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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 epidemiological and clinical studies. While allergic asthma and non-allergic asthma are frequently combined into one disease category in observational research and clinical trials, few studies have investigated the extent to which these two separate phenotypes are associated with distinct cytokine immunological profiles in a representative young adult population.

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

Methods—Subjects included 18–21 year-old members (n=540) of a suburban Detroit birth cohort study, the Childhood Allergy Study. PMA-stimulated whole blood IL4, IL5, IL10, IL12, IL13, IL17A, IL17F, IL22 and IFNγ secretory responses were analyzed for associations comparing participants with allergic versus non-allergic asthma phenotypes to those without asthma.

Results—Th2-polarized responses, measured as higher mean IL5 and IL13 secretion and lower ratios of IFNγ and IL12 to three Th2 cytokines IL4, IL5, or IL13, were observed only in allergic asthmatics. Non-allergic asthma was associated with Th1-polarized responses including higher adjusted IFNγ secretion compared to both allergic asthmatics and surprisingly, to those without asthma [OR=2.5 (1.2–5.1), p<0.01].

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Conclusions—As expected, young adults with a history of an allergic asthma phenotype exhibit a Th2-polarized cytokine response after polyclonal stimulation. However, Th1-polarization was observed in subjects with a history of non-allergic asthma. Allergic and non-allergic asthma are associated with etiologically distinct immune endotypes underscoring the importance of discriminating these endotypes in research analyses and clinical management.

Keywords

asthma; allergy; atopy; cytokine; Th1; Th2; Th17; Treg; Phenotype; IgE; birth cohort

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 phenotypical features of asthma are manifestations of a spectrum of disorders that may be driven by distinctive pathophysiological 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 as a precise disease definition is a fundamental tenet necessary for adequate design and analysis of both epidemiological 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]

Varying immune responses, that are further modulated by an individual's genetic susceptibility, are major influences as to whether and how an individual may 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 Th17 secreted cytokines including IL17 and IL22.[19] In contrast, immune responses to specific allergens that are characterized by IgE production, eosinophilia and the generation of Th2 polarized cytokine profiles (ie. high levels of IL4, IL5 and IL13) have been long-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 may also exhibit an "overall" tendency to produce a polarized cytokine profile as reflected by responses to stimulation by mitogen. In fact, some studies suggest 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]

In this report, we investigated the relationship of mitogen-induced, whole-blood cytokine production (polyclonal cytokine profiles) in association with allergic asthma versus non-allergic asthma and non-asthma clinical phenotypes among young adults from a general-risk population-based birth cohort.

METHODS

Study Population

Recruitment details for the Childhood Allergy Study (CAS) 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 and belonging to a health maintenance organization, seeing a Henry Ford Health System (HFHS) provider and having an estimated date of confinement between April 1987 and 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 prior to 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 a total of 772 eligible to complete study activities. Of these, 540 (69.9%) completed a phone interview and had secreted cytokine levels measured from a blood sample at age 18–21 years. The samples were obtained at a clinical evaluation for 453 (84%) of the teens with 87 obtained through home visits or by mail. The HFHS Institutional Review Board approved all aspects of this study.

Asthma Phenotype Categorization

Participants were classified as ever having a history of physician-diagnosed asthma based on review of longitudinal data collected at prior study time points including caregiver questionnaires during childhood, caregiver and participant interviews performed at age 18–21 years and systematic medical record abstraction and query of electronic claims data indicating a diagnosis of asthma a (ICD-9 493.XX) performed as part of the planned study evaluation. Participants were then categorized into one of two phenotypes: (1) atopic asthma (ever asthma and sIgE ≥ 0.35 kU/L for at least 1 of 6 inhalant allergens at age 18–21); (2) non-atopic asthma (ever asthma and no sIgE ≥ 0.35 kU/L to any of 6 inhalant allergens) with the remaining subjects classified as having no history of asthma.

Allergen-Specific IgE (sIgE) 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 following the standard manufacturer's protocols using the Phadia UniCAP (Phadia AB U.S., Portage, MI). A test was considered positive if sIgE ≥ 0.35 kU/L. One percent of assays were repeated in a different assay run on a different day to provide

estimates of interassay reliability. The geometric mean inter-assay 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 PMA, 1 μM ionomycin and 5 μl of the costimulatory antibodies CD28/CD49d for 6 h at 37°C to stimulate cytokine production. At the conclusion of the 6 hours incubation, samples were centrifuged to isolate the plasma fraction, which was stored at −80°C until the cytokines were assayed. Secreted cytokines (IL4, IL5, IL10, IL12p70, IL13, and IFNγ) were assayed by flow cytometry using BD Biosciences Cytometric Bead Array (CBA) Flex Set according to manufacturer's instructions on a BD LSR (BD Biosciences, San Jose, CA). 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 (MFI) relative to diluent MFI. 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. IL17A, IL17F and IL22 were assayed with the Bio-Plex 200 System (Bio-Rad, Hercules, CA) using a custom-plex kit according to manufacturer's instructions. Analyte concentrations (pg/ml) were calculated from standard curves using Bio-Plex Manager 6.0 software. Concentrations of each analyte below the Lower Limit of Quantitation (LLOQ) (1.2, 3.0, and 3.9 for IL17A, IL17F, IL22 respectively) were set to half the LLOQ. The geometric mean coefficient of interassay variation was 10.9% for the Th17 cytokines.

Statistical Methods

Comparison of characteristics between allergic and non-allergic asthma participants were performed with chi-square tests for binary variables such as gender, two-sample t-tests for age and eosinophils, and Wilcoxon rank sum tests for total IgE. Due to the skewness of the secreted cytokine data, non-parametric methods or log transformation of the data were used in all analyses. Geometric means (GM) and accompanying 95% confidence intervals (95% CI) were used as summary statistics. Variables were log-transformed prior to inclusion in any modeling.

To properly model the relationship between the 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, collinearity was assessed through correlations and comparisons of the standard errors in multiple models. All variance inflation factors were found to be < 5 and therefore we concluded there was no significant degradation to the fit of the models due to collinearity.

All analyses were performed by using SAS software (Version 9.2; SAS Institute, Cary, NC).

RESULTS

Participant Characteristics

The population included 540 young adults, 94% were White and a slight majority were female (52.6%). 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% (93/124) were atopic as were 81% (51/63) of those with current asthma.

Table 1 compares selected characteristics among the two asthma phenotypes: 93 subjects with allergic asthma and 31 non-allergic asthmatics. As expected, those with allergic asthma had elevated total IgE and higher eosinophil counts compared to non-allergic asthmatics. There were no differences with respect to recent medication use, age at first attack or age at most recent asthma attack.

Cytokine profiles among participants with the allergic asthma phenotype versus the non-allergic asthma phenotype

A univariate analysis of cytokine profiles comparing participants categorized into the three phenotypes, “no asthma”, “allergic asthma” and “non-allergic asthma” is presented in Table 2. Compared to participants with no asthma, those with allergic asthma had elevated IL5 secretion (OR= 1.3 (1.1–1.7), $p<0.05$) and exhibited a relatively Th2- polarized cytokine response as reflected by lower IFNγ/ Th2 cytokine ratios ($p<0.01$ each for IFNγ/IL4, IL5 and IL13) and a similar pattern of IL12-based calculations with significantly lower measures for the IL12/IL4 and IL12/IL5 ratios.

A comparison of responses between subjects with non-allergic asthma and no asthma is also displayed in Table 2. Although cytokine responses were comparable for most indicators, subjects with non-allergic asthma exhibited a relatively Th1-polarized response compared to non-asthmatics as reflected by a higher IFNγ / IL13 ratio [OR 2.1 (1.2–3.6), $p<0.01$]. These patterns were reflected in contrasting the two phenotypes in Table 2. Those with allergic asthma had Th2 polarized response in comparison to those with non-allergic asthma as reflected by significantly lower IFNγ / Th2 cytokine ratios ($p<0.01$ each for IFNγ / IL4, IL5 and IL13). The polyclonal responses for the Treg-associated cytokine IL10, and the Th17-associated cytokines did not differ among the non-asthma, allergic asthma and non-allergic asthma phenotypes.

Multivariate analyses, adjusting for all other cytokines in the model, are presented in Table 3. Participants with allergic asthma had higher levels of IL5 secretion than both non-allergic asthmatics (aOR=2.9 (1.1–7.3), $p <0.05$) and participants without history of asthma (aOR=2.3(1.3–3.8), $p<0.01$). Furthermore, IFNγ secretion after adjustment for the other cytokines was highest among subjects with non-allergic asthma. This distinguished them not only from the allergic asthma group as might be expected, but also those without asthma, adjusted OR=2.5 (1.2–5.1), $p<0.01$].

DISCUSSION

In a suburban population-based cohort of young adults, we found mitogen-induced whole blood cytokine responses to be Th2-polarized among those with the phenotype of allergic asthma. In contrast, the participants with non-allergic asthma exhibited Th1-biased responses when compared to those with allergic asthma and unexpectedly, when compared to subjects without a history of asthma. We also found that in comparison to analyzing concentrations of single cytokines, analyzing the ratios of key Th1-cytokines (IFNγ and IL12) to Th2 cytokines were more strongly correlated with the biomarkers of allergy and asthma examined in this study.

Recognition that lymphocytes have the ability to selectively produce distinct patterns of cytokines and the association of these Th1 and Th2 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 Th2 cytokines IL4, IL5 and IL13 were linked to the development of atopy and allergy-related disorders,[24;32] as well as asthma-related biomarkers such as eosinophilia[33] and IgE.[34] However, it is now well recognized that such biomarkers of atopic asthma are not universally associated with all asthma phenotypes and cytokine profiles more closely reflect the biomarkers versus clinic expression of disease.[35]

The importance of accounting for heterogeneity in the pathogenesis of different asthma phenotypes and unexpected associations of Th1 cytokines to some forms of asthma has been more recently emphasized.[2;36;37] Our data not only extends observations of the absence of Th2-skewed immunity in non-allergic asthma but suggests Th1-polarized responses among these patients in comparison to 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. The majority of studies still collapse these two phenotypes into one category. Combined analysis of asthma endotypes greatly increases the likelihood of masking risk estimates that would otherwise lead to the discovery of potentially important relationships that vary by endotype in opposite directions.[28;38;39] For instance, medications that have therapeutic effects on Th2-driven symptoms may have little benefit on those driven by Th1-related processes and may even have potential for detrimental effects. Studies have underscored the contention that therapeutics targeting Th2-driven inflammation such as anti-IL13,[40] anti-IL5,[41] and persistent eosinophila[42] are selectively effective among asthmatics exhibiting their respective cytokine-related biomarkers. Difficult-to-control or severe asthma clearly exhibits two distinct inflammatory subtypes that have implications for tailored management strategies.[43]

We did not find differences in polyclonally stimulated whole-blood secreted Th17 associated cytokines between participants without asthma, with allergic asthma or with non-allergic asthma. Several studies have suggested that Th17 effector cell driven inflammation may be important in some asthma phenotypes and may be particularly relevant in non-allergic severe asthma that manifests with neutrophil-predominant pulmonary inflammation.[44] There are previous reports of elevated serum IL-17 in severe asthmatics in comparison to those with less severe disease.[45] and recently a report showing higher IL17

levels among allergic asthmatics compared to healthy controls.[46] Our assessment of polyclonally stimulated Th17 production did not parallel these findings in plasma possibly secondary to the methodological differences. This may also be partially explained by the fact that severe asthma variants represent a much less common phenotype and therefore may have been minimally represented in our cohort.

We also did not find between-group differences in the key Treg cell associated cytokine IL10. Allergen-specific regulatory T cells (Tregs) are important in maintaining immunological tolerance and suppressing allergic inflammatory responses but their role in the pathogenesis of allergic asthma is uncertain. In particular, it has been difficult to distinguish whether decreased functional Treg activity is primarily a characteristic of allergy versus asthma per se.[47] In our cohort, there was a lack of correlation of IL10 production to either the presence of asthma, or among asthmatics to the allergic or non-allergic phenotypes. Prior reports have included both decreased[48] and increased circulating levels[49] of IL10 in allergic asthmatics compared to non-asthmatic controls. A prior and recent report demonstrated lower capacity of polyclonal (LPS and anti-CD3, respectively) stimulated PBMCs to produce IL10 among children with moderate and severe asthma compared to non-asthmatic controls although allergy was substantially overrepresented in the asthmatic subjects.[48;50] It is unclear why this finding was not reproduced in our cohort when comparing those asthmatics with and without allergy. Perhaps the discrepancy but may be related to results using PBMCs versus the whole-blood stimulation done in our 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 relationship of asthma biomarkers to polyclonal cytokine responses. Our methods roughly parallel two reports from the University of Western Australia.[26;27] In the first report, dust mite allergen and mitogen (PHA) stimulated cytokine responses from cryobanked PBMCs were reported among 172 school aged children comprised of an antenatally recruited birth cohort and their siblings.[26] They identified asthmatic phenotypes characterized by bronchial hyperresponsiveness and absence of atopy to be associated with polyclonal Th1 cytokine (IFNγ and TNFα) production. We consider these results consistent with our finding although compared to single cytokines, Th1/Th2 cytokine ratios appeared more informative in our study but were not reported in the Australian study. Others groups have also 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 1380 Australian teenagers (mean age of 14.1 years) was evaluated.[27] Cytokine secretion was again assessed in cryopreserved PBMCs after dust mite allergen or mitogen (PHA) incubation. Among teens with atopy (defined by dust mite sensitivity), the presence of asthma was associated not only with PHA induced IL5 production but also with enhanced IFNγ production in a univariate comparison. Our findings differed in that IFNγ was significantly reduced in atopic asthmatics in the multivariate analysis. while detecting Th1-polarized responses in non-atopic asthma as reflected by higher IFNγ-based ratios. Furthermore, we found higher polyclonal IFNγ / IL13 ratios among non-atopic subjects with asthma compared to subjects without asthma.

Arguably, localized airway cytokine production may be more relevant to asthma than stimulated cells from peripheral blood. Investigators have long noted distinct cytokine production patterns in airways of subjects with asthma.[21;52–54] Some reports suggest a similar Th2-polarized pattern in both allergic and non-allergic asthmatics.[52] Our findings in peripheral blood partially mirror those reported in the airway by Woodruff et al.[53] In their study, qPCR and microarrays from bronchial epithelial cells were compared among 42 asthmatics and 28 controls. Approximately half of the asthmatics exhibited a "Th2-high" signature (elevated IL5 and IL13) in comparison to a "Th2-low" classification. The Th2 low group was indistinguishable from the healthy controls in terms of Th1 and Th2 cytokine signatures, even when Th1/Th2 cytokine ratios were analyzed. Similar to our findings, IL10 signatures from the airway cells did not distinguish either of the asthma phenotypes nor the control group. However, striking clinical differences were noted with Th2-high asthmatics including higher IgE, enhanced responsiveness to methacholine, higher response to inhaled corticosteroids, increased degree airway remodeling and a trend toward a greater number of positive skin tests when compared to the Th2-low group.

There are several limitations related to our study. The classification of our participants as allergic or non-allergic asthma was based on the results of *in vitro* testing with 6 inhalant allergens in young adulthood leaving open the possibility that some participants classified as non-atopic would be found to have sensitization if we used a larger panel of allergens. However, data from the 2005–2006 US National Health and Nutrition Examination Survey suggests that using a panel of 6 common allergens would detect approximately 92% of sensitized individuals that were identified using a panel of 15 allergens.[55] We were not able to limit analyses to those with current asthma due to the small numbers and consequent inadequate statistical power; an inherent difficulty even with relatively large population-based cohorts where clinical outcomes like current non-allergic asthma in young adults are present in a small proportion of the population. In addition, the CAS cohort is a relatively homogeneous population of white, middle class, young adults. This may be considered a strength by minimizing variation attributable to ethnicity or socioeconomic status, but a weakness since it limits the application of our findings to other ethnic populations.

Also our assessment of Treg capacity included only the evaluation of IL10. Transforming growth factor beta has also 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 may be more relevant than mitogen-induced cytokine stimulation. Indeed, there are reports that the two responses do not parallel each other.[56] However, as we were interested in studying non-allergic and allergic asthma, mitogen stimulated responses were employed. Finally, we did not systematically obtain data regarding the presence of current infections or rare chronic diseases (e.g., type 1 diabetes, multiple sclerosis) at time of blood draw that could affect cytokine measures. However, most of the subjects had their blood draws at a clinic visit where they were deemed to be healthy with respect to spirometry testing, and the chronic immunological diseases are uncommon enough that few if any of our participants would be affected.

There are also several notable strengths related to our study. We report on a well-characterized population that have 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 among those with asthma in a cohort of population-based participants as a method of reducing the bias that may arise in selected high-risk populations. Finally, polyclonal stimulation of whole blood, compared to several studies restricted to PBMC responses, may provide unique information reflecting maximal immune cytokine production from a broadened spectrum of cell types.

In conclusion, among a population-based cohort of young adults, polyclonal Th2-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 and non-allergic asthma, with the latter representing a substantial proportion of those with asthma in young adults. Careful consideration of this heterogeneity is critically important in etiological studies and when determining efficacy in clinical trials that enroll a broad spectrum of asthma phenotypes that may exhibit opposing responses to potential therapeutic agents targeting specific inflammatory pathways.[57]

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Abbreviations

CAS	Childhood Allergy Study
GM	geometric mean
HFHS	Henry Ford Health System
ICD-9	International Statistical Classification of Disease and Related Health Problems version 9
IL	interleukin
IFNγ	interferon gamma
MFI	mean fluorescence intensity
OR	odds ratio
sIgE	allergen-specific IgE
Th1	T-helper cell Type 1
Th2	T-helper cell Type 2
Th17	T-help cell Type 17
Treg	regulatory T cell
WRS	Wilcoxon Rank Sum Test

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Table 1

Comparison of asthma characteristics among participants classified as having allergic versus non-allergic asthma

	Ever Asthma Subjects		
	Allergic (N=93)	**Non-Allergic (N=31)**	**p-value**
Male (n, %)	44 (47.3%)	10 (32.3%)	0.14
Total IgE, GM (95% CI)	102.0 (79.1, 131.5)	18.1 (12.3, 26.6)	<0.001
Asthma med use in past month (n, %)	20 (21.5%)	6 (19.4%)	0.80
Asthma med use in past 12 months (n, %)	39 (41.9%)	10 (32.3%)	0.34
Inhaled steroid use in past month (n, %)	20 (21.5%)	4 (12.9%)	0.29
Inhaled steroid use in past 12 months (n, %)	29 (31.2%)	5 (16.1%)	0.10
Eosinophil count, mean (s.d.)	0.2 (0.2)	0.1 (0.1)	0.008
Eosinophil %, mean (s.d.)	3.3 (2.6)	1.9 (1.3)	0.010
Neutrophil count, mean (s.d.)	4.0 (1.7)	3.7 (1.3)	0.28
Neutrophil %, mean (s.d.)	57.4 (9.7)	57.9 (8.0)	0.81
Age (years) of first asthma attack, mean (s.d.)[1]	9.5 (4.3)	9.5 (4.6)	0.99
Age (years) of most recent asthma attack, mean (s.d.)[2]	15.4 (3.4)	15.4 (3.4)	0.98

[1] missing data for 19 allergic asthmatics and 8 non-allergic asthmatics

[2] missing data for 17 allergic asthmatics and 9 non-allergic asthmatics

Table 2

Univariate analysis: Secreted cytokines[1] by asthma phenotype[2]

				Unadjusted Odds Ratio (95% CI) for higher cytokine MFI[3]		
	No Asthma N=416 GM (95% CI)	**Allergic Asthma N=93 GM (95% CI)**	**Non-Allergic Asthma N=31 GM (95% CI)**	**Allergic Asthma vs No Asthma**	**Non-Allergic Asthma vs No Asthma**	**Allergic Asthma vs Non-Allergic Asthma**
Th17 cytokine						
IL17A	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)
IL17F	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)
IL22	687 (622, 758)	724 (595, 881)	768 (583, 1010)	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						
IL10	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)
Th2 cytokine						
IL4	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)
IL5	20.6 (18.8, 22.6)	27.4 (22.5, 33.3)	18.6 (13.4, 25.7)	1.3 (1.1–1.7)*	0.9 (0.6–1.3)	1.5 (1.0–2.3)
IL13	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)
Th1 cytokine						
IFNγ	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)
IL12	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)
Th1 / Th2 ratio						
IFNγ/IL4	18.1 (16.8, 19.5)	14.2 (12.2, 16.5)	22.8 (17.9, 29.1)	0.7 (0.5–0.9)**	1.5 (0.9–2.5)	0.4 (0.3–0.8)**
IFNγ/ IL5	13.4 (12.3, 14.6)	9.1 (7.7, 10.7)	18.3 (13.7, 24.6)	0.6 (0.5–0.8)**	1.6 (1.0–2.4)	0.4 (0.2–0.6)**
IFNγ/ IL13	5.7 (5.4, 6.1)	4.7 (4.0, 5.5)	8.2 (6.6, 10.1)	0.7 (0.5–0.9)**	2.1 (1.2–3.6)**	0.3 (0.2–0.6)**
IL12 / IL4	0.30 (0.28, 0.33)	0.25 (0.22, 0.30)	0.30 (0.22, 0.40)	0.7 (0.5–1.0)*	0.9 (0.6–1.6)	0.8 (0.5–1.4)
IL12 / IL5	0.23 (0.21, 0.25)	0.16 (0.14, 0.20)	0.23 (0.16, 0.32)	0.7 (0.6–0.9)**	1.0 (0.7–1.6)	0.7 (0.4–1.1)
IL12 /IL13	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)

[1] cytokine variables were log-transformed prior to inclusion in the regression model

[2] sample size for phenotype groups the same for all cytokines except for IL12 (n=385, 90 and 28 for no history of asthma, atopic asthma and non-atopic asthma respectively.)

3 mean fluorescence intensity

** p <0.01;

* p <0.05

Table 3

Multivariate analysis: Secreted cytokines by asthma phenotype[1]

				Adjusted[2] OR for higher cytokine MFI[3]		
	No Asthma N=385 GM (95% CI)	**Allergic Asthma N=90 GM (95% CI)**	**Non-Allergic Asthma N=28 GM (95% CI)**	**Allergic Asthma vs No Asthma**	**Non-Allergic Asthma vs No Asthma**	**Allergic Asthma vs Non- Allergic Asthma**
Th17 cytokine						
IL17A	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)
IL17F	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)
IL22	673 (606, 747)	726 (593, 888)	773 (570, 1048)	1.4 (0.9–2.0)	1.0 (0.6–1.7)	1.4 (0.7–2.6)
Treg associated cytokine						
IL10	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)
Th2 cytokine						
IL4	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)
IL5	21.4 (19.4, 23.6)	28.3 (23.2, 34.5)	21.1 (15.3, 29.1)	2.3 (1.3–3.8)**	0.8 (0.3–1.8)	2.9 (1.1–7.3)*
IL13	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)
Th1 cytokine						
IFNγ	270 (245, 297)	251 (204, 308)	364 (282, 469)	0.6 (0.4–1.0)*	2.5 (1.2–5.1)**	0.3 (0.1–0.6)*
IL12	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)

[1] cytokine variables were log-transformed prior to inclusion in the regression model; sample size reduced due to inclusion of IL12 in multivariable model

[2] adjusted for all other cytokines in table

[3] mean fluorescence intensity

** p <0.01;

* p <0.05