)	0.685	1 (0.6)	5 (2.9)	0.216	0 (0)	1 (1.5)	0.42

Definition of abbreviations: HMPV = human metanepneumovirus; HRV = human rhinoviruses; ICU = intensive care unit; PIV = parainfluenza virus; RSV = respiratory syncytial virus.

No differences were observed when comparing breastfeeding, number of siblings, and smoking among groups.

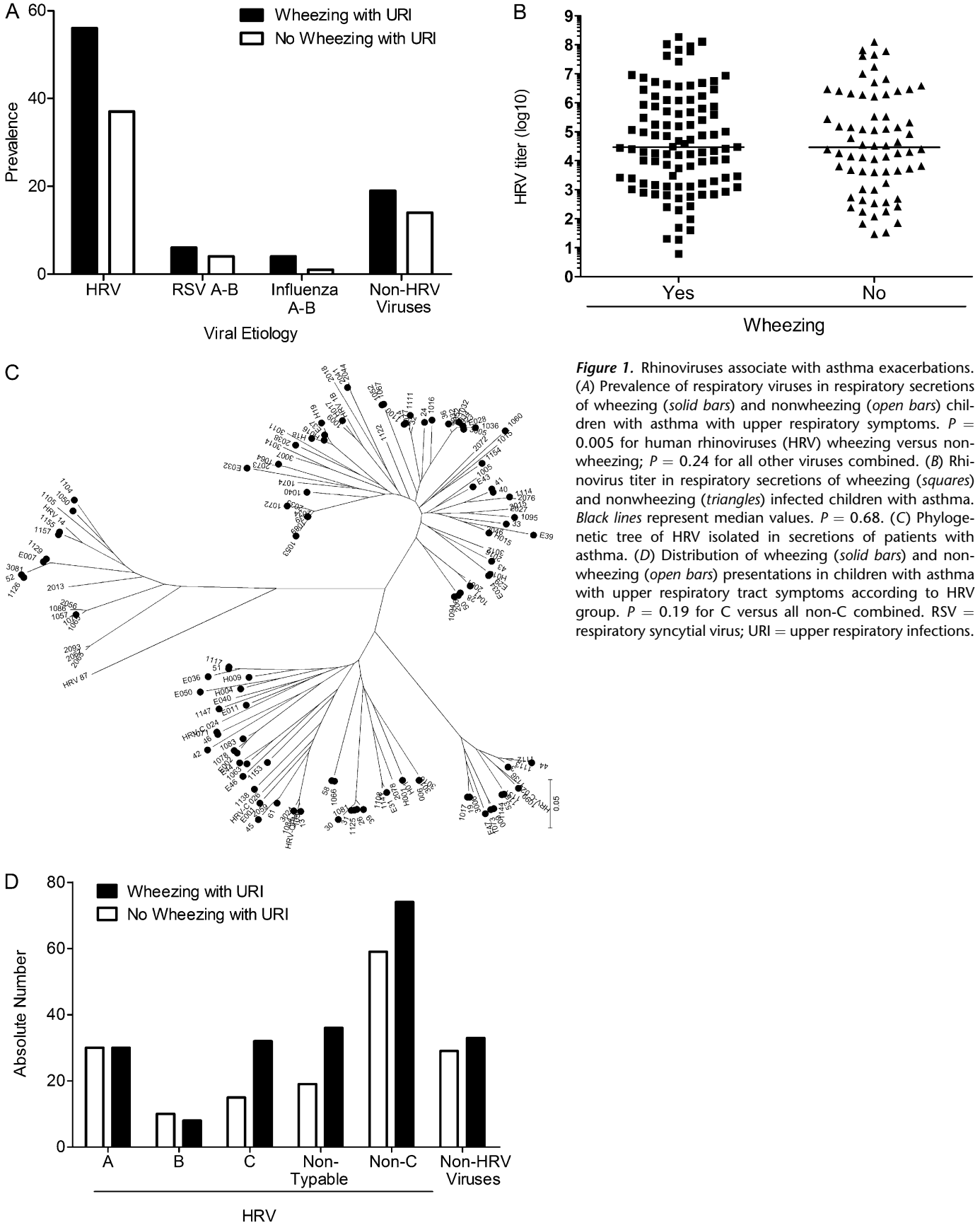

Figure 1. Rhinoviruses associate with asthma exacerbations. (*A*) Prevalence of respiratory viruses in respiratory secretions of wheezing (*solid bars*) and nonwheezing (*open bars*) children with asthma with upper respiratory symptoms. $P = 0.005$ for human rhinoviruses (HRV) wheezing versus nonwheezing; $P = 0.24$ for all other viruses combined. (*B*) Rhinovirus titer in respiratory secretions of wheezing (*squares*) and nonwheezing (*triangles*) infected children with asthma. *Black lines* represent median values. $P = 0.68$. (*C*) Phylogenetic tree of HRV isolated in secretions of patients with asthma. (*D*) Distribution of wheezing (*solid bars*) and nonwheezing (*open bars*) presentations in children with asthma with upper respiratory tract symptoms according to HRV group. $P = 0.19$ for C versus all non-C combined. RSV = respiratory syncytial virus; URI = upper respiratory infections.

TABLE 2. FEATURES ASSOCIATED WITH WHEEZING, MULTIVARIATE ANALYSES

	Odds Ratio	95% Confidence Intervals	*P* Value
HRV positive	1.90	1.21–2.99	0.005
Age, yr	1.05	0.97–1.13	0.243
Female	1.28	0.82–2	0.284
Baseline severity			
Mild intermittent	1		
Mild persistent	0.76	0.42–1.39	0.376
Moderate-to-severe	0.16	0.09–0.29	<0.001
Family history of asthma	0.81	0.46–1.42	0.457
Maternal asthma	1.07	0.52–2.2	0.86
Prior asthma hospitalizations			
0	1		
1	1.56	0.76–3.23	0.228
⩾2	2.6	1.59–4.25	<0.001

production did not differ in HRV-infected and -uninfected patients ($P = 0.19$ for IL-6 and $P = 0.90$ for IL-8, respectively) (Figures 2A and 2B).

We then speculated that differential levels of the regulatory cytokine IL-10 could affect the inflammatory profile of HRV-mediated wheezing in the respiratory tract (5). However, IL-10 levels in respiratory secretions were similar between wheezing and nonwheezing children with HRV URI ($P = 0.48$) (Figure 2C), and between HRV-infected and -uninfected subjects ($P = 0.57$) (Figure 2C). All these observations fail to support an association between innate inflammation and HRV-associated wheezing in our patients.

Th2 Bias and HRV-mediated Asthma Exacerbation

Several studies ascribe asthma exacerbations to Th2 cytokines, IL-13 in particular (29, 30). Comparison of IL-13 levels between cases and control subjects revealed no differences ($P = 0.35$) (Figure 2D). No differences were observed when comparing IL-13 levels in HRV-positive and HRV-negative patients with asthma ($P = 0.69$) (Figure 2D).

Another mechanism of Th2 polarization is the suppression of the Th1 response, which prior studies associated with the inception of reactive airways disease in children (31, 32). Levels of IFN-γ in the respiratory tract of children infected with HRV did not differ between cases and control subjects ($P = 0.36$) (Figure 2E) nor between HRV-infected and -uninfected children ($P = 0.93$) (Figure 2E).

Finally, we investigated the role of atopy in predisposing for HRV-mediated wheezing. For this purpose, we analyzed specific IgE levels against 10 common food allergens and aeroallergens (Table E4) in a subset of 89 children, 49 with HRV and 40 without HRV (Figure 2F). Specific IgE levels were similar in wheezing and nonwheezing children infected with HRV (P = NS) regardless of HRV infection. All these findings fail to support an association between Th2 bias and HRV-mediated wheezing in our population.

Type III IFN-λ_1 as a Determinant of Disease Severity

Recently, a study ascribed a role in asthma exacerbations to down-modulation of type III IFN-λ (14). Comparison of volunteers with and without asthma experimentally infected with an attenuated HRV showed lower type III IFN-λ_1 and type III IFN-λ_2 levels in respiratory secretions of patients with asthma (14). Conversely, other studies have shown elevated IFN-λ_1 in people with asthma compared with people without asthmas (33, 34).

In our study, baseline production of type III IFN-λ_1 in children with asthma without respiratory symptoms was lower than in healthy control subjects (Figure E1A). Moreover, in line with previous observations in adults (14), *in vitro* analysis of type III

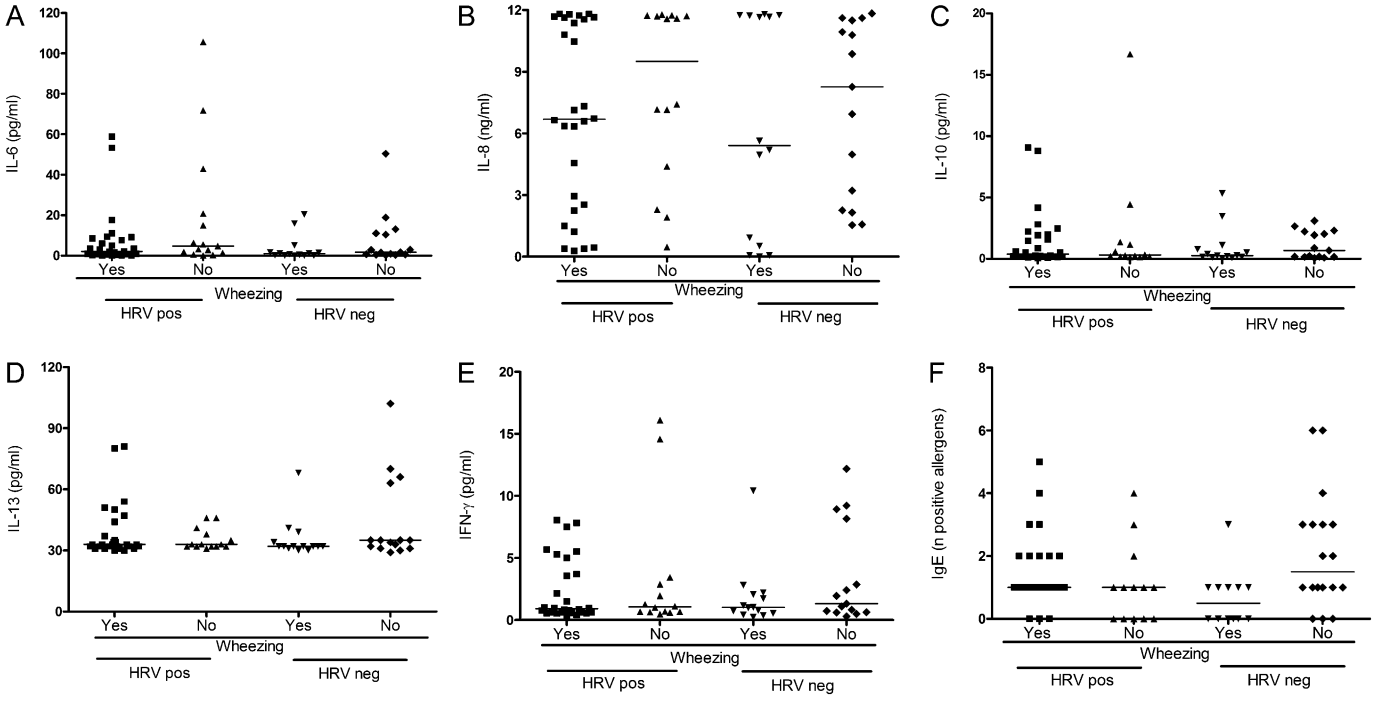

Figure 2. Cytokine responses in the respiratory tract of children with asthma. Cytokine responses in respiratory secretions of human rhinoviruses (HRV)–infected and non–HRV-infected children with asthma with upper respiratory infections symptoms and with or without wheezing (n = 80). Cytokine determinations include (*A*) IL-6, (*B*) IL-8, (*C*) IL-10, (*D*) IL-13, and (*E*) IFN-γ. Comparisons of all cytokines between wheezing and nonwheezing children infected with HRV, P = NS. (*F*) Number of allergens with positive serum-specific IgE titers in children with asthma (n = 89) with upper respiratory infections plus wheezing (yes/no) and HRV infection (yes/no), P = NS for all comparisons. *Black lines* represent median values.

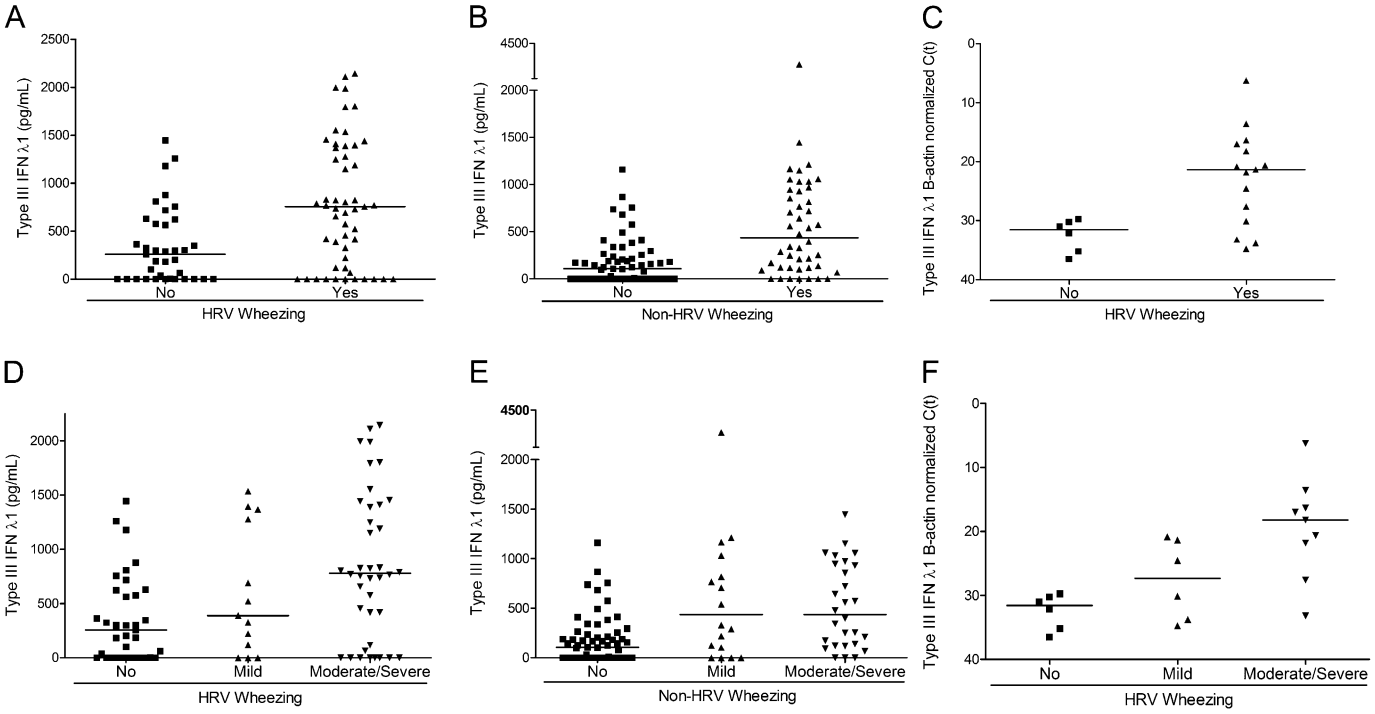

Figure 3. Type III IFN-λ_1 as a determinant of severity in asthma crises caused by human rhinoviruses (HRV). (*A*) Type III IFN-λ_1 production in respiratory secretions of HRV-infected nonwheezing (*squares*) versus wheezing (*triangles*) children with asthma with upper respiratory infection (URI) symptoms, $P < 0.001$. *Black lines* represent median values. (*B*) Type III IFN-λ_1 production in respiratory secretions of non–HRV-infected nonwheezing (*squares*) versus wheezing (*triangles*) children with asthma with URI symptoms, $P < 0.001$. *Black lines* represent median values. (*C*) Type III IFN-λ_1 mRNA levels in respiratory secretions of HRV-infected nonwheezing (*squares*) versus wheezing (*triangles*) children with asthma with URI symptoms, $P < 0.011$. *Black lines* represent median values. (*D*) Type III IFN-λ_1 production in respiratory secretions of HRV-infected children with asthma with nonwheezing (*squares*), mild wheezing (*up-pointing triangles*), and moderate-to-severe wheezing (*down-pointing triangles*), $P < 0.001$. *Black lines* represent median values. (*E*) Type III IFN-λ_1 production in respiratory secretions of non–HRV-infected children with asthma with nonwheezing (*squares*), mild wheezing (*up-pointing triangles*), and moderate-to-severe wheezing (*down-pointing triangles*), $P = 0.36$. *Black lines* represent median values. (*F*) Type III IFN-λ_1 mRNA levels in respiratory secretions of HRV-infected children with asthma with nonwheezing (*squares*), mild wheezing (*up-pointing triangles*), and moderate-to-severe wheezing (*down-pointing triangles*), $P = 0.008$. *Black lines* represent median values.

IFN-λ_1 production in primary nasal epithelial cells of children with asthma post-HRV infection was suppressed compared with cytokine production in nasal epithelial cells of healthy children (Figure E1B).

To explore whether type III IFN-λ_1 levels intervene in a pathway triggering asthma exacerbations associated with HRV, we compared responses in wheezing versus nonwheezing children presenting HRV URI in a sample of 197 patients participating in our study balancing cases and control subjects (Figure 3A) (*see* Table E5 for a comparison of subgroup with total population). Interestingly, wheezing children infected with HRV had higher levels of type III IFN-λ_1 than nonwheezing children infected with HRV with asthma ($P < 0.001$; because seven cytokines were tested, to control the family-wise type I error rate at 5%, "statistically significant" implies $P < 0.007$) (Figure 3A).

Children with asthma with URI not caused by HRV also showed a difference in type III IFN-λ_1 levels between those who wheezed and those who did not wheeze ($P < 0.001$) (Figure 3B). Among all wheezing children, those with HRV had higher levels of type III IFN-λ_1 than those without HRV ($P < 0.01$). Analysis of type III IFN-λ_1 mRNA levels confirmed our observations ($P = 0.011$) (Figure 3C).

Interestingly, higher type III IFN-λ_1 levels associated with worsening illness. Patients with HRV URI and no wheezing had lower median type III IFN-λ_1 levels than those with mild HRV wheezing, and these had lower levels than those with moderate and severe HRV disease ($P < 0.001$) (Figure 3D). These observations were confirmed by assaying mRNA levels of the antiviral cytokine by real-time RT-PCR ($P = 0.008$) (Figure 3F). Children with wheezing resulting from triggers other than HRV also mirrored this trend ($P < 0.001$) (Figure 3E).

Given that type III IFN-λ_1 levels correlated with wheezing and severity of acute exacerbation during HRV infection, we examined whether type III IFN-λ_1 is the mechanism by which HRV elevates the risk of wheezing. Mediation was assessed by seeing if the inclusion of type III IFN-λ_1 weakened the association of the virus with asthma crises (35). Among the subset of 197 children with IFN-λ_1 measurements, HRV was associated with asthma exacerbations (OR, 1.90; 95% CI, 1.08–1.35; $P = 0.027$). After adjusting for IFN-λ_1, those who were HRV-positive were no longer more likely to wheeze than those who were HRV-negative (OR, 1.31; 95% CI, 0.70–2.47; $P = 0.399$). IFN-λ_1 remained a strong predictor of wheezing, independent of HRV status (OR, 1.22 per 100-unit increase; 95% CI, 1.13–1.32; $P < 0.001$). When the effect of IFN-λ_1 was analyzed including age, sex, baseline severity, family history of asthma, and prior asthma hospitalizations, our observations were confirmed: IFN-λ_1 remained a strong predictor of wheezing (OR, 1.2 per 100-unit increase in IFN-λ_1; 95% CI, 1.11–1.31; $P < 0.001$) and the association between HRV and wheezing was significantly weakened by the cytokine (OR, 1.18; 95% CI, 0.57–2.46; $P = 0.66$). Note that in this subset of 197 patients, prior hospitalization and baseline severity of asthma also weakened the association between HRV and wheezing ($P = 0.14$ in absence of IFN-λ_1).

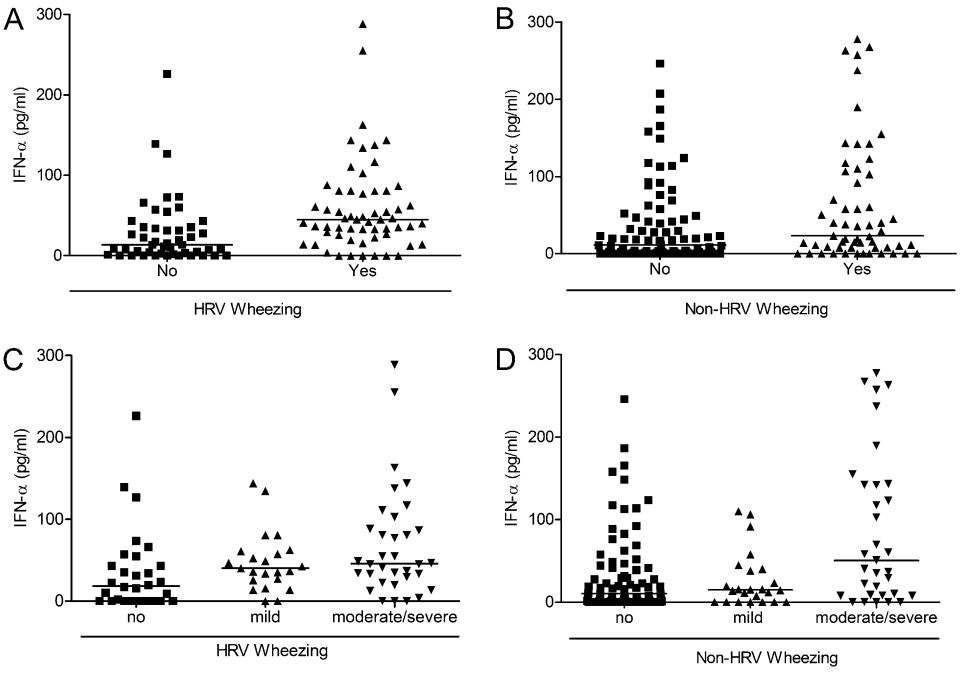

Figure 4. Type I IFN-α in human rhinoviruses (HRV)–positive or -negative wheezing severity. (*A*) Type I IFN-α production in respiratory secretions of HRV-infected nonwheezing (*squares*) versus wheezing (*triangles*) children with asthma with upper respiratory infection symptoms, $P < 0.001$. *Black lines* represent median values. (*B*) Type I IFN-α production in respiratory secretions of non–HRV-infected nonwheezing (*squares*) versus wheezing (*triangles*) children with asthma with upper respiratory infection symptoms, $P = 0.02$. *Black lines* represent median values. (*C*) Type I IFN-α production in respiratory secretions of HRV-infected children with asthma with nonwheezing (*squares*), mild wheezing (*up-pointing triangles*), and moderate-to-severe wheezing (*down-pointing triangles*), $P < 0.05$. *Black lines* represent median values. (*D*) Type I IFN-α production in respiratory secretions of non–HRV-infected children with asthma with nonwheezing (*squares*), mild wheezing (*up-pointing triangles*), and moderate-to-severe wheezing (*down-pointing triangles*), $P < 0.05$. *Black lines* represent median values.

Other IFNs and Asthma Exacerbations

IFN-$\lambda_{2/3}$ has an amino acid sequence similar to IFN-λ_1. Levels of type III IFN-$\lambda_{2/3}$ were similar in wheezing and nonwheezing children infected with HRV (*see* Figures E2 and E3).

Given that type III IFN-λ_1 levels correlated with wheezing and severity of acute exacerbation during HRV infection, and previous studies suggest that type I IFN and IFN-sensitive genes play an essential role in asthma exacerbations (36, 37), we examined whether type I IFN-α was also associated with asthma crises. Interestingly, type I IFN-α levels were higher in respiratory secretions of wheezing compared with nonwheezing people with asthma with URI ($P < 0.001$) (Figure 4) but did not explain mediation of exacerbations by type III IFN-λ_1 (type III IFN-λ_1 effect in the model did not change when adjusted for type I IFN-α; $P < 0.001$). The mediating role of type I IFN-α in HRV-associated crises was small ($P = 0.027$ for the relationship between HRV and exacerbations weakened to $P = 0.08$ when adjusted by type I IFN-α).

DISCUSSION

This study shows that HRV infections specifically associate with pediatric asthma exacerbations, and that type III IFN-λ_1 levels increase with worsening acute asthma symptoms during HRV infections. More importantly, our findings suggest that type III IFN-λ_1 responses contribute to HRV asthma attacks, and offer a potential explanation for divergent results in earlier manuscripts (14, 30): although type III IFN-λ_1 levels were deficient at baseline compared with healthy control subjects (14), they were hyperexpressed during asthma exacerbations in our population.

Type III IFN-λ_1 may play different roles in the pathogenesis of asthma. Low baseline type III IFN-λ_1 levels in people with asthma (14) may enhance susceptibility to HRV-infection and consequent virus-mediated asthma attacks in children. Potential markers of these low levels of defensive cytokines in the respiratory tract of people with asthma include early nasopharyngeal colonization with bacteria (38), and evidence for impaired viral clearance in these patients (13). In addition, IFN-λ_1 promotes production of inflammatory chemokines and cytokines (39), and modulates proliferation of regulatory T cells and Th1/Th2 bias (40, 41). However, no evidence was found to support a role for virus titer or these innate and adaptive responses in HRV-mediated crises in our study. Importantly, IFN-λ is produced by alveolar type II cells (42), and affects receptors in epithelial cells (43). Pathways in lung epithelial cells can trigger exacerbations (44). In line with this pathogenic role for the cytokine, a study of patients with asthma described higher serum IFN-λ_1 levels in dust mite–sensitized individuals and allergic people with asthma during peak pollen season compared with healthy control subjects or allergic people with asthma out of season (34). However, the mechanism by which IFN-λ_1 production after HRV infection leads to wheezing is unclear and needs further investigation. It may be of interest to study whether low baseline levels of IFN-λ_1 lead to higher expression levels of its receptor in respiratory epithelium and, consequently, a heightened, more exuberant cytokine effect during HRV infection. Interestingly, activation in IFN signaling pathways was significantly elevated during acute exacerbations compared with convalescence in a recent pediatric study, and subsequent baseline lung function in children with asthma varied as a function of the degree of activation of these IFN-dependent pathways (45).

Certain limitations are inherent to a study of these characteristics and stress that results should be interpreted with caution. First, in studying human populations we recognize the possibility that other environmental factors or unmeasured confounders may affect the results (35). Second, even though we examined highly representative inflammatory cytokines (28), other unmeasured inflammatory molecules could influence disease severity. Third, untypable HRV strains may obscure a species (perhaps C viruses, for which less sequence information is available) or group of viruses associated with wheezing. Fourth, our research question (determinants of wheezing in people with asthma with URI) did not include ascertainment of viral

shedding in completely asymptomatic children with asthma (no URI symptoms) and because shedding in asymptomatic patients can be prevalent could overestimate the role of HRV in URI. Moreover, sampling a single time point for viral replication and the potential variability of sample dilution during lavage suggest that the lack of association between wheezing and HRV replication should be interpreted with caution. In addition, studying children during wild-type infections requires extrapolation of immune manifestations in the lungs through the analysis of upper respiratory tract secretions. The direct correlation of cytokine responses and virus titer in the upper and lower respiratory tract during viral infections has been described in other publications (46, 47).

However, this study has important strengths. First, it presents hundreds of children with asthma with URI symptoms, with and without HRV-infection or asthma exacerbations, for analysis of virus-specific triggers of acute disease. Second, its design addresses whether HRV has a specific association with asthma crises by including nonwheezing children with asthma with URI symptoms as control subjects. Third, it evaluates several leading hypotheses on immune mechanisms of asthma crises. Finally, it contextualizes the interaction between HRV and type III IFN-λ_1 during asthma attacks and suggests a mechanistic role for type III IFN-λ_1. Moreover, it emphasizes the potential importance of IFN, in this case λ and α, in the disease.

More than 150 HRV serotypes have been recognized (48, 49), stressing the challenges for the design of HRV-specific vaccines. Modulation of IFN-λ_1 should be studied as a preventive or therapeutic target against HRV-associated asthma crises.

Author disclosures are available with the text of this article at www.atsjournals.org.

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