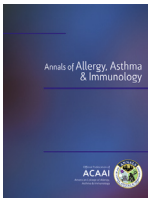
Ann Allergy Asthma Immunol xxx (2014) 1–6

Contents lists available at ScienceDirect



Differentiating asthma phenotypes in young adults through polyclonal cytokine profiles

Edward Zoratti, MD [*]; Suzanne Havstad, MA [†]; Ganesa Wegienka, PhD [†]; Charlotte Nicholas, MPH [†]; Kevin R. Bobbitt, PhD [†]; Kimberley J. Woodcroft, PhD [†]; Dennis R. Ownby, MD [‡]; and Christine Cole Johnson, PhD [†]

[*] Division of Allergy and Immunology, Department of Internal Medicine, Henry Ford Hospital, Detroit, Michigan
[†] Department of Public Health Sciences, Henry Ford Hospital, Detroit, Michigan
[‡] Division of Allergy and Immunology, Department of Pediatrics, Georgia Regents University, Augusta, Georgia

ARTICLE INFO

Article history:
Received for publication October 17, 2013.
Received in revised form April 15, 2014.
Accepted for publication April 16, 2014.

ABSTRACT

Background: Recent research has emphasized the need to better discriminate asthma phenotypes and consider underlying mechanistic endotypes in epidemiologic and clinical studies. Although allergic asthma and nonallergic asthma are frequently combined into 1 disease category in observational research and clinical trials, few studies have investigated the extent to which these 2 separate phenotypes are associated with distinct cytokine immunologic profiles in a representative young adult population.

Objective: To investigate the cytokine production-based endotypes underlying the clinical phenotypes of allergic and nonallergic asthma in a population-based birth cohort evaluated as young adults.

Methods: Participants included 18- to 21-year-old members (n = 540) of a suburban Detroit birth cohort study, the Childhood Allergy Study. Phorbol myristate acetate–stimulated whole blood interleukin (IL)-4, IL-5, IL-10, IL-12, IL-13, IL-17A, IL-17F, IL-22, and interferon-γ secretory responses were analyzed for associations comparing participants with allergic vs nonallergic asthma phenotypes with those without asthma.

Results: T-helper cell type (T_H) 2-polarized responses, measured as higher mean IL-5 and IL-13 secretions and lower ratios of interferon-γ and IL-12 to 3 T_H2 cytokines (IL-4, IL-5, or IL-13), were observed only in participants with allergic asthma. Nonallergic asthma was associated with T_H1-polarized responses, including higher adjusted interferon-γ secretion compared with participants with allergic asthma and, surprisingly, those without asthma (odds ratio 2.5, confidence interval 1.2–5.1, $P < .01$).

Conclusion: As expected, young adults with a history of an allergic asthma phenotype exhibited a T_H2–polarized cytokine response after polyclonal stimulation. However, T_H1 polarization was observed in patients with a history of nonallergic asthma. Allergic and nonallergic asthma are associated with etiologically distinct immune endotypes, underscoring the importance of discriminating these endotypes in research analyses and clinical management.

Introduction

Clinicians frequently diagnose asthma after recognizing a constellation of signs and symptoms, including recurrent episodic dyspnea, wheezing, or coughing. Additional supporting clinical data typically include airflow obstruction that is responsive to bronchodilators and the presence of bronchial hyperresponsiveness. However, it is becoming increasingly clear that the common phenotypic features of asthma are manifestations of a spectrum of disorders that might be driven by distinctive pathophysiologic mechanisms.[1–3] Recent work using study populations, patient groups, and some birth cohorts has begun to discriminate asthma endotypes using characteristics, such as patterns of sensitization and symptoms, and obesity[4–11] or parameters, such as biomarkers, severity, and medication use.[12–14] This work is of paramount importance because a precise disease definition is a fundamental tenet necessary for adequate design and analysis of epidemiologic studies and clinical trials. Failure to distinguish clinically distinct asthma subtypes introduces substantial risk for missing key associations of variables that are present only in specific asthma phenotypes.[15] Further, elucidating different asthma endotypes and their distribution has the potential to improve clinical decision making when selecting interventions that will be most likely to be successful in individual patients.[16,17]

Reprints: Edward Zoratti, MD, 1 Ford Place, 4B, Detroit, MI 48202; E-mail: ezoratt1@hfhs.org.

Disclosure: Authors have nothing to disclose.

Funding: This work was funded by the Fund for Henry Ford Hospital and the National Institute of Allergy and Infectious Diseases (grant R01AI051598).

1081-1206/14/$36.00 - see front matter
http://dx.doi.org/10.1016/j.anai.2014.04.013

Changing immune responses, which are further modulated by an individual's genetic susceptibility, are major influences as to whether and how an individual might respond to express various manifestations of the asthma syndrome phenotype.[18] For instance, forms of asthma that often manifest with neutrophil-predominant bronchial inflammation that is poorly responsive to corticosteroid treatment have recently been linked to high levels of T-helper cell type (T_H) 17-secreted cytokines, including interleukin (IL)-17 and IL-22.[19] In contrast, immune responses to specific allergens that are characterized by IgE production, eosinophilia, and the generation of T_H2-polarized cytokine profiles (ie, high levels of IL-4, IL-5, and IL-13) have long been considered a defining feature of the more common "allergic asthma" phenotype.[20] Although response patterns to defined allergens are important, patients with the allergic asthma phenotype also can exhibit an "overall" tendency to produce a polarized cytokine profile as reflected by responses to stimulation by mitogen. In fact, some studies have suggested that such polyclonal cytokine responses vary significantly among different asthma phenotypes and that these patterns correlate with other biologic characteristics, or endotypes, that underlie the varied clinical phenotypes.[21–28]

This study investigated the relation of mitogen-induced whole-blood cytokine production (polyclonal cytokine profiles) in association with allergic vs nonallergic asthma and nonasthmatic clinical phenotypes in young adults from a general-risk population-based birth cohort.

Methods

Study Population

Recruitment details for the Childhood Allergy Study have been previously described in detail.[29] Briefly, pregnant women at least 18 years of age residing in a geographically defined suburban area of Detroit, Michigan, belonging to a health maintenance organization, seeing a Henry Ford Health System provider, and having an estimated date of confinement from April 1987 to August 1989 were eligible. Enrollment included a prenatal interview and cord blood collection at delivery. Women were asked to complete annual telephone questionnaires until the child's sixth birthday. At a clinical visit near the child's sixth birthday, a venous blood sample was obtained. Participants were contacted after their 18th birthday to obtain updated health information and to request that they complete a study visit, including a venous blood sample. Of 835 teens originally eligible after 6 years of age, 15 withdrew, died, or otherwise became ineligible before age 18 years. Of the remaining 820, 40 were missing valid telephone numbers, 3 were in the military and unable to participate, 3 had disabilities precluding participation, and 2 were incarcerated, leaving 772 eligible to complete study activities. Of these, 540 (69.9%) completed a telephone interview and had secreted cytokine levels measured from a blood sample at 18 to 21 years of age. Samples were obtained at a clinical evaluation for 453 teens (84%), with 87 obtained through home visits or by mail. The Henry Ford Health System institutional review board approved all aspects of this study.

Asthma Phenotype Categorization

Participants were classified as ever having physician-diagnosed asthma based on a review of longitudinal data collected at prior study time points, including caregiver questionnaires during childhood, caregiver and participant interviews performed at 18 to 21 years of age, and systematic medical record abstraction and query of electronic claims data indicating a diagnosis of asthma (*International Statistical Classification of Diseases, Ninth Revision,* code 493.XX), performed as part of the planned study evaluation. Then, participants were categorized into 1 of 2 phenotypes: (1) atopic asthma (ever asthma and allergen-specific IgE [sIgE] $\geq$0.35 kU/L for $\geq$1 of 6 inhalant allergens at 18–21 years of age) or (2) nonatopic asthma (ever asthma and no sIgE $\geq$0.35 kU/L to any of the 6 inhalant allergens); the remaining participants were classified as having no history of asthma.

Allergen-Specific IgE Levels

Venous blood was collected and plasma was isolated and stored at −80°C until assayed. Measurements of sIgE (*Dermatophagoides farinae*, dog, cat, grass, ragweed, *Alternaria alternata*) were performed according to the manufacturer's standard protocols using the Phadia UniCAP (Phadia AB, Portage, Michigan). A test result was considered positive if the sIgE level was at least 0.35 kU/L. One percent of assays was repeated in a different assay run on a different day to provide estimates of interassay reliability. The geometric mean interassay coefficient of variation was 5.9% for all 6 allergens.

Secreted Cytokines

Whole blood (0.5 mL) was incubated within 22 hours of collection with 20 ng/mL of phorbol 12-myristate 13-acetate, 1 μmol/L of ionomycin, and 5 μL of the costimulatory antibodies CD28/CD49d for 6 hours at 37°C to stimulate cytokine production. At the conclusion of the 6-hour incubation, samples were centrifuged to isolate the plasma fraction, which was stored at −80°C until the cytokines were assayed. Secreted cytokines (IL-4, IL-5, IL-10, IL-12p70, IL-13, and interferon-γ [IFN-γ]) were assayed by flow cytometry using the BD Biosciences Cytometric Bead Array Flex Set, according to the manufacturer's instructions, on a BD LSR (BD Biosciences, San Jose, California). Three hundred events were collected per cytokine examined. Data were analyzed using BD CellQuest Pro software. Data are presented as fold increase in sample mean fluorescence intensity relative to diluent mean fluorescence intensity. Ten percent of all assays were repeated in a separate assay run on a different day to provide estimates of interassay reliability. The geometric mean coefficient of interassay variation was 11.6% for all 6 cytokines. IL-17A, IL-17F, and IL-22 were assayed with the Bio-Plex 200 System (Bio-Rad, Hercules, California) using a custom plex kit according to the manufacturer's instructions. Analyte concentrations (picograms per milliliter) were calculated from standard curves using Bio-Plex Manager 6.0 software. Concentrations of each analyte below the lower limit of quantitation (1.2, 3.0, and 3.9 for IL-17A, IL-17F, and IL-22 respectively) were set to half the lower limit of quantitation. The geometric mean coefficient of interassay variation was 10.9% for the T_H17 cytokines.

Statistical Methods

Comparison of characteristics between participants with allergic asthma and those with nonallergic asthma were performed using χ^2 tests for binary variables such as sex, 2-sample *t* tests for age and eosinophil levels, and Wilcoxon rank sum tests for total IgE. Owing to the skewness of the secreted cytokine data, nonparametric methods or logarithmic transformation of the data were used in all analyses. Geometric means and accompanying 95% confidence intervals (CIs) were used as summary statistics. Variables were logarithmically transformed before inclusion in any modeling.

To properly model the relation between cytokines and phenotypes and adjust for potential confounders, multinomial logistic regression was used. Multivariable (multinomial) logistic regression was used to test the independent effects of each cytokine. For multivariable models, colinearity was assessed through correlations and comparisons of the SEs in multiple models. All variance inflation factors were found to be less than 5, and therefore the authors