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Asthma and antibodies to pneumococcal virulence proteins

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Abstract

Purpose—We previously reported that asthmatics had lower anti-serotype-specific pneumococcal polysaccharide antibody levels than non-asthmatics, and T-helper 2 (Th2)-immune profile was associated with suboptimal pneumococcal polysaccharide antibody. Our objective was to determine the influence of asthma status on anti-pneumococcal protein antigen antibody levels.

Methods—We conducted a cross-sectional study, which enrolled 16 children and adults with asthma and 14 subjects without asthma. Asthma was ascertained by predetermined criteria. Serum IgG antibody levels to pneumococcal surface protein A (PspA), pneumococcal surface protein C (PspC), pneumococcal choline-binding protein A (PcpA), and pneumolysin (PLY) were measured by ELISA assays. These antibody levels were compared between asthmatics and non-asthmatics. Th2-immune profile was determined by IL-5 secretion from PBMCs cultured with house dust mite (HDM) and staphylococcal enterotoxin B (SEB) at day seven. The correlation between the anti-pneumococcal antibody levels and Th2-HDM and SEB-responsive immune profile was assessed.

Results—Of the 30 subjects, 16 (53%) were male and the median age was 26 years. There were no significant differences in anti-PspA, anti-PspC, anti-PcpA, and anti-PLY antibody levels between asthmatics and non-asthmatics. Th2-immune profile was inversely correlated with anti-PspC antibody levels ($r = -0.53$, $p = 0.003$). This correlation was significantly modified by asthma status ($r = -0.74$, $p = 0.001$ for asthmatics vs. $r = -0.06$, $p = 0.83$ for non-asthmatics). Other pneumococcal protein antibodies were not correlated with Th2-immune profile.

Conclusion—No significant differences in anti-pneumococcal protein antigen antibody levels between asthmatics and non-asthmatics were found. Asthma status is an important effect modifier determining the negative influence of Th2-immune profile on anti-PspC antibody levels.

Keywords

Asthma; Atopy; Risk; Humoral immunity; and Pneumococcal virulence proteins

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Introduction

Streptococcus pneumoniae is a leading cause of bacterial pneumonia, meningitis, and sepsis in children worldwide, and it continues to present a major public threat associated with significant morbidity and mortality. In 2000, there was an estimated 14.5 million episodes of serious pneumococcal disease. Worldwide, pneumococcal disease causes more than 800,000 deaths each year among children under age 5 years [1]. Overall, yearly child deaths attributed to *S pneumoniae* range from 700,000 to 1 million worldwide[2]. In the U.S., pneumococcal diseases were responsible for 4 million illness episodes, 445,000 hospitalizations, and 22,000 fatal cases caused by *S. pneumoniae* have been reported in 2004.[3, 4] Our previous study suggested that individuals with asthma have a significantly increased risk of serious pneumococcal diseases (pneumococcal pneumonia and/or invasive pneumococcal disease) compared to those without asthma [5]. These results confirmed similar study findings reported by Talbot et al [6]. Thus, the Advisory Committee on Immunization Practices (ACIP) issued a recommendation for all adults with asthma to receive 23-valent pneumococcal polysaccharide vaccine (PPV23) for the prevention of invasive pneumococcal disease (IPD)[7].

Little is known about the mechanisms underlying the higher risk of serious pneumococcal diseases in individuals with asthma. Several studies have identified impaired innate immune function in bronchial epithelial cells among asthmatics [8–11]. Other studies suggest asthma or its-associated Th2 immune environment might result in suboptimal adaptive immune function. [12–15] We recently reported that T-helper 2 (Th2)-predominant immune responses (e.g., IL-4) to OVA sensitization was a significant risk factor for pneumococcal pneumonia, and Khan et al.[16] reported an association between Th2 cytokines and suppressed anti-pneumococcal antibody responses[17]. In our previous work we found significantly lower serotype-specific antibody to 23 pneumococcal polysaccharide antigens among individuals with asthma compared to those without [18]. This was true for vaccine serotypes for heptavalent pneumococcal conjugate vaccine (PCV-7). These results suggest that the underlying Th-2-immune environment seen in asthma may promote suboptimal humoral immune function, especially T-cell independent type II immunity against pneumococcal polysaccharide (T cells help maturation of antibody response). However, it is unknown whether this is true for humoral immune responses against pneumococcal surface or cytosolic (virulence) protein antigens (i.e., T-cell dependent immune response, which are known to elicit protective immunity for pneumococcal infections)[19–25].

To address whether a Th2-immune environment affects humoral immune response to pneumococcal protein antigens, and also whether asthma is associated with a differential immune response to pneumococcal protein antigens, we conducted a cross-sectional study to compare anti-pneumococcal protein antigen antibodies between asthmatics and non-asthmatics and to determine the correlation between Th2 immune profile and humoral immune responses to pneumococcal protein antigens.

Materials and Methods

Study Design

This was a cross-sectional study examining the correlation between Th2 immune profile (predictor variable) and pneumococcal protein antigen antibody levels (response variable). We also compared pneumococcal protein antigen antibody levels between asthmatics and non-asthmatics. Asthma status was assessed using predetermined criteria delineated in Table 1. Th2 immune profile was assessed using cytokine secretion patterns after PBMC stimulation with dust mite antigen or SBE.

Study Subject

Details on study subjects, and study eligibility and exclusion criteria were previously reported [18]. Briefly, a convenience sample of 16 children and adults with asthma and 14 subjects without asthma were enrolled from a list of subjects who had received medical care at Mayo Clinic and who resided in the community (Olmsted County, MN). Because random sampling is logistically difficult and costly, a convenience sample reasonably representing our clinic population was used for this exploratory lab-based study. Subjects with asthma were free of acute asthma symptoms at the time of enrollment. Because inhaled corticosteroids are unlikely to affect antibody response [26, 27], asthmatics on these at the time of enrollment were included in the study. The sample size of the present study as an exploratory study was based on feasibility and empirical power calculation (1 standard deviation as an effect size). Based on our original study finding (difference in the number of positive serotype-specific pneumococcal antibodies between asthmatics and non-asthmatics), [18] the present study had 83% power to detect the difference ($\mu_1 - \mu_2$) of 6 or 0.9 standard deviation.

The study was approved by the Institutional Review Board at Mayo Clinic.

Measurement of anti-pneumococcal protein antigen antibodies

We measured subjects' natural serum antibody levels ($\mu\text{g/mL}$) in response to pneumococcal surface protein A(PspA), pneumococcal surface protein C(PspC), pneumococcal choline-binding A(PcpA), and Pneumolysin(PLY) using standard enzyme-linked immunosorbent assay (ELISA) methods. PspA, PspC, and PcpA antigens were made at the University of Alabama, Birmingham [28–30], and PLY was provided by Dr. Rodney Tweten at the University of Oklahoma Health Sciences Center. Separate ELISA plates were used to measure reactivity of patients' serum to either 1 mg/ml RX1-PspA, 1 mg/ml PcpA, 1mg/ml PLY, or 1mg/ml PspC. All assays were run with a BSA coated plate to be used as a control to subtract out any titer that might exist to BSA blocking. Plates were washed with ELISA wash buffer (PBS-1.05% Tween-100mM NaCL-0.5mM NaH₂ PO₄ -1.5mM Na₂HPO₄), blocked with 1% bovine serum albumin PBS, incubated with immune sera overnight at 40°C, and then washed with ELISA wash buffer. Plates were then incubated for 2 hours at room temperature with biotin-conjugated goat anti-human immunoglobulin antiserum IgG(H+L), goat anti-human immunoglobulin antiserum IgM, goat anti-human immunoglobulin antiserum IgA, mouse anti-human immunoglobulin antiserum IgG1, or mouse anti-human immunoglobulin antiserum IgG2 (Southern Biotechnology Associates, Birmingham, AL), and washed with ELISA wash buffer. Plates were then incubated for 1 hour at room temperature with streptavidin-alkaline phosphatase (Southern Biotechnology Associates, Birmingham, AL), washed with ELISA wash buffer, and developed with p-Nitrophenyl phosphate (Sigma, St. Louis, MO). Absorbance was read at 405 nm. Data is reported in log reciprocal titer for the 33% maximal titer.

Ascertainment of asthma

Asthma as an independent (predictor) variable was assessed using predetermined criteria as summarized in Table 1. Only subjects who met criteria for definite asthma were included in this study.

House dust mite (HDM) and Staphylococcal enterotoxin B (SEB) extract-induced IL-5 production by cultured PBMCs

Th2 immune profile (IL-5 secretion in pg/mL) was assessed using cytokine secretion patterns after PBMC stimulation with HDM or SBE.. Details of this method were previously described [18]. HDM and SEB have been used to determine Th2 immune profiles in both

asthmatics and non-asthmatics [31, 32]. A recent study showed SEB-induced IL-5 was associated with atopic tendency [33].

Other variables

We determined atopic sensitization using allergen specific IgE levels (> 0.35 kU/L). Specific IgE levels of five allergens [cat (hair/dander), Short ragweed, June grass, house-dust mite (*D. pteronyssinus* and *D. farinae*), and *Alternaria*] common in our region were measured at the Mayo Clinic Clinical Immunology Laboratory using the Phadia immunoCAP system (Phadia, Uppsala, Sweden).

To assess the influence of pneumococcal colonization on serotype-specific pneumococcal antibody levels, we performed nasal swabs on all subjects at the time of enrollment and serotyped pneumococcal isolates. Serotyping was performed by Dr. Moon Nahm's laboratory at the University of Alabama, Birmingham.[34] In addition, we collected sociodemographic characteristics (age, gender, ethnicity, and socioeconomic status), history of pneumococcal vaccination (PCV-7 or PPV23) as documented in medical records, corticosteroid and other asthma medication use at the time of enrollment, and exposure to household tobacco smoke ascertained through medical record review and subject interview.

Data analysis

We used Student's t-test to compare pneumococcal protein IgG antibody levels (after natural-log transformation) in response to PspA, PcpA, PLY and PspC between subjects with and without asthma. Pearson's correlation coefficients assessed the relationship between log-transformed SEB-induced IL-5 secretion by PBMCs and levels of anti-pneumococcal protein antibody (IgG). For significant correlations, we determined whether adjustment by asthma status modified the relationship.

Results

Subject Characteristics

Characteristics of subjects are summarized in Table 2. Of the 30 subjects, 16 (53%) were male, 14 (47%) were children, 21 (70%) were Caucasians, and 16 (53%) were asthmatic. The median age for children and adults was 10.5 and 47 years, respectively. Of the 30 subjects, 7 had received PCV-7 (2 had received 3 doses and 5 received 4 doses) and 5 subjects had received PPV23. At the time of study enrollment, six subjects with asthma were on inhaled corticosteroid and one subject with asthma was on both inhaled and short-course systemic corticosteroids.

Relationship between pneumococcal protein antibody levels and asthma status

We compared the median levels of pneumococcal protein antigen IgG antibodies, including anti-PspA, anti-PcpA, anti-PspC, and anti-PLY, between individuals with and without asthma. The results are summarized in Table 3. There were no significant differences in antibody concentrations between asthmatics and non-asthmatics. This was also true for age-stratified results (adults vs. children). There were no significant differences in these antibodies between asthmatics with or without inhaled or systemic corticosteroid therapy (data not shown).

Correlation between Th2-immune profiles and pneumococcal protein antibody levels

We analyzed the correlation between pneumococcal protein antibody levels and HDM-induced IL-5 secretion by PBMC at day 7. There were no significant correlations between HDM-induced IL-5 levels and anti-PspA, anti-PspC, anti-PcpA, and anti-PLY levels.

However, SEB-induced IL-5 levels secreted by PBMC at day 7 was inversely correlated with anti-PspC antibody levels ($r = -0.53$, $p = 0.003$). Other pneumococcal protein antibodies were not correlated with the SEB-induced Th2-immune response. The results are summarized in Table 4 and Figure 1.

Effect modification of the correlation between Th2-immune profiles and anti-PspC antibody level by asthma status

We determined whether the correlation between anti-PspC antibody level and SEB-induced IL-5 secretion by PBMC was modified by asthma status. We found that the inverse relationship between IL-5 levels and anti-PspC antibody levels was significantly modified by asthma status ($r = -0.74$, $p = 0.001$ for asthmatics vs. $r = -0.06$, $p = 0.83$ for non-asthmatics). These results are presented in Figure 2.

Discussion

Known risk factors account for only 50% of all invasive pneumococcal disease (IPD)[35]. The role of other factors, such as asthma, in the development of IPD has not been studied until recently. We have previously reported a population attributable risk percent (PAR%) of 17% for IPD in adults with asthma [4]. Based upon the data provided in their study, Talbot et al [5] found a PAR% of 11% in adults with asthma. Given that the PAR% for all combined ACIP vaccine-eligible conditions in adults is estimated at around 24% [5], these data suggest that asthma may be a significant unrecognized risk factor for IPD and pneumococcal pneumonia.

The specific mechanisms underlying the increased risk of IPD among asthmatics are unknown. Specifically, the role of asthma status in humoral immunity against pneumococci after pneumococcal vaccination has not been adequately investigated. Recently, we reported significantly lower serotype-specific pneumococcal polysaccharide antibody levels in asthmatics compared with non-asthmatics (8.5 vs. 15.5 out of 23 pneumococcal serotypes, $p = 0.034$), [18] a finding that is consistent with prior literature [13], although the underlying mechanism remains unknown. Pneumococcal vaccinations did not account for this association. In our previous study, there was a trend toward an inverse relationship between the ratio of log-transformed IL-5/IFN- and the number of positive serotype-specific antibodies ($r = -0.36$, $p = 0.052$). These findings raise an important question: whether subjects with asthma also have lower antibody responses to cell surface pneumococcal protein antigens (PspA, PspC, and PcpA) and intracytoplasmic pneumococcal antigen (PLY), given the reported protective effect of these antibodies against pneumococcal infections or colonization [19–25]. Antibodies to pneumococcal surface or cytosolic protein antigens are known to elicit protective immunity, including PspA, PspC, PcpA, and PLY. PspA inhibits classical pathway complement activation, while PspC binds human factor H and thereby inhibits the alternative (amplification) complement pathway. Together they minimize opsonophagocytosis. PspC also inhibits the binding of secretory IgA; antibodies to PcpA are protective against lung infection and sepsis in mice. PLY can interfere with phagocyte function and slows ciliary beating; it also disrupts the surface integrity of the human respiratory epithelium. [19, 20, 36–40] Our study finding that subjects with asthma are able to make responses to these proteins suggests that they may be adequately responsive to some of the pneumococcal protein vaccines that are now being considered [41] and those pneumococcal conjugate vaccines currently available [42].

Although our previous study identified lower pneumococcal polysaccharide antibody levels in asthmatics than non-asthmatics, in the present study we found no significant differences in pneumococcal protein antigen antibody levels between the two groups. While this finding may have been due to limited statistical power, it may also imply that pneumococcal protein

antigens confer better immunogenicity or durability in overcoming the Th2-immune profile-mediated immune incompetence found in asthma than polysaccharide antigens. Inhaled or systemic corticosteroid therapy did not influence antibody levels. Marra SM et al reported that IL-5 levels were higher in induced sputum from patients with asthma and acute rhinitis compared with non-atopic subjects, which further suggests the role of the Th2-type immune response in upper and lower respiratory airways[43]. To examine the influence of the Th2 immune environment on pneumococcal protein antibody levels, we assessed the correlation between pneumococcal protein antibody levels and IL-5 secretion using cultured PBMC with SEB at day 7[33]. We found that the correlation varied depending on the type of pneumococcal protein antibodies: no correlation was found between IL-5 levels secreted by cultured PBMC with SEB and anti-PspA anti-PcpA levels, however, a significant inverse correlation was noted between IL-5 secretion by PBMC cultured with SEB at day 7 and anti-PspC antibody levels ($r = -0.53$, $p = 0.003$). The same inverse correlation with anti-PLY approached statistical significance ($r = -0.34$, $p = 0.069$). Additionally, we found that the inverse relationship between IL-5 levels and anti-PspC antibody levels was significantly modified by asthma status ($r = -0.74$, $p = 0.001$ for asthmatics vs. $r = -0.06$, $p = 0.83$ for non-asthmatics). This data suggests that the potential negative impact of a Th2 immune profile on anti-PspC antibody levels differentially impacts subjects with asthma.

Khan et al. demonstrated that endogenous IL4, a Th2 cytokine, inhibits formation of IgG2a and IgG2b antibody in a mouse model [17]. In support of this finding, Hales et al. reported that children with asthma or house-dust mite sensitization have significantly lower IgG₁ titers against H. influenza antigens (P4 and P6) and PspC than non-asthmatics or those without house-dust mite sensitization at 5 years of age[44]. In addition, children with house-dust mite sensitization had lower PspA titers at 3 years of age. These findings confirm previous reports on lower anti-P6 antibody[45] and lower serotype-specific pneumococcal titers[13, 18]. However, the clinical significance of our findings, the specific mechanisms by which Th2 cytokines or immune responses affect humoral immune responses to pneumococcal antigens, and the role of asthma status in modifying this relationship all require further investigation. However, our study findings may provide some explanation for the association between asthma and IPD/pneumococcal pneumonia. PspC (also called choline-binding protein A) is a cell-surface associated protein and a major adhesin to polymeric immunoglobulin receptor (PIgR). It enables pneumococci to bind to nasopharyngeal epithelial cells, resulting in colonization and invasion through epithelial reverse transcytosis [36, 46, 47]. Mice infected with a PspC-deficient strain of *S. pneumonia* were found to have 100-fold reduction in colonization[48]. Importantly, PIgR expression was able to be up-regulated in subjects with asthma, allowing increased colonization and invasion through reverse transcytosis since Th2-cytokines, such as IL-4, upregulate PIgR expression[49]. PspC has been reported to be immunogenic and antibody to PspC has been shown to provide passive protection in a mouse model[48]. Therefore, it will be important to replicate our study findings in a larger epidemiologic study, looking specifically at the relationship between asthma, Th2 immune profile, and anti-PspC antibody responses, and to determine the clinical significance of a Th2 immune profile on anti-PspC antibody levels.

The main strength of our study is the use of the same criteria for the definition of asthma as our previous study, in which we found an increased risk of serious pneumococcal diseases in patients with asthma and a suboptimal serotype-specific pneumococcal polysaccharide antibody levels in asthmatics.

Our study has several limitations. First, the sample size was relatively small. Second, because it was cross-sectional in nature, had only baseline outcome measurements. Third, no immunoglobulin or complement levels and other cytokine data (e.g., IL-33 or 22) were available to our study.

In conclusion, we found no significant differences in anti-pneumococcal protein antigen antibody levels between subjects with and without asthma. Asthma status, however, was found to be an important effect modifier of the negative influence of the Th2-immune response on anti-PspC antibody levels.

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Figure 1.
Correlation between natural log-transformed anti-PspC antibody level ($\mu\text{g/mL}$) and SEB-induced IL-5 secretion by PBMCs in pg/mL ($r = -0.53$, $p = 0.003$)

Figure 2.
Correlation between natural log-transformed anti-PspC antibody level ($\mu\text{g/mL}$) and SEB-induced IL-5 secretion by PBMCs in pg/mL by asthma status

Table 1**Criteria for asthma**

Patients were considered to have <i>definite</i> asthma if a physician had made a diagnosis of asthma as well as two of the following conditions or if each of the following three conditions were present:	
1	History of cough and/or dyspnea, plus wheezing, OR history of cough and/or dyspnea plus wheezing on examination
2	Substantial variability in symptoms from time to time or periods of weeks or more when symptoms were absent, and
3	Two or more of the following <ul style="list-style-type: none"> • Nocturnal cough • Nonsmoker (14 years or older) • Nasal polyps • Blood eosinophilia • Positive wheal and flare skin tests or elevated IgE • History of atopy (atopic conditions) • Pulmonary function tests showing one FEV₁ or FVC less than 70% predicted or methacholine challenge test showing greater than 20% or greater decrease in FEV₁ • Favorable clinical response to bronchodilator

Table 2

Demographic and clinical characteristics of subjects with asthma and those without asthma

	Asthmatics (N=16)	Non-asthmatics (N=14)
Age at index date (years)		
Median (Interquartile range)	20.0 (11.0 – 47.5)	28.0(12.0 – 47.0)
Gender		
Male	9 (56.3%)	7 (50.0%)
Ethnicity		
Caucasian	11 (69%)	10 (71%)
Non-Caucasian	5 (31%)	4 (29%)
Educational status *		
<High school education	1 (6.3%)	0 (0.0%)
High school graduate	5 (31.3%)	5 (35.7%)
College graduate	10 (62.5%)	9 (64.3%)
Allergic sensitization		
Yes	9 (56%)	4 (29%)
No	7 (44%)	10 (71%)
Pneumococcal colonization		
Yes	2 (12.5%)	2 (14.3%)
No	14 (87.5%)	12 (85.7%)
Pneumococcal vaccinations		
No vaccination	8 (50.0%)	10 (71.4%)
** PCV7 only	4 (25.0%)	3 (21.4%)
# PPV23 only	4 (25.0%)	1 (7.1%)
History of high-risk conditions † for IPD		
Immunosuppressive therapy > 2 years prior to enrollment ††	2 (12.5%)	1 (7.1%)
Diabetes mellitus - Type II	2 (12.5%)	0
Alcohol abuse	1 (6.3%)	0
Rheumatoid arthritis	0	1 (7.1%)

* For children, parents' educational status was used;

** heptavalent pneumococcal conjugate vaccine;

23-valent pneumococcal polysaccharide vaccine;

† High-risk conditions are based on the ACIP-recommended pneumococcal vaccine eligible conditions, and these conditions are not mutually exclusive because subjects can have more than one condition;

†† defined as a history of immunosuppressive drug intake for malignant neoplasms, leukemias, lymphomas, Hodgkin disease, and solid organ transplantation

Table 3

Asthma status and pneumococcal protein antibody levels

Serotype	Pneumococcal Protein Antibody Levels*		p-value**
	Individuals with asthma (n=16)	Individuals without asthma (n=14)	
Anti-PspA (µg/mL)	2.02±0.28	2.12±0.26	0.80
Anti-PcpA (µg/mL)	1.75±0.20	2.02±0.16	0.31
Anti-PspC (µg/mL)	1.97±0.35	1.92±0.30	0.92
Anti-PLY (µg/mL)	-0.93±0.34**	-0.70±0.29**	0.62

* Natural log-transformed antibody concentration and negative value indicates original lab values to be between 0 and 1.

** p-values were calculated by Student t-test.

Table 4

Correlation between Th2-immune response and pneumococcal protein antibody levels

	Correlation with IL-5 secretion by PBMC culture with HDM at day 7*	p-value	Correlation with IL-5 secretion by PBMC culture with SEB at day 7*	p-value
Anti-PspC (µg/mL)	0.33	0.080	−0.53	0.003
Anti-PLY (µg/mL)	0.23	0.225	−0.34	0.069
Anti-PspA (µg/mL)	0.27	0.156	−0.22	0.248
Anti-PcpA (µg/mL)	0.14	0.459	−0.19	0.326

Correlation was measured by Pearson's correlation coefficient; HDM: house-dust mite; SEB: staphylococcal enterotoxin B