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Early-life mold and tree sensitivity is associated with allergic eosinophilic rhinitis at 4 years of age

Christopher D. Codispoti, MD, PhD [*,†,§]; David I. Bernstein, MD [*]; Linda Levin, PhD [†]; Tiina Reponen, PhD [†]; Patrick H. Ryan, PhD [†,‡]; Jocelyn M. Biagini Myers, PhD [§]; Manuel Villareal, MD [*]; Jeff Burkle, BS [†]; Zana Lummus, PhD [*]; James E. Lockey, MD, MS [*,†]; Gurjit K. Khurana Hershey, MD, PhD [‡]; and Grace K. LeMasters, PhD [†]

[*] Department of Internal Medicine, Divisions of Immunology/Allergy and Pulmonary Medicine, University of Cincinnati, Cincinnati, Ohio
[†] Department of Environmental Health, University of Cincinnati, Cincinnati, Ohio
[‡] Division of Asthma Research, Children's Hospital Medical Center, Rush University Medical Center, Chicago, Illinois
[§] Division of Immunology and Microbiology, Rush University Medical Center, Chicago, Illinois

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ABSTRACT

Background: Nasal eosinophils are a biomarker for allergic rhinitis (AR) and are associated with increased symptom severity.
Objective: To identify predictors of allergic eosinophilic rhinitis (AER) in early childhood in children at higher risk for chronic allergic respiratory disorders.
Methods: In the Cincinnati Childhood Allergy and Air Pollution Study, infants born to aeroallergen-sensitized and symptomatic parents were examined and underwent skin prick testing (SPT) annually to 15 aeroallergens from 1 to 4 years of age. Wheal circumferences were traced and scanned and areas were determined by computer planimetry. At 4 years, AER was defined as (1) at least 1 positive aeroallergen SPT result, (2) presence of sneezing and runny nose without a cold or influenza, and (3) nasal eosinophilia of at least 5%. Wheal areas at 1 to 3 years were analyzed for an association with AER compared with children without AR.
Results: At 4 years, 487 children completed rhinitis health histories, SPT, and nasal sampling. Ninety-nine children (22.8%) had AR. Thirty-eight children had AER (8.8% of total sample and 38.4% of AR sample, respectively). At 3 years, for every 1-mm^2 increase in *Penicillium* species (adjusted odds ratio 1.18, 95% confidence interval 1.06–1.32, $P = .002$) and maple (adjusted odds ratio 1.07, 95% confidence interval 1.01–1.13, $P = .02$), wheal area significantly increased the risk of AER at 4 years of age.
Conclusion: Allergic eosinophilic rhinitis was identified in 8.8% of children at 4 years of age. Age 3 years was the earliest that aeroallergen SPT wheal areas were predictive of AER. Skin testing at 3 years identifies children at risk for an AR phenotype with nasal eosinophilia.

Introduction

Allergic inflammation is associated with tissue eosinophilia, which is a prominent finding in nasal mucosa of patients with allergic rhinitis (AR).[1] Nasal eosinophils correlate with nasal symptom severity in adults with seasonal AR.[2] In addition to reflecting inflammation within the upper airway, nasal eosinophilia is associated with sputum eosinophilia in patients with AR and concomitant asthma.[3] Nasal eosinophils can be objectively measured as a biomarker of allergic airway inflammation.[3] Nasal eosinophils correlated with chronic nasal symptoms in a cross-sectional study of Finnish children and adults, although their atopic status was unknown.[4] Currently, the percentage of young children with AR who have nasal eosinophilia is unknown.

It is unknown whether early skin prick testing (SPT) to aeroallergens can identify children with severe AR using an objective biomarker such as nasal eosinophils. Linking the magnitude of the wheal reaction younger in life to an objective biomarker, such as nasal eosinophilia, could be attractive in future intervention trials by identifying those children most susceptible to the later onset of

Reprints: Christopher D. Codispoti, MD, PhD, Division of Immunology, Microbiology and Allergy, Rush University Medical Center, 1725 W Harrison Ave, Suite 117, Chicago, IL 60612; E-mail: Christopher_D_Codispoti@rush.edu.
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severe AR symptoms. The hypothesis of this study was that specific aeroallergen wheal areas during the first 3 years of childhood would be associated with allergic eosinophilic rhinitis (AER) at 4 years of age. An association between wheal area by SPT at a young age and AER would reinforce a connection between early aeroallergen sensitization and childhood AR and provide important diagnostic information for earlier diagnosis of severe AR.

Methods

Study Population

The Cincinnati Childhood Allergy and Air Pollution Study (CCAAPS) strategy for recruiting infants at high risk for developing allergic disease has been published.[5,6] Birth records were obtained for infants born in greater Cincinnati and northern Kentucky. Parents were required to live nearer than 400 m or farther than 1,500 m from a major road to determine whether early traffic-related air pollution exposures were associated with allergic disease. However, the authors previously found that traffic-related air pollution is associated with wheezing but not with AR.[7,8] Of those parents living within the defined area, at least 1 parent reporting a symptom history of allergies or asthma was required for SPT eligibility. Symptomatic parents were invited to a screening visit and, after obtaining written informed consent that was approved by the University of Cincinnati institutional review board, underwent SPT to 15 aeroallergens. Aeroallergens in the screening SPT panel included eastern red cedar, American elm, maple mix, white oak, meadow fescue, timothy, short ragweed, house dust mite mix (*Dermatophagoides farinae* and *Dermatophagoides pteronyssinus*), German cockroach, cat, dog, and 4 mold allergens (*Alternaria alternata*, *Aspergillus fumigatus*, *Penicillium* species mix, and *Cladosporium* species; ALK-Abelló, Hørsholm, Denmark). These symptomatic parents who also were sensitive to at least 1 aeroallergen were invited to enroll their infant into the CCCAPS cohort.[5]

Clinical Visits

At 1 year of age, parents brought their infants to CCAAPS clinics for clinical evaluation. The CCAAPS clinical staff interviewed the parents using questionnaires to obtain details on the infant's medical history and the home environmental history. Infants were examined and underwent SPT to the same 15 aeroallergens used in the parental screening panel in addition to cow's milk and hen's egg. The children returned to the CCAAPS clinics annually at 2, 3, and 4 years of age for repeat physical examination, SPT, and parental interview. At the year 4 visit, nasal epithelial smears also were obtained.

Quantitative Skin Prick Testing

Skin prick testing was performed using a bifurcated needle coated with histamine dihydrochloride (10 mg/mL) as a positive control, 50% glycerinated human serum albumin–saline as a negative control, or 1 of the 17 test panel allergens.[9] Skin reactions were read 15 minutes after SPT. A positive reaction was noted if the diameter was at least 3 mm larger than the negative control in accordance with the most recent allergy diagnostic practice parameter published by the American Academy of Allergy, Asthma, and Immunology and American College of Allergy, Asthma, and Immunology.[9] All wheal and flare circumferences were traced with ink pen. The ink was absorbed by Transpore tape (3M, St Paul, Minnesota) and affixed to a labeled grid paper in the child's permanent record. These records were scanned and saved as true image files. The ink outlines of wheal circumferences were digitally retraced and the enclosed area was calculated using AutoCAD (Autodesk, Inc, San Rafael, California). For accuracy, these measurements were performed independently in duplicate by 2 independent individuals.

Nasal Cytology

At 4 years of age, each inferior nasal turbinate was swabbed with a separate cotton applicator. The sample processing was adapted from a previously published protocol.[2,10,11] Cells were stained with Nasal Cytology Stain (Volu-Sol, Inc).[12] Only cells with an intact nucleus and cytoplasm were counted. The number of eosinophils was counted using 40× or 100× magnification until a maximum of 400 cells was counted. For quality control, a second scientist counted 10% of samples using a random block sampling procedure of each quartile. There was no significant difference between the cell counts of each scientist.

Health Outcomes

At each annual visit, the parents were asked the International Study of Asthma and Allergies in Childhood (ISAAC) validated question, "In the past 12 months, has your child ever had a problem with sneezing, or a runny, or a blocked nose when he/she DID NOT have a cold or flu?"[13] AR was defined as a positive response to the ISAAC question and a positive SPT reaction to 1 of the 15 aeroallergens. AER, the primary outcome of this study, was defined as a positive response to the ISAAC question, a positive SPT reaction to 1 of the 15 aeroallergens, and more than 5% nasal eosinophils.[3] These AER cases were compared with children without nasal symptoms and negative SPT reactions to all 15 aeroallergens.

Exposure Assessments

Before 1 year of age, the CCAAPS research staff visited the infant's home. The home's general characteristics, basement, and the infant's primary activity room and sleeping room were inspected for visible mold, water damage, and the general state of repair of each room. To determine the greatest component of endotoxin, (1-3)-β-D-glucan, and indoor allergen exposure, the infant's primary activity room, a 2-m^2 area of floor space, was vacuumed at a standard rate of 2 min/m^2.[14,15] The collected dust samples were filtered, desiccated, and stored at −20°C.[16] The dust samples were separated for measuring house dust endotoxin (endotoxin units per milligram of settled dust) and (1-3)-β-D-glucan (micrograms per gram of dust) by the limulus amebocyte lysate assay (Associates of Cape Cod, Inc, East Falmouth, Massachusetts).[17] Separate aliquots of settled dust were used for analysis of major cat allergen (Fel d 1), major dog allergen (Can f 1), major dust mite allergen (Der f 1), and major cockroach allergen (Bla g 1) by monoclonal sandwich enzyme-linked immunosorbent assay.[18–21]

Covariates

Other covariates previously identified in the CCAAPS cohort as relevant for AR were evaluated for model inclusion and included ethnicity (non–African American vs African American), sex, annual household income (>$20,000 vs ≤$20,000), breastfeeding duration (months), number of children in the home (≥2 vs <2 children), season of birth, and the environmental covariates described earlier.[22] Hair cotinine levels were measured and used as an objective biomarker of tobacco smoke exposure at 2 years of age.[23]

Data Analysis

The aeroallergen wheal areas at 1, 2, and 3 years of age were analyzed for associations to AER using logistic regression. The odds ratios (ORs) and 95% confidence intervals (CIs) reported were obtained from the profile likelihood ratio. Any allergen wheal area or covariate significantly associated with AER ($\alpha < 0.2$) was further evaluated in multivariate logistic regression. Home environmental