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Differentiating asthma phenotypes in young adults through polyclonal cytokine profiles

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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.

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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]

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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

concluded there was no significant degradation to the fit of the models owing to colinearity.

All analyses were performed using SAS 9.2 (SAS Institute, Cary, North Carolina).

Results

Participant Characteristics

The population included 540 young adults; 94% were white and 52.6% were women. Of these, 23% (n = 124) had a prior diagnosis of asthma and 11.7% (n = 63) had current asthma. Of those with a history of asthma, 75% (93 of 124) were atopic, as were 81% (51 of 63) of those with current asthma.

Table 1 presents a comparison of selected characteristics between the 2 asthma phenotypes: 93 participants with allergic asthma and 31 with nonallergic asthma. As expected, those with allergic asthma had elevated total IgE levels and higher eosinophil counts compared with those with nonallergic asthma. There were no differences in recent medication use, age at first attack, or age at most recent asthma attack.

Cytokine Profiles in Participants with Allergic vs Nonallergic Asthma Phenotype

A univariate analysis of cytokine profiles comparing participants categorized into the 3 phenotypes (no asthma, allergic asthma, and nonallergic asthma) is presented in Table 2. Compared with participants with no asthma, those with allergic asthma had elevated IL-5 secretion (odds ratio [OR] 1.3, 95% CI 1.1–1.7, $P < .05$) and exhibited a relatively T_H2-polarized cytokine response as reflected by lower IFN-γ/T_H2 cytokine ratios ($P < .01$ each for IFN-γ/IL-4, IL-5, and IL-13) and a similar pattern of IL-12–based calculations with significantly lower measurements for the IL-12/IL-4 and IL-12/IL-5 ratios.

A comparison of responses between participants with nonallergic asthma and those with no asthma also is presented in Table 2. Although cytokine responses were comparable for most indicators, participants with nonallergic asthma exhibited a relatively T_H1-polarized response compared with those without asthma as reflected by a higher IFN-γ/IL-13 ratio (OR 2.1, 95% CI 1.2–3.6, $P < .01$). These patterns were reflected in contrasting the 2 phenotypes presented in Table 2. Those with allergic asthma had T_H2-polarized response compared with those with nonallergic asthma as reflected by significantly lower IFN-γ/T_H2 cytokine ratios ($P < .01$ each for IFN-γ/IL-4, IL-5, and IL-13). The polyclonal responses for the regulatory T cell (Treg)-associated cytokine IL-10 and the T_H17-associated cytokines did not differ among the nonasthmatic, allergic asthmatic, and nonallergic asthmatic phenotypes.

Multivariate analyses, adjusting for all other cytokines in the model, are presented in Table 3. Participants with allergic asthma had higher levels of IL-5 secretion than those with nonallergic asthma (adjusted OR 2.9, 95% CI 1.1–7.3, $P < .05$) and those without a history of asthma (adjusted OR 2.3, 95% CI 1.3–3.8, $P < .01$). Furthermore, IFN-γ secretion after adjustment for the other cytokines was highest in participants with nonallergic asthma. This distinguished them not only from the allergic asthmatic group, as might be expected, but also from the group without asthma (adjusted OR 2.5, 95% CI 1.2–5.1, $P < .01$).

Table 1
Comparison of asthma characteristics in participants classified as having allergic vs nonallergic asthma

	Participants who ever had asthma		*P* value
	Allergic (n = 93)	Nonallergic (n = 31)	
Men, n (%)	44 (47.3)	10 (32.3)	.14
Total IgE, GM (95% CI)	102.0 (79.1–131.5)	18.1 (12.3–26.6)	<.001
Asthma medication use in past month, n (%)	20 (21.5)	6 (19.4)	.80
Asthma medication use in past 12 mo, n (%)	39 (41.9)	10 (32.3)	.34
Inhaled steroid use in past month, n (%)	20 (21.5)	4 (12.9)	.29
Inhaled steroid use in past 12 mo, n (%)	29 (31.2)	5 (16.1)	.10
Eosinophil count, mean (SD)	0.2 (0.2)	0.1 (0.1)	.008
Eosinophil percentage, mean (SD)	3.3 (2.6)	1.9 (1.3)	.010
Neutrophil count, mean (SD)	4.0 (1.7)	3.7 (1.3)	.28
Neutrophil percentage, mean (SD)	57.4 (9.7)	57.9 (8.0)	.81
Age (y) at first asthma attack, mean (SD)[a]	9.5 (4.3)	9.5 (4.6)	.99
Age (y) at most recent asthma attack, mean (SD)[b]	15.4 (3.4)	15.4 (3.4)	.98

Abbreviations: CI, confidence interval; GM, geometric mean.
[a]Missing data for 19 participants with allergic asthma and 8 with nonallergic asthma.
[b]Missing data for 17 participants with allergic asthma and 9 with nonallergic asthma.

Discussion

In a suburban population-based cohort of young adults, mitogen-induced whole-blood cytokine responses were found to be T_H2 polarized in those with the phenotype of allergic asthma. In contrast, participants with nonallergic asthma exhibited T_H1-biased responses compared with those with allergic asthma and, unexpectedly, those without a history of asthma. In addition, compared with analyzed concentrations of single cytokines, analyzed ratios of key T_H1-cytokines (IFN-γ and IL-12) to T_H2 cytokines were more strongly correlated with the biomarkers of allergy and asthma examined in this study.

The recognition that lymphocytes can selectively produce distinct patterns of cytokines and the association of these T_H1 and T_H2 cytokine profiles to functional responses has generated intense clinical investigation since their discovery 25 years ago.[30,31] It rapidly became apparent that the production of the T_H2 cytokines IL-4, IL-5, and IL-13 was linked to the development of atopy and allergy-related disorders[24,32] and asthma-related biomarkers such as eosinophilia[33] and IgE.[34] However, it is well recognized that such biomarkers of atopic asthma are not universally associated with all asthma phenotypes and cytokine profiles more closely reflect the biomarkers vs clinical expression of disease.[35]

The importance of accounting for heterogeneity in the pathogenesis of different asthma phenotypes and the unexpected associations of T_H1 cytokines to some forms of asthma have been more recently emphasized.[2,36,37] The present data not only extend observations of the absence of T_H2-skewed immunity in nonallergic asthma but also suggest T_H1-polarized responses in these patients compared with those without a diagnosis of asthma. This lends further support to the contention that careful phenotypic classification is important not only for therapeutic decision making but also when conducting risk factor or outcome studies related to asthma. Most studies still collapse these 2 phenotypes into 1 category. Combined analysis of asthma endotypes greatly increases the likelihood of masking risk estimates that would otherwise lead to the discovery of potentially important relations that vary by endotype in opposite directions.[28,38,39] For instance, medications that have therapeutic effects on T_H2-driven symptoms might have little benefit on those driven by T_H1-related processes and might even have potential for detrimental effects. Studies have underscored the contention that therapeutics targeting T_H2-driven inflammation, such as anti–IL-13,[40] anti–IL-5,[41] and persistent eosinophila,[42] are selectively effective in patients with asthma exhibiting their respective cytokine-related biomarkers. Difficult-to-control or severe asthma clearly exhibits 2 distinct

Table 2
Univariate analysis: secreted cytokines[a] by asthma phenotype[b]

	GM (95% CI)			Unadjusted OR (95% CI) for higher cytokine MFI		
	No asthma (n = 416)	Allergic asthma (n = 93)	Nonallergic asthma (n = 31)	Allergic asthma vs no asthma	Nonallergic asthma vs no asthma	Allergic vs nonallergic asthma
T_H17 cytokine						
IL-17A	246 (218–277)	228 (178–291)	298 (208–425)	1.0 (0.8–1.1)	1.2 (0.8–1.6)	0.8 (0.6–1.2)
IL-17F	105 (95–116)	115 (91–144)	123 (92–164)	1.1 (0.9–1.4)	1.2 (0.8–1.7)	0.9 (0.6–1.4)
IL-22	687 (622–758)	724 (595–881)	768 (583–1,010)	1.1 (0.8–1.3)	1.1 (0.8–1.7)	0.9 (0.6–1.5)
	246 (218–277)	228 (178–291)	298 (208–425)	1.0 (0.8–1.1)	1.2 (0.8–1.6)	0.8 (0.6–1.2)
Treg-associated cytokine						
IL-10	13.8 (12.6–15.1)	15.0 (12.2–18.5)	12.4 (9.4–16.3)	1.1 (0.9–1.4)	0.9 (0.6–1.3)	1.3 (0.8–2.0)
T_H2 cytokine						
IL-4	15.3 (14.2–16.5)	17.6 (14.9–20.8)	14.9 (11.6–19.1)	1.3 (0.9–1.7)	1.0 (0.6–1.5)	1.3 (0.8–2.2)
IL-5	20.6 (18.8–22.6)	27.4 (22.5–33.3)	18.6 (13.4–25.7)	1.3 (1.1–1.7)[c]	0.9 (0.6–1.3)	1.5 (1.0–2.3)
IL-13	48.3 (44.2–52.7)	52.9 (43.4–64.5)	41.6 (33.1–62.2)	1.1 (0.9–1.4)	0.8 (0.6–1.2)	1.3 (0.9–2.1)
T_H1 cytokine						
IFN-γ	277 (252–303)	250 (205–305)	340 (267–434)	0.9 (0.7–1.1)	1.3 (0.8–2.0)	0.7 (0.4–1.1)
IL-12	4.8 (4.7–5.0)	4.6 (4.3–5.0)	4.8 (4.3–5.4)	0.7 (0.4–1.4)	1.0 (0.4–2.9)	0.7 (0.2–2.3)
T_H1/T_H2 ratio						
IFN-γ/IL-4	18.1 (16.8–19.5)	14.2 (12.2–16.5)	22.8 (17.9–29.1)	0.7 (0.5–0.9)[d]	1.5 (0.9–2.5)	0.4 (0.3–0.8)[d]
IFN-γ/IL-5	13.4 (12.3–14.6)	9.1 (7.7–10.7)	18.3 (13.7–24.6)	0.6 (0.5–0.8)[d]	1.6 (1.0–2.4)	0.4 (0.2–0.6)[d]
IFN-γ/IL-13	5.7 (5.4–6.1)	4.7 (4.0–5.5)	8.2 (6.6–10.1)	0.7 (0.5–0.9)[d]	2.1 (1.2–3.6)**	0.3 (0.2–0.6)[d]
IL-12/IL-4	0.30 (0.28–0.33)	0.25 (0.22–0.30)	0.30 (0.22–0.40)	0.7 (0.5–1.0)[c]	0.9 (0.6–1.6)	0.8 (0.5–1.4)
IL-12/IL-5	0.23 (0.21–0.25)	0.16 (0.14–0.20)	0.23 (0.16–0.32)	0.7 (0.6–0.9)[d]	1.0 (0.7–1.6)	0.7 (0.4–1.1)
IL-12/IL-13	0.10 (0.09–0.11)	0.09 (0.07–0.10)	0.11 (0.08–0.14)	0.8 (0.6–1.1)	1.1 (0.7–1.6)	0.8 (0.5–1.2)

Abbreviations: CI, confidence interval; GM, geometric mean; IFN, interferon; IL, interleukin; MFI, mean fluorescence intensity; OR, odds ratio; T_H, T-helper cell type; Treg, regulatory T cell.
[a]Cytokine variables were logarithmically transformed before inclusion in the regression model.
[b]Sample size for phenotype groups was the same for all cytokines except for IL-12 (n = 385, 90, and 28 for no history of asthma, atopic asthma, and nonatopic asthma, respectively.)
[c]$P < .05$.
[d]$P < .01$.

inflammatory subtypes that have implications for tailored management strategies.[43]

The authors did not find differences in polyclonally stimulated whole-blood secreted T_H17-associated cytokines in participants without asthma, those with allergic asthma, or those with nonallergic asthma. Several studies have suggested that T_H17 effector cell-driven inflammation could be important in some asthma phenotypes and might be particularly relevant in nonallergic severe asthma that manifests with neutrophil-predominant pulmonary inflammation.[44] There are previous reports of elevated serum IL-17 in patients with severe asthma compared with those with less severe disease,[45] and a recent report has shown higher IL-17 levels in patients with allergic asthma compared with healthy controls.[46] The authors' assessment of polyclonally stimulated T_H17 production did not parallel these findings in plasma, possibly secondary to the methodologic differences. This also might be explained in part by the fact that severe asthma variants represent a much less common phenotype and therefore might have been minimally represented in the present cohort.

The authors also did not find between-group differences in the key Treg-associated cytokine IL-10. Allergen-specific Tregs are important in maintaining immunologic tolerance and suppressing allergic inflammatory responses, but their role in the pathogenesis of allergic asthma is uncertain. In particular, it has been difficult to

Table 3
Multivariate analysis: secreted cytokines by asthma phenotype[a]

	GM (95% CI)			Adjusted[b] OR (95% CI) for higher cytokine MFI		
	No asthma (n = 385)	Allergic asthma (n = 90)	Nonallergic asthma (n = 28)	Allergic asthma vs no asthma	Nonallergic asthma vs no asthma	Allergic vs nonallergic asthma
T_H17 cytokine						
IL-17A	243 (215–274)	231 (179–297)	299 (206–435)	0.8 (0.5–1.1)	1.1 (0.6–1.8)	0.7 (0.4–1.3)
IL-17F	104 (94–115)	116 (91–147)	120 (87–166)	1.1 (0.9–1.4)	1.2 (0.8–1.8)	1.0 (0.6–1.5)
IL-22	673 (606–747)	726 (593–888)	773 (570–1,048)	1.4 (0.9–2.0)	1.0 (0.6–1.7)	1.4 (0.7–2.6)
Treg-associated cytokine						
IL-10	14.2 (12.9–15.6)	15.4 (12.4–19.0)	13.5 (10.2–17.9)	1.2 (0.8–1.8)	0.7 (0.4–1.4)	1.6 (0.8–3.4)
T_H2 cytokine						
IL-4	15.9 (14.7–17.2)	18.2 (15.3–21.5)	16.4 (12.8–21.1)	0.9 (0.4–1.7)	1.4 (0.5–4.0)	0.6 (0.2–2.0)
IL-5	21.4 (19.4–23.6)	28.3 (23.2–34.5)	21.1 (15.3–29.1)	2.3 (1.3–3.8)[d]	0.8 (0.3–1.8)	2.9 (1.1–7.3)[c]
IL-13	48.5 (44.1–53.3)	54.5 (44.5–66.6)	48.5 (44.1–53.3)	0.8 (0.5–1.4)	0.6 (0.2–1.4)	1.4 (0.5–3.7)
T_H1 cytokine						
IFN-γ	270 (245–297)	251 (204–308)	364 (282–469)	0.6 (0.4–1.0)[c]	2.5 (1.2–5.1)[d]	0.3 (0.1–0.6)[c]
IL-12	4.8 (4.7–5.0)	4.6 (4.3–5.0)	4.8 (4.3–5.4)	0.5 (0.3–1.2)	0.8 (0.2–2.7)	0.7 (0.2–3.8)

Abbreviations: CI, confidence interval; GM, geometric mean; IFN, interferon; IL, interleukin; MFI, mean fluorescence intensity; OR, odds ratio; T_H, T-helper cell type; Treg, regulatory T cell.
[a]Cytokine variables were logarithmically transformed before inclusion in the regression model; sample is smaller because of the inclusion of IL-12 in the multivariable model.
[b]Adjusted for all other cytokines in table.
[c]$P < .05$.
[d]$P < .01$.

distinguish whether decreased functional Treg activity is primarily a characteristic of allergy vs asthma per se.[47] In the present cohort, there was a lack of correlation of IL-10 production to the presence of asthma or in participants with asthma to the allergic or nonallergic phenotypes. Prior reports have included decreased[48] and increased[49] circulating levels of IL-10 in patients with allergic asthma compared with controls without asthma. A recent report has shown a lower capacity of polyclonal (lipopolysaccharide and anti-CD3, respectively) stimulated peripheral blood mononuclear cells (PBMCs) to produce IL-10 in children with moderate and severe asthma compared with controls without asthma, although allergy was substantially over-represented in the participants with asthma.[48,50] It is unclear why this finding was not reproduced in the present cohort when comparing participants with allergic asthma with those with nonallergic asthma. Perhaps the discrepancy is related to results using PBMCs vs the whole-blood stimulation performed in the present study or the assessment of a cohort with less severe asthma overall.

Few studies of population-based cohorts that are not at high risk of asthma have reported on the relation of asthma biomarkers to polyclonal cytokine responses. The present methods roughly parallel 2 reports from the University of Western Australia.[26,27] In the first report, dust mite allergen and mitogen (phytohemagglutinin) stimulated cytokine responses from cryobank-stored PBMCs were reported in 172 school-age children comprised of an antenatally recruited birth cohort and their siblings.[26] The investigators identified asthmatic phenotypes characterized by bronchial hyper-responsiveness and absence of atopy to be associated with polyclonal T_H1 cytokine (IFN-γ and tumor necrosis factor-α) production. The authors consider these results consistent with their finding, although compared with single cytokines, T_H1/T_H2 cytokine ratios appeared more informative in the present study but were not reported in the Australian study. Others groups also have noted the importance of considering cytokine combination patterns in asthma research.[51]

In a subsequent report from the same investigative group, a larger population-based cohort of 1,380 Australian teenagers (mean age 14.1 years) was evaluated.[27] Cytokine secretion was assessed in cryopreserved PBMCs after dust mite allergen or mitogen (phytohemagglutinin) incubation. In teens with atopy (defined by dust mite sensitivity), the presence of asthma was associated not only with phytohemagglutinin-induced IL-5 production but also with enhanced IFN-γ production in a univariate comparison. The present findings differed in that IFN-γ was significantly decreased in participants with atopic asthma in the multivariate analysis and T_H1-polarized responses were detected in participants with nonatopic asthma as reflected by higher IFN-γ–based ratios. Furthermore, higher polyclonal IFN-γ/IL-13 ratios were found in participants with nonatopic asthma compared with participants without asthma.

Arguably, localized airway cytokine production might be more relevant to asthma than stimulated cells from peripheral blood. Investigators have long noted distinct cytokine production patterns in airways of patients with asthma.[21,52–54] Some reports have suggested a similar T_H2-polarized pattern in patients with allergic asthma and those with nonallergic asthma.[52] The present findings in peripheral blood partly mirror those reported in the airway by Woodruff et al.[53] In their study, quantitative polymerase chain reaction and microarrays from bronchial epithelial cells from 42 patients with asthma and 28 controls were compared. Approximately half the patients with asthma exhibited a "T_H2-high" signature (elevated IL-5 and IL-13) compared with a "T_H2-low" classification. The T_H2-low group was indistinguishable from the healthy control group in T_H1 and T_H2 cytokine signatures, even when T_H1/T_H2 cytokine ratios were analyzed. Similar to the present findings, IL-10 signatures from the airway cells did not distinguish the asthma phenotypes or the control group. However, striking clinical differences were noted in the T_H2-high group, including higher IgE levels, enhanced responsiveness to methacholine, higher response to inhaled corticosteroids, increased degree airway remodeling, and a trend toward a larger number of positive skin test results, compared with the T_H2-low group.

There are several limitations related to the present study. The classification of participants as having allergic vs nonallergic asthma was based on results of in vitro testing with 6 inhalant allergens in young adulthood, leaving open the possibility that some participants classified as nonatopic would be found to have sensitization if a larger panel of allergens had been used. However, data from the 2005 to 2006 US National Health and Nutrition Examination Survey suggest that using a panel of 6 common allergens would detect approximately 92% of sensitized individuals who were identified using a panel of 15 allergens.[55] The authors could not limit analyses to those with current asthma owing to the small numbers and consequent inadequate statistical power, an inherent difficulty even with relatively large population-based cohorts in which clinical outcomes, such as current nonallergic asthma, in young adults are present in a small proportion of the population. In addition, the Childhood Allergy Study cohort is a relatively homogeneous population of white, middle-class, young adults. This might be considered a strength, by minimizing variation attributable to ethnicity or socioeconomic status, or a weakness, because it limits the application of the present findings to other ethnic populations.

In addition, the assessment of Treg capacity included only the evaluation of IL-10. Transforming growth factor-β also has emerged as an important regulatory factor and perhaps between-group differences in the production of this cytokines would have been detectable. One could argue that allergen-induced cytokine stimulation might be more relevant than mitogen-induced cytokine stimulation. Indeed, there are reports that the 2 responses do not parallel each other.[56] However, because the authors were interested in studying nonallergic and allergic asthma, mitogen-stimulated responses were used. Moreover, the authors did not systematically obtain data regarding the presence of current infections or rare chronic diseases (eg, type 1 diabetes, multiple sclerosis) at the time of blood draw that could affect cytokine measurements. However, most participants had their blood draws at a clinic visit, where they were deemed to be healthy by spirometric testing, and chronic immunologic diseases are uncommon enough that few, if any, participants would be affected.

There are also several notable strengths related to the present study. The authors report on a well-characterized population that has been followed longitudinally since birth, thereby minimizing recall bias regarding important outcomes for this study, such as physician-diagnosed asthma. Although not entirely unique, few studies have compared mitogen-induced cytokine production in those with asthma in a cohort of population-based participants as a method of decreasing the bias that can arise in selected high-risk populations. Further, polyclonal stimulation of whole blood compared with several studies restricted to PBMC responses might provide unique information reflecting maximal immune cytokine production from a broadened spectrum of cell types.

In conclusion, in a population-based cohort of young adults, polyclonal T_H2-polarized immune responses in peripheral blood were characteristic of asthma only in those with concomitant atopy, classified as an allergic asthma phenotype. Distinct polyclonal cytokine patterns are likely to indicate opposing endotypes of asthma pathogenesis between those with allergic asthma and those with nonallergic asthma, with the latter representing a substantial proportion of those with asthma in young adults. Careful consideration of this heterogeneity is critically important in etiologic studies and when determining efficacy in clinical trials that

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enroll a broad spectrum of asthma phenotypes that can exhibit opposing responses to potential therapeutic agents targeting specific inflammatory pathways.[57]

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