

Detection of Anti-*Chlamydia pneumoniae* IgE in Children with Reactive Airway Disease

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An association of *Chlamydia pneumoniae* infection and reactive airway disease has been demonstrated in children. To determine if *C. pneumoniae* infection triggers production of *C. pneumoniae*-specific IgE, sera were examined from 45 children with and without *C. pneumoniae* infection. Anti-*C. pneumoniae* IgE was demonstrated by immunoblotting in 12 (85.7%) of 14 culture-positive asthmatic patients with wheezing compared with only 1 (9.1%) of 11 culture-positive patients with pneumonia, 2 (18.2%) of 11 culture-negative asthmatic children with wheezing, and 2 (22.2%) of 9 culture-negative asymptomatic patients. The most commonly recognized proteins were at 98 (82.4%), 78 (58.8%), 58–60 (70.6%), and 36 kDa (64.7%). The presence of anti-*C. pneumoniae* IgE by immunoblotting was not associated with the presence of anti-*C. pneumoniae* IgG and IgM by microimmunofluorescence. These results suggest that production of specific IgE may be an underlying mechanism leading to reactive airway disease in some patients with *C. pneumoniae* infection.

The association between clinical symptoms of airway reactivity and respiratory tract infections in children has been well documented. Infections with viruses such as respiratory syncytial virus, parainfluenza virus, and rhinovirus have been identified in 40%–60% of children with acute exacerbations of asthma [1–3]. Infection with other organisms, such as *Mycoplasma pneumoniae*, have also been documented in up to 25% of children with wheezing [4]. A number of mechanisms have been proposed to explain airway hyperresponsiveness following infections, including the development of organism-specific IgE antibody.

We previously reported persistent *Chlamydia pneumoniae* infection in several adult patients for periods of up to 11 months, 1 of whom was thought to have asthmatic bronchitis and was treated accordingly [5]. In 1991, Hahn et al. [6] reported an association between serologic evidence of acute *C. pneumoniae* infection and adult-onset asthma and asthmatic bronchitis. Subsequently, we examined the prevalence of *C. pneumoniae* infection in children with acute exacerbations of reactive airway disease [7]. *C. pneumoniae* was isolated from 11% of children with acute episodes of wheezing compared with 4.9% of age- and sex-matched healthy controls. Clinical and laboratory improvement of the reactive airway disease was documented after successful treatment and eradication of *C. pneumoniae* infection in 75% of asthmatic children. To further investigate the mechanism of *C. pneumoniae*-associated reactive airway disease, we examined sera from these children and

from other patients with and without *C. pneumoniae* infection to determine whether there was a *C. pneumoniae*-specific IgE response.

Materials and Methods

Study population. Sera were obtained from children who were divided into groups based on their clinical and culture status. Group 1 had 14 children with asthma who presented with an acute episode of wheezing associated with culture-proven *C. pneumoniae* infection. Group 2 included 11 children with community-acquired pneumonia who had cultures positive for *C. pneumoniae* and were not wheezing. Group 3 had 11 asthmatic children with acute exacerbations but cultures negative for *C. pneumoniae*, and group 4 contained 9 asymptomatic patients with cultures negative for *C. pneumoniae*.

Chlamydial serology. Serum samples for determination of antibody to *C. pneumoniae* were collected and frozen at -70°C . Serologic studies were done with the microimmunofluorescence (MIF) method at the University of California, San Francisco, against the prototype strain TW183 with use of anti-IgG and anti-IgM conjugates.

Chlamydial strains. *C. pneumoniae* strains used in this study were TW183 and 2023 (ATCC VR1356). *Chlamydia trachomatis* strains used were serovars C (ATCC VR578) and D (ATCC VR885). Organisms were grown in HEp-2 cells, harvested, and purified on a Percoll gradient [8]. The final product contained 1.0×10^6 ifu/mL. A modified Laemmli buffer system to reduce and denature proteins (Bio-Rad Laboratories, Melville, NY) was used [9].

Separation of *C. pneumoniae* antigens by SDS-PAGE. Proteins of purified elementary bodies were separated by discontinuous SDS-PAGE with 12% polyacrylamide gels [10]. About 10 μg of protein was added to gels. Protein bands were visualized by staining with fast Coomassie stain (Promega, Madison,

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Table 1. *C. pneumoniae* IgE in *C. pneumoniae* culture-positive and culture-negative patients.

Group (n)	No. (%) with <i>C. pneumoniae</i> IgE	P*
1, culture-positive asthmatic (14)	12 (85.7)	
2, culture-positive pneumonia (11)	1 (9.1)	<.001
3, culture-negative asthmatic (11)	2 (18.2)	<.001
4, culture-negative asymptomatic (9)	2 (22.2)	<.006

* Fisher's exact test vs. group 1.

WI). Molecular weights were determined by using protein standards (Bio-Rad).

Immunoblot analysis. Western blots were done after *C. pneumoniae* proteins were transferred to nitrocellulose membranes. Before IgE immunoblotting, sera were immunoabsorbed in an anti-human IgG-agarose system (Sigma, St. Louis) to remove IgG. Subsequently, blots were blocked overnight at 4°C with 5% nonfat dry milk, washed with 1× PBS/Tween 20 (six times for 2 h), incubated overnight with human immune serum (diluted 1:10 in 3% bovine serum albumin), washed again with 1× PBS/Tween 20 (six times for 2 h), incubated overnight with goat anti-human IgE (diluted 1:1000) conjugated to alkaline phosphatase (Sigma), and washed with 1× PBS/Tween 20 followed by two washes with PBS. Color development was observed on the addition of nitroblue tetrazolium/5-bromo-4-chloro-3-indolyl phosphate (Kirkegaard & Perry, Gaithersburg, MD). Protein transfer was monitored by staining the blots with Ponceau S (Sigma), and prestained molecular weight markers were used (Bio-Rad).

Statistical analysis. Statistical analysis for the comparison of *C. pneumoniae*-specific IgE was done using Fisher's exact test.

Results

The ages of the asthmatic children (groups 1 and 3) ranged from 5 to 15 years (means ± SEs, 7.8 ± 3.35 and 10.5 ± 3.4 , respectively). The ages of nonasthmatic children (groups 2 and 4) ranged from 6 to 17 years (means ± SEs, 7.0 ± 3.1 and 12.1 ± 3.2 , respectively). Serologic evidence of acute *C. pneumoniae* infection, defined as a 4-fold rise in IgG, a single IgG titer of $\geq 1:512$, or an IgM titer of $\geq 1:16$, was present in 1 (7.1%) of 14 and none of 11 culture-positive children (groups 1 and 2) and in 1 (9.1%) of 11 and 1 (11.1%) of 9 culture-negative children (groups 3 and 4). Preexisting infection, defined as an IgG titer of 16–512, was present in 4 (28.6%) and 3 (27.3%) of the culture-positive children and 2 (18.2%) and 1 (11.1%) of the uninfected children, respectively. The majority of the culture-positive children did not have any detectable anti-*C. pneumoniae* IgE antibody by MIF (group 1, 9 [64.3%]; group 2, 7 [77.8%]).

As shown in table 1, anti-*C. pneumoniae* IgE was detected with immunoblotting in the acute-phase sera of 12 (85.7%) of

the 14 asthmatic children in group 1 compared with 1 (9.1%) of 11 children with community-acquired pneumonia in group 2. Among the culture-negative children, 2 (18.2%) of 11 asthmatic children and 2 (22.2%) of 9 asymptomatic children had anti-*C. pneumoniae* IgE. The presence of *C. pneumoniae*-specific IgE by immunoblotting was not associated with the presence of *C. pneumoniae* IgG and IgM by MIF in these children.

The patterns of reactivity against TW183 and 2023 antigens were similar in all patient groups. The most commonly recognized proteins were at 98 (82.4%), 78 (58.8%), 58–60 (70.6%), and 36 kDa (64.7%). Reactive bands at 106, 52, 46–48, 40, and 29–32 kDa were observed in 31.3%–43.8% of the sera. Representative blots are shown in figure 1.

Sera from 9 children who were seropositive for anti-*C. pneumoniae* IgE were also tested for IgE against *C. trachomatis* (serovar C) by immunoblotting. Six (66.7%) showed reactivity to *C. trachomatis* proteins, including 29–32, 48, and 58–60 kDa (figure 1). Sera from 5 negative control patients negative for anti-*C. pneumoniae* IgE were also negative for anti-*C. trachomatis* IgE.

Discussion

In 1981, Welliver et al. [2] demonstrated the development of respiratory syncytial virus-specific IgE using ELISA in 65.7% of infants with wheezing and 9.1% of infants without wheezing. The patients with wheezing also had significantly higher virus-specific IgE titers than did patients with no wheezing. Subsequently, the same investigators found a similar relationship in children with parainfluenza virus infections [3]. Patients experiencing wheezing with or without croup had significantly greater virus-specific IgE responses than did patients with upper respiratory tract illness. We were able to demonstrate the presence of anti-*C. pneumoniae* IgE by immunoblotting in 85.7% of culture-positive children with wheezing, in contrast to only 9.1% of culture-positive patients with community-acquired pneumonia who were not wheezing. Two (18.2%) of 11 culture-negative asthmatic patients also had anti-*C. pneumoniae* IgE. The possibility of inadequate nasopharyngeal sample collection or previous *C. pneumoniae* infection could not be ruled out in these patients. Thus, there was a strong association between the presence of anti-*C. pneumoniae* IgE and the presence of wheezing during *C. pneumoniae* infection. As reported with respiratory syncytial virus and parainfluenza virus infections, wheezing in *C. pneumoniae* infection may be related to the development of an organism-specific IgE response that may result in the release of chemical mediators leading to bronchospasm, airway inflammation, and airway reactivity. In contrast to viral infections, which may last for only 2–3 weeks, *C. pneumoniae* infection can be chronic, lasting for a year or longer [5]. Thus, unless *C. pneumoniae* is eradicated with antibiotic therapy, antigenic stimulation leading to specific IgE production may be persistent. This may explain

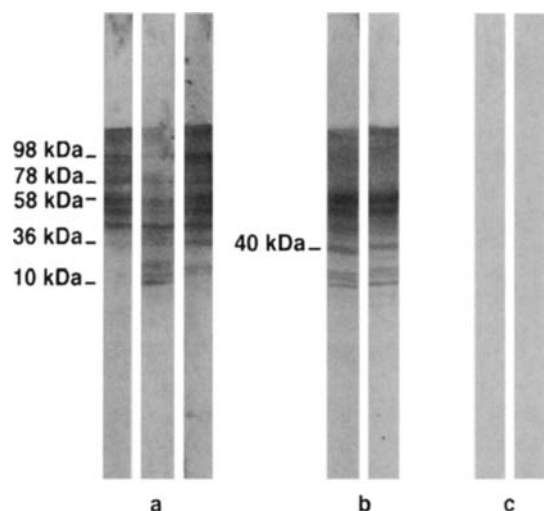


Figure 1. Immunoblot analysis of whole cell lysates of *Chlamydia* elementary bodies with human sera. Lanes a, 3 examples of sera from patients with asthma and an acute episode of wheezing associated with culture-documented *C. pneumoniae* infection, demonstrating IgE reactivity to 98-, 78-, 58-, and 36-kDa proteins of *C. pneumoniae*. Lanes b, 2 sera from *C. pneumoniae*-positive asthmatic children blotted against *C. trachomatis* proteins. Lanes c, sera from children with community-acquired pneumonia with positive *C. pneumoniae* cultures blotted against *C. pneumoniae* proteins.

the protracted clinical course of asthma in some patients despite intensive therapy with bronchodilators and steroids.

Reactivity to IgE was similar to that previously reported for IgG by Campbell et al. [10] and Iijima et al. [11]. We also found that the 98-kDa protein was the most commonly recognized antigen; however, the intensity of the bands was rather weak. The 36-kDa protein, which was recognized in 64.7% of sera, had the strongest reactivity. Similar to results of other studies, we found the sera to react with some proteins of both *C. pneumoniae* and *C. trachomatis*. Although sera reacted to similar chlamydial proteins, there seems to be a clear difference between the pattern of reactivity between these two species. However, *C. trachomatis* infection sometime in the past cannot be ruled out.

Transient elevation of total serum IgE levels have been demonstrated during the acute phase of viral as well as *M. pneumoniae* infections, even in the absence of wheezing [1, 12–14]. Low titers of respiratory syncytial virus- and parainfluenza virus-specific IgE have also been detected in some children with limited upper respiratory tract infections due to these agents [2, 3]. Thus, the IgE antibody response, along with IgG

and IgM antibody development, seems to be an integral part of the host response to a variety of infections. The demonstration of anti-*C. pneumoniae* IgE in 2 (22.2%) of 9 asymptomatic children may well be a marker of *C. pneumoniae* infection that we failed to demonstrate by culture. One of these 2 patients also had anti-*C. pneumoniae* IgG of 1:512, which further supports this hypothesis.

The results of this study support our clinical observations on the association of *C. pneumoniae* infections and reactive airway disease. Airway reactivity seems to be mediated through a specific IgE response. The role of host factors as well as other immune mechanisms in the pathogenesis of *C. pneumoniae* infections needs to be further investigated.

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