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Research article

Chlamydia pneumoniae immunoglobulin E antibody levels in patients with asthma compared with non-asthma

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ABSTRACT

Chlamydia pneumoniae is an obligate intracellular bacterium that causes respiratory infection in adults and children. There is evidence for an association between atypical bacterial pathogens and asthma pathogenesis. We sought to determine whether past *C. pneumoniae* infection triggers *C. pneumoniae*- IgE antibodies (Abs) in asthmatics and non-asthmatics, who had detectable IgG titers. *C. pneumoniae* IgE Abs were quantified using enzyme immunoassay (EIA). *C. pneumoniae* IgE Ab levels were higher in asthmatics compared with non-asthmatics. There was no correlation found between total serum IgE levels and specific *C. pneumoniae* IgE Ab levels. *C. pneumoniae* infection may trigger IgE-specific responses in asthmatics.

1. Introduction

Chlamydia pneumoniae, an obligate intracellular organism, causes acute respiratory infection in both adults and children [1]. *C. pneumoniae* has been implicated in the pathogenesis and exacerbation of asthma [2]. *C. pneumoniae* infection *in vitro* activates monocytes/macrophages to produce cytokines that may contribute to the pathology observed in asthma [1], but may diminish protective immune responses against respiratory infection [3, 4]. The prevalence of asthma is increasing in the United States, especially in inner city minority populations [5]. Asthma causes significant excess morbidity in children especially in those living in inner city areas [5]; exposure to environmental factors and infections, including *C. pneumoniae,* can trigger asthma symptoms.

Two studies from our laboratory found specific *C. pneumoniae* immunoglobulin (Ig) E antibody (Ab) responses in human sera [6, 7]. Using a Western blot technique, Emre *et al* found that *C. pneumoniae* IgE Abs were detected in 12 of 14 (85.7%) *C. pneumoniae* culture-positive children with wheezing compared with 1 of 11 (9.1%) culture-positive children with pneumonia, 2 of 11 (18.2%) culture-negative asthmatic children with wheezing and 2 of 9 (22.2%) culture-negative asymptomatic patients [6]. A subsequent study in children with stable allergic asthma, who did not have acute symptoms and negative upper respiratory polymerase chain reaction (PCR) for *C. pneumoniae*, demonstrated that *C. pneumoniae* specific IgE Abs were detected in 12/45 (27.0%) stable allergic asthmatics also using Western blotting [7]. *C. pneumoniae* T lymphocyte memory responses were detected only in patients who had *C. pneumoniae* specific-IgE Abs [7]. The presence of *C. pneumoniae* specific IgE Abs in stable asthmatics (without acute airway infection) with *C. pneumoniae* provides further evidence for ongoing stimulation of allergic responses by *C. pneumoniae* [7].

IgE use as a biomarker can be helpful in diagnosis, treatment, and predicting the outcome of allergic disease [8]. In asthma, IgE has an established role in defining different asthma phenotypes and its treatment [8]; however, specific IgE is important for identification of allergens that trigger asthma [8]. Lux *et al* studied test performance parameters of specific IgE tests for diagnosing occupational asthma and evaluated the impact of allergenic components and the implementation of measures for test validation [9]; specific IgE test performance was satisfactory for a range of allergens [9].

Others have studied the immunologic response in respiratory infections caused by *Mycoplasma pneumoniae (M. pneumoniae),* which is an extracellular pathogen [10]. Increased total serum IgE levels were higher in hospitalized children diagnosed with different *M. pneumoniae*-related extra-pulmonary diseases compared with children that developed only classical respiratory illness due to *M. pneumoniae* [10]. The authors concluded that IgE probably had no role in the pathogenesis of these clinical manifestations, but the immune imprinting to produce IgE (i.e. cellular processes and cytokine environment) [10].

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The purpose of this study was to develop a modified direct EIA to quantify *C. pneumoniae* IgE Abs in serum specimens. Using this assay, we investigated whether past *C. pneumoniae* infection triggers production of *C. pneumoniae*-IgE Abs in adult subjects with and without asthma, who had positive *C. pneumoniae*-IgG titers.

2. Materials and methods

2.1. Participants

De-identified serum samples were selected from a cohort study of adult subjects with a diagnosis of asthma or no asthma who were enrolled at an inner-city primary care clinic (Brooklyn, NY). The subjects classified were based on physician-based diagnosis of asthma, and the Global Initiative for Asthma (GINA) guidelines were followed [11]. Known variables included age, gender, total serum IgE, and *C. pneumoniae* IgG status. The SUNY Downstate Medical Center Institutional Review Board (IRB) approved the protocol, and informed consent was obtained from all subjects. Study procedures were followed in accordance with guidelines involving human subjects

2.2. Ig determination: total serum IgE

Total serum IgE levels were determined using the UniCap Total IgE fluoroenzyme immunoassay (Pharmacia and Upjohn Diagnostics, Freiburg, Germany), according to manufacturer's recommendation. Reference range for adult serum; 20–100 IU/mL. ImmunoCAP IgE is a solid-phase immunoassay. Allergen components that are immobilized on a solid substrate in a microarray format are incubated with human serum or plasma samples to detect specific IgE antibodies. Binding of the specific IgE antibodies to the immobilized allergen components is detected by the addition of a secondary fluorescence-labeled anti-human IgE antibody. The procedure is followed by image acquisition using an appropriate microarray scanner. The units for total IgE are determined and the test results are analyzed using Microarray Image Analysis Software. Excitation wavelength is 532 nm and/or 635 nm

2.3. Detection of C. pneumoniae IgG Abs

Detection of *C. pneumoniae* IgG Abs was performed using an EIA (Labsystems Diagnostics; Vantaa, Finland). *C. pneumoniae*-specific IgG Abs were measured in serum according to manufacturer's recommendations. The quantification of IgE *C. pneumoniae* Abs in serum was determined using a modification of the LabSystems EIA. Our assay detected IgE against native *C. pneumoniae* outer membrane protein (CpOMP). It is prepared from native elementary bodies (EBs) originating in infected human cells in culture. The suspension is then inactivated by gamma irradiation

2.4. Detection of C. pneumoniae IgE Abs

Briefly, samples (100 ul) were added to plates and incubated 1 h at room temperature. Goat polyclonal antihuman IgE (ICN Biomedicals, Aurora, OH) diluted 1:100 in tris buffered saline (TBS) wash buffer (100 ul) was added and incubated 1 h. Wells were washed 3X in wash buffer. Rabbit anti-goat peroxidase labeled antibody (ICN Biomedicals), diluted 1:1000 in wash buffer was then added (100 ul), and incubated for 1 h. The wells were washed 3X in wash buffer and developed in 3, 3′, 5, 5′-tetramethylbemzidine (TMB) substrate solution (100 ul) (Bio-Quant) for 5–10 min. The reaction was stopped by adding 1N H_2SO_4 (100 ul). The negative control was a serum sample that was negative for *C. pneumoniae* Ab. Samples were run in duplicate. The plates were read using an automated microplate reader (Model Elx800; Bio-Tek Instruments, Winooski, VT); optical density (O.D.) measurements were read at 450 nm. For determination of *C. pneumoniae* IgE Ab levels, data were reported as Abs 450 (O.D. value) (range: $\geq$ 0.464 OD value, positive). Final O.D. value reported was subtracted from chromogen blank O.D. value (background). Calculation of the cutoff value: negative control mean absorbance value +0.025

2.5. Statistical analysis

The results are presented as the mean ± standard deviation (SD) unless otherwise specified. Rank sum tests were performed for statistical comparisons between the two groups. Pearson correlations were used to compare total serum IgE levels and IgE *C. pneumoniae* Ab levels. All statistical analyses were performed using SAS 9.4 software (SAS Institute, Cary N.C.). A *P*-value <0.05 was considered statistically significant. Patient clinical information was stored using Microsoft Excel

3. Results

3.1. Study population

The demographics are shown in Table 1. A total of 44 patients were enrolled: 22 with asthma and 22 non-asthmatic controls. The mean age of the asthmatics was 43.0 ± 7.0 (range: 28–53 y/o), and 31.0 ± 5.9 (range: 21–38 y/o) for the non-asthmatics controls ($P = 0.009$). As shown in the table, there were 77.0% female and 23.0% males versus 72.0% female and 28.0% males in the control group (*P*=NS). Total serum IgE Ab levels were similar in asthmatic compared with non-asthmatic controls (186 IU/mL± 159 vs. 170 IU/mL ± 142; $P = 0.720$) (Table 1)

3.2. C pneumoniae IgE Ab responses

C. pneumoniae IgE Abs were detected in both groups. Ninety-five percent (21/22) asthmatics had positive *C. pneumoniae* IgE Ab responses levels compared with 45.0% (10/22) of controls. *C. pneumoniae* IgE Ab levels (Ab450) were significantly higher in asthmatics compared with non-asthmatic controls (1.10 ± 0.30 vs. 0.39 ± 0.34; $P < 0.001$) (Figure 1). There was no correlation found between total serum IgE levels and specific *C. pneumoniae* IgE Ab levels (Pearson correlation, R = –0.004, $P = 0.981$) (Data not shown).

4. Discussion

We developed a modified EIA to quantify *C. pneumoniae* IgE Abs in human sera. Using this assay, we demonstrated that *C. pneumoniae* IgE Ab levels were significantly higher in adult asthmatic patients compared with non-asthmatic controls. These results are similar to those previously reported in children using Western blotting [6]. There was no significant correlation between levels of total serum IgE and specific *C. pneumoniae* IgE Ab responses. Our central finding was that 95% of asthmatic patients with detectable *C. pneumoniae* IgG Abs also had IgE *C. pneumoniae* Abs and these Ab levels were higher in asthmatics compared with non-asthmatic controls.

Table 1. Participant characteristics.

Characteristic	Asthma (N = 22)	Non-Asthma (N = 22)	*P* value
Age (y)	43 ± 7.0	31 ± 5.9	0.009*
	(Range: 28–53)	(Range: 21–38)	
Gender (N, %)			
Female	17 (77)	16 (72)	NS
Male	5 (23)	6 (28)	NS
Total serum IgE (IU/mL)	186 ± 159	170 ± 142	0.720#

* *P*-value statistically significant.
\# Fisher's exact test.

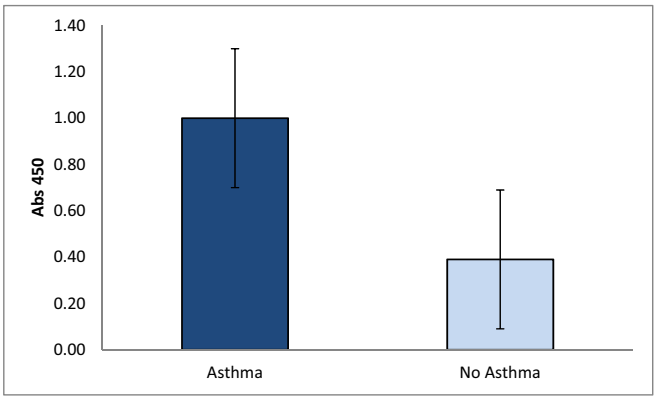

Figure 1. IgE *C. pneumoniae* Ab levels in serum of asthmatic patients compared with non-asthmatic controls. Asthma (N = 22), non-asthma (N = 22). $P < 0.001$. Data are represented as Abs 450 (OD value).

As stated above, Emre, *et al* in our previous study, used whole cell lysates of elementary bodies (EBs) from *C. pneumoniae* isolates TW183 and 2023 for the Western blots [6]. However, no specific *C. pneumoniae* proteins were identified consistently [6]. Most children with culture documented *C. pneumoniae* infection are seronegative using micro immunofluorescence. However, Kutlin, *et al*, found that these children did have Ab that was detectable by Western blotting [12]. The *C. pneumoniae* isolates used were TW-183, 2023, BAL-14 and 379CJM [12]. As with the *C. pneumoniae* IgE Ab study [6], there was no specific pattern of reactivity to *C. pneumoniae* proteins in sera from culture-positive or culture-negative children [12].

A published study of *C. pneumoniae* IgE Abs was by Hahn, *et al* [13], who examined *C. pneumoniae* IgE Abs in children also using a Western blot technique. Proteins of purified EBs from *C. pneumoniae* TW183 were used as the antigen [13]. Hahn, *et al* found that *C. pneumoniae*-specific IgE was present in 50% (33/66) adult asthmatic patients and appeared to be associated with disease severity [13]. *C. pneumoniae* IgE was detected in 21.0% of mild intermittent asthma vs 79.0% of severe persistent asthmatics [13]. No specific *C. pneumoniae* proteins were identified consistently, which was similar to our findings [6, 12].

Similar to studies of Hahn, *et al* [13], Patel, *et al* [14] demonstrated the prevalence and identity of *Chlamydia*-specific IgE in children with asthma and other respiratory symptoms [14]. *Chlamydia* specific IgE was isolated from bronchoalveolar lavage (BAL) fluid and serum samples. The authors developed a Western blot assay to determine the presence, prevalence, and identity of *Chlamydia*-specific IgE in serum and BAL fluid [14]. Proteins of purified EBs from *C. pneumoniae* TW183 and *C. trachomatis* (serovar E) were used as the antigen [14]; protein content was normalized using the Bradford protein assay [14]. *Chlamydia*-specific IgE was detected in the serum and/or BAL of 54% patients suffering from chronic respiratory disease, but in none of the healthy control sera [14]. Of the 74 BAL culture-positive patients, 68 (92%) tested positive for *Chlamydia* specific IgE [14]. Only three chlamydial antigens induced *C. pneumoniae* specific IgE responses in BAL (lipopolysaccharides (LPS), 250 kDa and major outer membrane protein (MOMP), while five chlamydial antigens induced *C. trachomatis* specific IgE responses in BAL (LPS, C reactive protein (Crp A), MOMP, and 250 kDa, 64-6 kDa) [14]. Five chlamydial antigens induced *C. pneumoniae* specific IgE responses in serum (LPS, Crp A, heat shock protein (HSP) 60, putative outer membrane protein (POMP), and 250 kDa), while five chlamydial antigens induced *C. trachomatis* specific IgE responses in serum (MOMP, HSP 60, POMP, 250 kDa and CrpA). Thus, similar to the other *C. pneumoniae* IgE studies listed above [6, 12, 13], no specific *C. pneumoniae* proteins were identified consistently.

Wheezing in *C. pneumoniae* infection may be related to the development of an organism specific IgE response that leads to the release of chemical mediators that cause airway inflammation [6]. Thus, in some patients with *C. pneumoniae* infection, production of specific *C. pneumoniae* IgE may be an underlying mechanism leading to asthma [6]. This may indicate persistent infection that can lead to ongoing or worsening of asthma symptoms. Treating the underlying *C. pneumoniae* infection may therefore improve symptoms.

The modified EIA used in this study detected IgE against *C. pneumoniae* OMP, one antigen, compared to multiple proteins present in Western blots. The advantage of using an EIA is quantification, greater specificity, sensitivity and reproducibility. Given the potential relevance of a positive IgE test against *C. pneumoniae* for diagnosing infection as a co morbidity in patients with asthma, it would be of great benefit to have an automated test available. It would be feasible to monitor patients for *C. pneumoniae*-IgE using this assay.

The ages of our asthmatic patients were higher than the ages of the non-asthmatic controls. It could be that repeated infections occur with increasing age and may lead to higher levels of specific-IgE. However, we did not find a trend with age to higher specific IgE levels in this study population. Therefore, we believe that the difference in specific-IgE responses between subjects with or without asthma reflects their disease status and not their age. Studies of Tanaka, *et al* found that the mean age of patients with a longitudinal increase in IgE was higher than that of patients with a decrease or no change in IgE, suggesting that an increase in total IgE is associated with aging among patients with uncontrolled asthma [15]. In contrast, other studies reported that mean total IgE levels in patients with asthma decreased with age [16].

Our last finding was there was no significant correlation between total serum IgE levels and specific-*C. pneumoniae* IgE Ab levels in asthmatic patients compared with non-asthmatic controls. This is in accordance with our previous study [17], and may be due to the different specificity of the immune response or demographic differences.

Total serum IgE levels were similar in asthmatic and non-asthmatics ($P = 0.720$). A possible explanation may be the role of IgE in different asthmatic endotypes/phenotypes [18]. A distinct subgroup of patients present with asthma without evidence of Th2/eosinophilic inflammation, which are referred to as "low Th2" asthma phenotype; these patients are less atopic and have low IgE levels [18]. In addition, asthma can be divided into two types, extrinsic and intrinsic; mean IgE levels were significantly different between the two groups [19]. Longitudinal changes in total IgE levels and asthma status can also contribute to the pathophysiology of severe asthma [15]. Data suggest that a longitudinal increase in total IgE is associated with both poor asthma control and *Aspergillus*-specific IgE in patients with adult asthma [15].

5. Conclusions

In summary, we demonstrated that adults with asthma have increased specific *C. pneumoniae* IgE Ab responses, using a direct EIA. Deficient host response mechanisms in asthmatics may contribute to a higher prevalence of these specific Ab responses and increased susceptibility to *C. pneumoniae* infection.

Declarations

Author contribution statement

T. Smith-Norowitz: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

S. Kohlhoff: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

J. Loeffler and Y. Huang: Performed the experiments; Analyzed and interpreted the data.

Y. Norowitz and E. Klein: Performed the experiments.

M. Hammerschlag: Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

R. Joks: Contributed reagents, materials, analysis tools or data.

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Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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