High titers of IgE antibody to dust mite allergen and risk for wheezing among asthmatic children infected with rhinovirus

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Background: The relevance of allergic sensitization, as judged by titers of serum IgE antibodies, to the risk of an asthma exacerbation caused by rhinovirus is unclear.
Objective: We sought to examine the prevalence of rhinovirus infections in relation to the atopic status of children treated for wheezing in Costa Rica, a country with an increased asthma burden.
Methods: The children enrolled (n = 287) were 7 through 12 years old. They included 96 with acute wheezing, 65 with stable asthma, and 126 nonasthmatic control subjects. PCR methods, including gene sequencing to identify rhinovirus strains, were used to identify viral pathogens in nasal washes. Results were examined in relation to wheezing, IgE, allergen-specific IgE antibody, and fraction of exhaled nitric oxide levels.
Results: Sixty-four percent of wheezing children compared with 13% of children with stable asthma and 13% of nonasthmatic control subjects had positive test results for rhinovirus ($P < .001$ for both comparisons). Among wheezing subjects, 75% of the rhinoviruses detected were group C strains. High titers of IgE antibodies to dust mite allergen (especially *Dermatophagoides* species) were common and correlated significantly with total IgE and fraction of exhaled nitric oxide levels. The greatest risk for wheezing was observed among children with titers of IgE antibodies to dust mite of 17.5 IU/mL or greater who tested positive for rhinovirus (odds ratio for wheezing, 31.5; 95% CI, 8.3-108; $P < .001$).
Conclusions: High titers of IgE antibody to dust mite allergen were common and significantly increased the risk for acute wheezing provoked by rhinovirus among asthmatic children.
(J Allergy Clin Immunol 2012;129:1499-505.)

Key words: *Acute asthma, dust mite–specific IgE, emergency department visits, viral respiratory tract infections, rhinovirus strain C, total serum IgE, inhaled allergens, exhaled nitric oxide*

In countries with temperate climates (eg, North America, Europe, and Australia), viral respiratory tract infections are associated with 80% to 90% of wheezing attacks in the pediatric population, especially during early childhood.[1-4] After 3 years of age, rhinovirus accounts for 75% to 80% of the virus-induced attacks leading to hospitalizations and emergency department (ED) visits, and the majority of these children are atopic.[1,5] Evidence to date also indicates that a rhinovirus infection together with sensitization and exposure to inhaled allergens increases the risk for acute symptoms, suggesting that these risk factors might act synergistically to provoke asthma exacerbations.[1,5,6] Whether these observations can be generalized to children living in countries with tropical climates is not clear. In studies from Brazil and Trinidad, the prevalence of infections with rhinovirus among children treated for asthma was less than half of what has been reported in countries with temperate climates.[7,8] Recognizing that additional studies are needed from tropical environments to gain a better understanding of the role of viral infections in the cause of asthma exacerbations worldwide, the purpose of this investigation was to examine the prevalence of viral respiratory tract infections among children treated for acute wheezing in Costa Rica and to evaluate the results in relation to their atopic status.

Costa Rica is a small country (19,730 square miles) in Central America with a diverse ecosystem. Similar to other countries near the equator, Costa Rica has 2 seasons: a "dry" season (generally December through April) and a "rainy" season (May through November). Exposure to Helminthes, which, like environmental allergens, can stimulate the production of IgE, is common in economically deprived regions of Hispanic America. However, anti-helminth medications are given annually to children in Costa Rica starting at 1 year of age. Thus active parasitic and enteric infections during childhood are not frequent in that country (eg, ≤2% for infections with *Ascaris lumbricoides*).[9,10]

According to school surveys and studies using the International Study of Asthma and Allergies in Childhood questionnaire, the

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Supported by the Cove Point Foundation, National Institutes of Health grants R01 AI020565 and U19 AI070364, and the University of Virginia Children's Hospital Research Fund.

Disclosure of potential conflict of interest: T. A. E. Platts-Mills has consultant arrangements with IBT/Viracor Labs and has received research support and honorarium from Phadia/Thermo Fisher. J. W. Steinke has received payment as a Board Review Course Speaker for the American Academy of Allergy, Asthma & Immunology; has received research support from the National Institutes of Health and Medtronic; and is the AIR Committee Chair for the American Academy of Allergy, Asthma & Immunology. J. Kennedy has received research support from the National Institutes of Health and the University of Virginia. P. W. Heymann has received research support from the National Institutes of Health, the Cove Point Foundation, and the University of Virginia Children's Hospital. The rest of the authors declare that they have no relevant conflicts of interest.

Received for publication January 11, 2012; revised February 27, 2012; accepted for publication March 27, 2012.

Available online May 8, 2012.

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0091-6749
doi:10.1016/j.jaci.2012.03.040

Abbreviations used
ED: Emergency department
ETS: Environmental tobacco smoke
FENO: Fraction of exhaled nitric oxide
GM: Geometric mean
ICAM-1: Intercellular adhesion molecule 1
RSV: Respiratory syncytial virus

prevalence of asthma among school-aged children in Costa Rica (approximately 23% to 27%) is higher than in North America,[11,12] and sensitization to allergens, especially to allergens produced by house dust mites, is common.[13] At present, however, there is little information about the relevance of viral infections to acute attacks of asthma in this country. Additionally, recent evidence from countries with temperate climates suggest that group C strains of rhinovirus might be strongly related to asthma exacerbations compared with other strains.[14-16] This information is also lacking in studies of asthma in tropical countries. Thus our objective was to investigate the relationship between respiratory tract viruses, including different strains of rhinovirus, and episodes of asthma requiring acute treatment. Additionally, we took advantage of the dominance of dust mite sensitization in Costa Rica to investigate the relationship of titers of IgE antibodies to exacerbations of asthma with or without evidence of a recent viral infection.

METHODS

Study population

This was a cross-sectional case-control investigation of 287 children aged 7 to 12 years enrolled in the ED of the Hospital Nacional de Niños, the main tertiary care hospital for children in San José, Costa Rica, where the majority of children are seen for trauma, wheezing illnesses, vomiting, diarrhea, abdominal pain, and fever. Subjects included 96 children referred to the acute care nebulization room for wheezing by a triage physician. They required at least a nebulized bronchodilator (albuterol) for treatment. Children seen in the ED for nonwheezing disorders (n = 191) were enrolled as control subjects. They included 65 children who had been hospitalized or treated in the ED or who had been using medications prescribed by a physician for asthma during the last 12 months. Data from the latter group, who were defined as having "stable asthma" at the time of enrollment, were compared with data obtained from the actively wheezing children and other nonwheezing control subjects in a *post hoc* analysis. Children with chronic lung disease, congenital heart disease, or immunodeficiency or oncologic disorders were not enrolled.

The subjects included 137 children (44 with wheezing) enrolled in February 2009 during the dry season, when children in Costa Rica begin the school year, and 150 children (51 with wheezing) enrolled in October 2009 during the rainy season, 1 month before the end of the school year. Demographic information and subjects' characteristics were obtained from questionnaires administered to parents. The questionnaires focused on each child's history for asthma treatments, family history for allergic disorders, and environmental tobacco smoke (ETS) exposure at home. Informed consent was obtained from parents, and informed assent was obtained from children who participated. The study was approved by the Ethics Committee at the Hospital Nacional de Niños and by the Institutional Review Board at the University of Virginia.

Virus detection

Nasal washes were obtained for viral analyses, as described in the Methods section in this article's Online Repository at www.jacionline.org. Initially, they were evaluated for rhinovirus by using RT-PCR, as described previously.[17,18] Other respiratory viral pathogens were evaluated by using real-time PCR assays obtained from the Centers for Disease Control and Prevention, according to published procedures.[19,20] These assays included tests for rhinovirus, as well as tests for influenza A (including H1N1) and B; respiratory syncytial virus (RSV); human metapneumovirus; parainfluenza viruses 1, 2, and 3; coronaviruses (229E, OC43, NL63, and HKU1 species); and adenovirus. A high degree of concordance was observed between RT-PCR and real-time PCR methods for detecting rhinovirus (percentage of absolute agreement, 93.3%; 95% CI, 89.8% to 95.9%). Additionally, strains of rhinovirus and enterovirus were identified by means of PCR and sequencing of a region comprising the VP4 and partial VP2 capsid protein genes.[21,22]

Measurements of total serum IgE, allergen-specific IgE antibody, and fraction of exhaled nitric oxide levels

Blood (5 mL) was obtained by means of venipuncture, and serum from each sample was analyzed for the total IgE level by using the Phadia ImmunoCAP assay (Phadia, Uppsala, Sweden). Each sample was also analyzed for allergen-specific IgE antibody to dust mite (*Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, and *Blomia tropicalis*), *Alternaria* species, *Aspergillus* species, cockroach (*Periplaneta americana* and *Blattella germanica*), Bahia grass, cat and dog allergens, and *A lumbricoides*. Sera with 0.35 IU/mL or greater IgE antibody to any of the allergens tested were considered positive for allergen sensitization. Fraction of exhaled nitric oxide (FENO) levels were measured with a portable FENO analyzer (NIOX MINO; Aerocrine, Inc, New Providence, NJ).

Statistical analysis

Questionnaire data and frequencies for positive test results for viral pathogens and allergen sensitization were analyzed by using robust exact binomial and exact multinomial contingency table methods. Binomial contingency table hypotheses were evaluated by using the exact binomial test, whereas multinomial contingency table hypotheses were evaluated by using the Pearson exact goodness-of-fit test. For both the binomial and multinomial contingency table analyses, the 2-sided null hypothesis rejection rule was set at a P value of .05 or less. Multivariate logistic regression was used to determine whether a child's wheezing status was associated with rhinovirus infection and atopic status. Tests of association were based on the type III Wald χ^2 statistic, and a P value of .05 or less was used to identify significant associations. Total serum IgE levels, titers of allergen-specific IgE antibody, and FENO levels were analyzed on a logarithmic scale by using 2-way ANOVA. The 2 sources of variation considered in the ANOVA were the study group and the season of data collection. The rejection rule for hypothesis testing was based on a P value of .05 or less, and 95% CI construction for the ratio of the geometric means (GMs) was based on the Student t test distribution. The statistical software package SAS version 9.2.2 (SAS Institute, Inc, Cary, NC) was used to conduct statistical analyses.

RESULTS

Demographics and subjects' characteristics

Among the control children enrolled who presented to the ED with a diagnosis that did not involve breathlessness, 34% (65/191) had stable asthma, as judged by parental report of treatment regimens. The percentages of the 96 wheezing children and those with stable asthma who had required hospitalization or treatment in the ED or who used medications (bronchodilator, controller, or both) for asthma during the last 12 months were similar (Table I). A minority of children in this study were exposed to ETS at home (23%), more often from the father. Children with stable asthma had less exposure to ETS at home, and they used inhaled and nasal steroids daily more often than children enrolled for wheezing (Table I). More detailed comparisons for children enrolled in