The Bronchial Lavage of Pediatric Patients with Asthma Contains Infectious Chlamydia

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There has been a worldwide increase in the incidence of asthma, and the disease has greatly impacted the public health care system. Chlamydia pneumoniae has been reported as a possible contributing factor in asthma. The organism has been detected by polymerase chain reaction (PCR) in bronchial tissue, but there has been no direct evidence of viability. To determine the frequency of viable Chlamydia in children, blood and bronchoalveolar lavage were collected from 70 pediatric patients undergoing flexible fiberoptic bronchoscopy. Forty-two of these patients had asthma, whereas the remaining patients had various respiratory disorders. Fifty-four percent (38) of the bronchoalveolar lavage samples were PCR-positive for Chlamydia, and 31% (22) of the PCR-positive samples were positive when cultured on macrophages. Twenty-eight samples (40%) and 14 samples (20%) of the PCR- and culture-positive samples, respectively, were from patients with asthma. Culture of the blood samples revealed that 24 (34.3%) of 70 were positive for Chlamydia compared with 8 (11%) of 70 matched nonrespiratory control subjects (p < 0.01); 17 (24%) of the positive blood cultures from the respiratory group were from patients with asthma. Elevation of total IgE was strongly associated with lavage culture positivity for Chlamydia. We therefore conclude that viable Chlamydia pneumoniae organisms are frequently present in the lung lavage fluid from this cohort of predominantly asthmatic pediatric patients.

Keywords: asthma; bronchial lavage; Chlamydia pneumoniae; culture

Chlamydia pneumoniae has long been associated with community-acquired pneumonia, bronchitis, and pharyngitis (1–3). The organism has recently been associated with other chronic illnesses, including Alzheimer’s disease, multiple sclerosis, and atherosclerosis (4–6). The worldwide increase in the incidence of asthma and the impact of the disease on public health care has led to new investigations into the etiology of the disease. Accumulating evidence suggests that infections of the respiratory tract may influence asthma pathogenesis in several ways, including disease inception, exacerbation, and severity (7–9). C. pneumoniae has been linked with both the exacerbation and increased incidence of chronic pulmonary conditions, including chronic obstructive pulmonary disease and bronchial asthma in adults (10–13). It also appears that acute C. pneumoniae infections can initiate asthma in some previously asymptomatic patients (11).

The majority of research linking Chlamydia to asthma has been conducted with adult populations. However, there is evidence suggesting that C. pneumoniae may play a role in pediatric asthma onset (8, 14, 15). A limitation to definitively linking the presence of infectious organisms and asthma is the lack of effective diagnostic methods. Antibody titers, although indicative of past or ongoing infection, cannot be used to establish a direct link because C. pneumoniae infects the majority of humans at some point in our lifetime (16). Several permutations of the polymerase chain reaction (PCR) have been successfully used to study the association of C. pneumoniae with various clinical manifestations and chronic illnesses, including asthma. However, PCR cannot establish whether the amplified products derive from residual DNA fragments, nonviable elementary bodies, or viable inclusions. Therefore, in this respect, culture is the desired method for isolation and identification. C. pneumoniae, however, grows poorly in cultures using epithelial or fibroblast cells, and the inclusions formed are smaller than those seen for other Chlamydia species (16).

This study used a combination of modified tissue culture media and macrophage cells to successfully isolate and culture viable Chlamydia from blood and bronchoalveolar lavage (BAL) samples. The data presented establish the presence of infectious C. pneumoniae organisms in pediatric asthma. These organisms were cultured from both BAL and peripheral blood specimens of patients with asthma in a cohort of pediatric patients undergoing bronchoscopic examination for a variety of respiratory disorders. This is the first report of live culture from BAL in children. Some of the results of these studies have been previously reported in the form of an abstract (17).

METHODS

Specimens

The approval for using excess BAL fluid and peripheral blood was obtained from the Institutional Review Board at Baystate Medical Center, a community-based hospital setting. Written, informed consent was obtained from the patients or their guardians. From July 2002 to July 2003, samples were obtained from 70 pediatric patients with chronic respiratory symptoms for diagnostic purposes in the course of clinical practice, and diagnoses were only made after analysis of the BAL. BAL fluid was collected from the right middle lobe under general anesthesia using a flexible fiberoptic bronchoscope through a laryngeal mask airway, as described previously (18) by one investigator (P.S.S.). Samples of 3 to 5 ml of ethylenediaminetetraacetic acid blood were also collected. Control blood samples were collected from nonrespiratory patients at the University of Massachusetts Health Services Department. All patient identifiers were removed, and the specimens were given code numbers. Patients were not contacted with the results of the investigation.

Smear Examination

The buffy coat from whole blood and BAL cell pellets were smeared onto glass slides and fixed with methanol. The slides were stained with guinea pig polyclonal anti-Chlamydia antibody and a fluorescein isothiocyanate-conjugated secondary antibody (Biomedia Corp., Foster City, CA). Slides then were examined using an epifluorescence microscope.

Culture of BAL and Blood Samples

Buffy coats and BAL for culture were washed, and the cells lysed with sterile glass beads assisted by vortexing in a sucrose phosphate...
glutamate buffer. Lysates were centrifuged, and the Chlamydia-containing supernatants were collected. THP-1 or 3774A.1 cell monolayers grown in Eagle’s minimum essential medium with insulin (Irvine Scientific, Santa Ana, CA), supplemented with 10% fetal bovine serum (Atlanta Biologicals, Lawrenceville, GA), were inoculated. The inoculum was removed after 4 hours and cycloheximide/Eagle’s minimum essential media added. Plates were incubated at 37°C for 96 hours, rinsed with phosphate-buffered saline, fixed, and then stained as previously described. All samples were examined in duplicate, and 20 randomly selected samples were retested in duplicate for repeatability.

**PCR Analysis**

Genomic DNA was isolated from BAL or blood samples using the QiAamp DNA Blood Mini Extraction Kit (Qiagen, Inc., Valencia, CA). DNA was amplified using a 16S signature sequence (16SIG-5’ CGGC GTGGATGAGGCTC 3’ 16SIG-5’ TCAGTCCCAGTGTGCCC 3’) to detect all Chlamydiales strains, resulting in a 298-bp product (19). C. pneumoniae-specific PCR was performed using the primer pair Cpn A and B (5’TGACAACCTGTAGAAATACGCG 3’ and 5’ATTGA TTAGGAGTAGGCC 3’) to generate a 463-bp product (20). PCR was performed using a Bio-Rad MyCycler (Bio-Rad Laboratories, Hercules, CA), and the PCR products (10 μl) were separated by electrophoresis on a 2% agarose gel and visualized with ethidium bromide staining.

**Evaluation of Total IgE**

Determination of total IgE was performed using the Elecsys IgE kit (Roche Diagnostics, Indianapolis, IN), which uses the electrochemiluminescence immunosay according to the manufacturer’s instructions, and the plates were read on the Roche Elecsys 1020 analyzer. The analyzer automatically calculated the IgE concentration of each sample based on a standard curve. Elevated IgE levels were determined based on the manufacturer’s recommended threshold by age range (neonates, 3.6 ng/ml; infants in first year of life, 36 ng/ml; children aged 1-5 years, 144 ng/ml; children aged 6-9 years, 216 ng/ml; children aged 10-15 years, 480 ng/ml; adults, 240 ng/ml).

**Statistics**

Data were analyzed using the SPSS 11.5 Graduate Pack (SPSS, Inc., Chicago, IL) statistics program. Cross-tabs with the Fisher exact test and χ² test were used to determine significance. For all analyses, tests were two-sided, and the level of significance was p ≤ 0.05.

**RESULTS**

**Demographics of Patient Population**

The average age of this group of community-based patients was 7.5 years, with an age range of 1 month to 19 years. There were 38 males and 32 females from four different ethnic groups: white (48 patients), Hispanic (14 patients), black (7 patients), and Asian (1 patient; Table 1). The single Asian patient did not have asthma. Asthma was diagnosed by family and/or personal history of atopy, elevated IgE, positive skin or RAST testing, reversible flow limitation on spirometry, the presence of increased cosinophils and basement membrane thickening on bronchial biopsy, or positive methacholine challenge. All of the black patients in this study had diagnosed asthma. Eight Hispanics (57%) and 30 whites (63%) also had diagnosed asthma. The remaining patients had various respiratory disorders, including aspiration bronchitis (17 patients), structural and airway anomalies (5 patients), gastroesophageal reflux disease (15 patients), cystic fibrosis (1 patient), and recurrent pneumonia of unknown etiology (1 patient).

Many patients were diagnosed with a combination of asthma, gastroesophageal reflux disease, and airway aspiration bronchitis. There was no significant relationship between race or sex and BAL or blood culture positivity for infectious Chlamydia. Thirty of the 70 patients whose samples were analyzed were taking medications at the time of testing. The most common medications included fluticasone/salmeterol, budesonide, triamcinolone, montelukast, fluticasone, and prednisolone. Fourteen of the patients with asthma were on medications; however, there was no significant correlation between the finding of infectious Chlamydia and a course of medication.

**Visualization of Chlamydial Inclusions on Blood and BAL Smears**

Smears were made from the isolated cells and stained with an anti-Chlamydia polyclonal antibody. Fluorescence microscopy examination of the stained smears revealed that 28 (40%) of the 70 samples tested had Chlamydia in the blood smears and/or in the BAL smears (Table 2). The chlamydial organisms could be seen inside lymphocytes and monocyte-like cells of the peripheral blood samples. Inclusions were seen in alveolar macrophages as well as epithelial cells in the BAL smears. Cellular differentiations were based solely on morphologic observations (Figure 1). These results demonstrate that C. pneumoniae can infect and be transported in cells of the peripheral blood as well as in cells of the respiratory tract.

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**TABLE 1. PATIENT CHARACTERISTICS**

<table>
<thead>
<tr>
<th>Description</th>
<th>Diagnosed Asthma</th>
<th>Nonasthma</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diagnosed disease</td>
<td>42</td>
<td>28</td>
<td>70</td>
</tr>
<tr>
<td>Average age, yr</td>
<td>7.8</td>
<td>7.0</td>
<td>7.5</td>
</tr>
<tr>
<td>Age range, yr</td>
<td>0-2.0</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>2.1-5.0</td>
<td>8</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>5.1-10.0</td>
<td>14</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>10.1-15.0</td>
<td>10</td>
<td>5</td>
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<td></td>
<td>15.1-20.0</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Sex</td>
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<td>Male</td>
<td>23</td>
<td>15</td>
<td>38</td>
</tr>
<tr>
<td>Female</td>
<td>19</td>
<td>13</td>
<td>32</td>
</tr>
<tr>
<td>Ethnicity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>29</td>
<td>20</td>
<td>49</td>
</tr>
<tr>
<td>Black</td>
<td>6</td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td>Hispanic</td>
<td>7</td>
<td>7</td>
<td>14</td>
</tr>
<tr>
<td>Asian</td>
<td>0</td>
<td>1</td>
<td>1</td>
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<tr>
<td><strong>Medication</strong></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>14</td>
<td>16</td>
<td>30</td>
</tr>
<tr>
<td>No</td>
<td>28</td>
<td>12</td>
<td>40</td>
</tr>
</tbody>
</table>

*Represents patients who were on medication at the time of sample collection.

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**TABLE 2. CROSS-TABULATION OF BRONCHOALVEOLAR LAVAGE/BLOOD CULTURE AND SMEARS STAINED FOR CHLAMYDIA**

<table>
<thead>
<tr>
<th></th>
<th>Positive</th>
<th>Negative</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>BAL culture</td>
<td>22 (31.4%)</td>
<td>48 (68.6%)</td>
<td>70</td>
</tr>
<tr>
<td>BAL smear</td>
<td>21 (30%)</td>
<td>49 (70%)</td>
<td>70</td>
</tr>
<tr>
<td>Buffy coat culture</td>
<td>24 (34%)</td>
<td>46 (66%)</td>
<td>70</td>
</tr>
<tr>
<td>Buffy coat smear</td>
<td>21 (30%)</td>
<td>49 (70%)</td>
<td>70</td>
</tr>
<tr>
<td>Both blood and BAL culture-positive</td>
<td>17 (59% of total positive)</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>Both blood and BAL smear-positive</td>
<td>28 (40% of total positive)</td>
<td>NA</td>
<td></td>
</tr>
</tbody>
</table>

*Definition of abbreviations: BAL = bronchoalveolar lavage; NA = not applicable. Cells for smear or culture analysis were made from the buffy coat of peripheral blood or the cells from BAL of pediatric respiratory patients. Coverslips containing macrophage monolayers of cultivated Chlamydia cultured or fixed smears were stained with a guinea pig anti-Chlamydia antibody and visualized by adding a fluorescein isothiocyanate–conjugated secondary antibody. Data analysis was performed using the Fisher χ² statistics from the SPSS Graduate Pack 11.5 statistics program (SPSS Inc., Chicago, IL).
Isolation of Infectious C. pneumoniae Organisms from Blood and BAL Using Tissue Culture

All BAL and blood samples were cultured on human or mouse macrophage cells to determine if the organisms present were infectious. The results demonstrated that 22 (31%) of the 70 samples were positive for Chlamydia on BAL culture and 24 samples (34%) were positive when the peripheral blood was cultured (Table 2). Seventeen samples (59% of all culture-positive BAL or blood samples) were culture-positive in both blood and BAL. On many of the coverslips with infected monolayers, there were foci of infection, indicating that the majority of the inclusions in any given area resulted from progeny elementary bodies that infected neighboring cells (Figure 2). Organisms at all stages of development could be seen at 96 hours postinfection of culture monolayers. For comparison, nonrespiratory, age-matched blood samples were also similarly tested, and of the 70 tested, 8 (11%) samples were positive for cultivable Chlamydia (p < 0.01 compared with the respiratory study group, Fisher exact test). There was no difference in the finding of infectious Chlamydia between sexes; 11 of the BAL culture-positive samples were from female patients and 11 from males. Likewise, for the finding of the organism by blood smear and culture, the percentage positivity per sex was similar to the results obtained for BAL. Eight of the BAL culture-positive samples were from patients between the ages of 1 month and 5 years, whereas the age groups of 5.1 to 10.0 and 15.1 to 22 years accounted for four each. The age group of 10.1 to 15.0 years accounted for the remaining six culture-positive patients.

Samples from Patients with Asthma Had Viable C. pneumoniae in Culture

Forty-two of the 70 samples analyzed (60%) were from patients with asthma, as defined by clinical diagnosis and/or laboratory testing. The remaining patients constituting a disease reference control subgroup without asthma (n = 28) exhibited various respiratory disorders, including aspiration bronchitis, structural and airway anomalies, and recurrent pneumonia of unknown etiology. Of 22 BAL samples positive by culture, 14 (64%) samples were from patients with asthma (Table 3). Of the 42 patients with asthma, one-third (14 patients) were BAL culture-positive. The remaining eight positives were from the respiratory disease population without asthma. Four of these culture-positive samples were from patients between the ages of 0 and 2 years. The age ranges of 2.1 to 5.0 and 5.1 to 10.0 years also accounted for four and five BAL culture-positive patients, respectively. The age group of 10.1 to 15.0 years accounted for the majority of BAL culture-positive samples (6). There were six patients with diagnosed asthma in age range of 0 to 2.0 years and eight in the age range of 2.1 to 5.0 years. Thirteen patients in the age range of 5.1 to 10.0 years had asthma, whereas 10 patients with asthma were in the age range of 10.1 to 15.0 years. Four patients in the age range of 15.1 to 20.0 years had asthma (Table 1). Seventeen of the 42 patients with asthma had culture-positive blood. In addition, using a specific peptide from the major outer membrane protein (MOMP) porin of C. pneumoniae, ELISAs were performed on all samples to assess the anti-C. pneumoniae antibody titers. Twenty-nine percent of all samples tested had positive antibody titers (> 200; data not shown). Sixty-five percent of the C. pneumoniae antibody-positive samples derived from patients with diagnosed asthma.

IgE Antibody Positivity, Viable C. pneumoniae, and Disease Association

Previous reports that sought to determine if C. pneumoniae infection triggers the production of C. pneumoniae-specific IgE...
in reactive airway disease in children detected 85.7% of patients with culture-positive asthma with wheezing compared with only 9.1% of patients with culture-positive asthma with pneumonia (21). We measured total serum IgE and defined an elevated level based on the Roche Diagnostics kit–recommended threshold values for age ranges. In the present cohort of respiratory patients, elevated total IgE was present in 17 (77.3%) of the 22 patients with positive BAL cultures compared with 9 (18.8%) of the 48 patients with negative BAL cultures (p < 0.0001). Elevated total IgE was also present in 20 (47.6%) of 42 patients with asthma versus 6 (21.4%) of 28 patients without asthma (p < 0.05; Table 4). C. pneumoniae-specific IgE was not evaluated.

**PCR Amplification of BAL Samples**

Chlamydia organisms frequently revert to a persistent state soon after infection and are therefore often not easily cultivable. We performed PCR on the BAL samples using a 16S signature ribosomal DNA sequence to determine the infectious and noninfectious levels of chlamydial organism carriage. Amplification of the target sequence produced a 298-bp product when the PCR products were electrophoresed. Fifty-four percent of the BAL samples tested were positive by PCR (Figure 3). Of the 42 patients with asthma, 28 (67%) were PCR-positive. All samples that were BAL culture- or smear–positive also tested positive by PCR. This confirms the specificity of our culture technique and suggests that the culture-negative, PCR-positive samples could contain noninfectious elementary bodies or that the elementary body level was too low for detection by culture. We confirmed that the organisms were C. pneumoniae by using a specific 16S rRNA primer pair to yield a 463-bp product. Thirty-four of the 38 samples positive for *Chlamydia* using the general primers were also *C. pneumoniae*-positive. The four negative samples were subsequently shown to contain *C. trachomatis* using specific primers (data not shown).

**DISCUSSION**

In adults and children, various atopic pathogens have either been implicated or demonstrated to play definitive roles (8, 22–24) as agents of lower respiratory tract infections. Asthma is characterized by airway hyperreactivity, inflammation, and atopy. Recently, it has been shown that respiratory infections caused by various bacterial and viral organisms play an important role in the exacerbation of asthma (7, 8, 25). In particular, *C. pneumoniae* has been shown to exacerbate asthma symptoms and might play a role in the initiation of adult disease (10, 26). The association of *C. pneumoniae* with pediatric asthma, however, has not been extensively studied. Moreover, none of the current studies investigated the viability, or infectivity, of the organisms present in BAL samples from young children with asthma.

Our study used a combination of professional phagocytic cells in the form of transformed human monocyte/macrophage cell.

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**TABLE 3. CULTURE-POSITIVE BRONCHOALVEOLAR LAVAGE SAMPLES ANALYZED BY DISEASE PRESENTATION**

<table>
<thead>
<tr>
<th>Disease Diagnosis</th>
<th>Asthma</th>
<th>Other</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>BAL culture Positive</td>
<td>14</td>
<td>8</td>
<td>22</td>
</tr>
<tr>
<td>63.6% (33.3% of patients with asthma)</td>
<td>36.4% (38.6% of patients without asthma)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>28</td>
<td>20</td>
<td>48</td>
</tr>
<tr>
<td>58.3% (66.6% of patients with asthma)</td>
<td>41.7% (71.4% of patients without asthma)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>42</td>
<td>28</td>
<td>70</td>
</tr>
</tbody>
</table>

*Definition of abbreviation: BAL = bronchoalveolar lavage.*

**TABLE 4. ELEVATED IgE LEVELS, DISEASE DIAGNOSIS, AND BRONCHOALVEOLAR LAVAGE CULTURE**

<table>
<thead>
<tr>
<th>BAL Culture</th>
<th>Disease Diagnosis</th>
<th>Asthma</th>
<th>Other</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive IgE</td>
<td>17</td>
<td>9</td>
<td>20</td>
</tr>
<tr>
<td>65.4%</td>
<td>34.6%</td>
<td></td>
<td>23.1%</td>
</tr>
<tr>
<td>Negative IgE</td>
<td>5</td>
<td>39</td>
<td>22</td>
</tr>
<tr>
<td>11.4%</td>
<td>88.6%</td>
<td></td>
<td>50.0%</td>
</tr>
<tr>
<td>Total</td>
<td>22</td>
<td>48</td>
<td>42</td>
</tr>
</tbody>
</table>

For definition of abbreviation, see Table 3.

Total IgE levels were evaluated and correlated with the frequency of positive BAL culture and disease diagnosis. Twenty-six samples had elevated IgE levels: 20 of 42 (47.6%) asthmatics versus 6 of 28 (21.4%) nonasthmatics, p < 0.05.
line (THP-1), as well as a mouse macrophage cell line (J774A.1), together with modified culture medium to successfully isolate infectious organisms from the blood and BAL of these pediatric patients. The medium used was a minimum essential medium supplemented with 4 g/L of insulin. The quantities of calcium and other ions as well as several amino acids were sometimes more than two times the concentration present in media such as RPMI 1640, which is traditionally used to grow cells for isolating the organism. In normal passages, we observed larger inclu-
sions and more efficient infection than with RPMI 1640 (W.C.W. and E.S.S., unpublished data). The results revealed a high prevalence of the infectious organisms in diagnosed asthma in this cohort of consecutive patients requiring diagnostic bronchoscopy to assess a variety of respiratory disorders. Our analysis indicates that 33% of patients diagnosed with asthma had infectious chlamydial organisms in their BAL samples, and overall, 67% of patients with asthma were PCR-positive. Fifty-seven percent (17) of the patients with PCR-positive asthma also car-
rried infectious Chlamydia in their peripheral blood as seen by culture. PCR data confirmed the presence of chlamydial DNA in the BAL samples and, when specific primers were used, PCR definitively showed that the majority of the organisms were Chlamydia pneumoniae. There were four patient samples that tested positive using the general Chlamydiae primers but were nega-
tive using C. pneumoniae-specific primers. These samples were subsequently shown to contain Chlamydia trachomatis. There were also samples with both C. pneumoniae and Chlamydia trachomatis organisms. The finding of C. trachomatis in the lungs of children with respiratory problems is not unusual because data suggest that C. trachomatis can cause neonatal lung infections that can persist (27–29). All samples that tested positive by BAL culture and/or smear were also PCR-positive; however, an additional 13 (18.5%) samples, negative on culture and/or smear, were positive by PCR. It is possible that these samples contained persistent Chlamydia pneumoniae and therefore were not readily cul-
tured. It is also likely that there were nonviable elementary bodies in some samples subjected to PCR, and this accounted for their lack of growth when cultured.

Previous studies performed on both adults and children dem-
strated an elevated anti-C. pneumoniae-specific IgE titer in patients with culture-positive asthma with wheezing (21). In this study, total IgE levels were assessed, and 26 (37%) of the 70 samples tested exhibited elevations. C. pneumoniae-specific IgE was not evaluated here. However, there was a significant associ-
ation (p < 0.001) between the presence of infectious organisms shown by culture of BAL and elevated total IgE levels, which was quantitatively and statistically stronger than the association between IgE and asthma diagnosis. The data presented here from a cohort of pediatric patients demonstrate the presence of infectious C. pneumoniae organisms in both BAL and peripheral blood samples of patients with asthma. A more extensive study that would span the full range of seasonal environmental conditions could further add significant insights into the implications of the presence of infectious C. pneumoniae organisms in this multifactorial disease.

Conflict of Interest Statement: W.C.W. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript; P.S.S. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript; C.A. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript; F.C. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript; C.A.W. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript; Y.T. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript; E.S.S. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript.

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Clinical Use of Noninvasive Measurements of Airway Inflammation in Steroid Reduction in Children 171:1077-1082

The use of noninvasive methods of monitoring airway inflammation, such as exhaled nitric oxide (eNO) and induced sputum, has been shown to improve asthma monitoring and optimize treatment in adult patients with asthma. There is a lack of comparable data in children. Forty children with stable asthma eligible for inhaled steroid reduction were reviewed every 8 weeks, and their inhaled steroid dose halved if clinically indicated. eNO, sputum induction combined with bronchial hyperreactivity testing, and exhaled breath condensate collection were performed at each visit to predict success or failure of reduction of inhaled steroids. Thirty of 40 (75%) children tolerated at least one dose reduction, 12 of 40 (30%) were successfully weaned off, and in total, 15 of 40 (38%) children experienced loss of asthma control. Treatment reduction was successful in all children who had no eosinophils in induced sputum before the attempted reduction. Using multiple logistic regression, increased eNO (odds ratio, 6.3; confidence interval, 3.75–10.58) and percentage of sputum eosinophils (odds ratio, 1.38; confidence interval, 1.06–1.81) were significant predictors of failed reduction. These findings suggest that monitoring airway inflammation may be useful in optimizing treatment in children with asthma. AM J RESPIR CRIT CARE MED 2005.

The Bronchial Lavage of Pediatric Patients with Asthma Contains Infectious Chlamydia 171:1083–1088

There has been a worldwide increase in the incidence of asthma, and the disease has greatly impacted the public health care system. Chlamydia pneumoniae has been reported as a possible contributing factor in asthma. The organism has been detected by polymerase chain reaction (PCR) in bronchial tissue, but there has been no direct evidence of viability. To determine the frequency of viable Chlamydia in children, blood and bronchoalveolar lavage were collected from 70 pediatric patients undergoing flexible fiberoptic bronchoscopy. Forty-two of these patients had asthma, whereas the remaining patients had various respiratory disorders. Fifty-four percent (38) of the bronchoalveolar lavage samples were PCR-positive for Chlamydia, and 31% (22) of the PCR-positive samples were positive when cultured on macrophages. Twenty-eight samples (40%) and 14 samples (20%) of the PCR- and culture-positive samples, respectively, were from patients with asthma. Culture of the blood samples revealed that 24 (34.3%) of 70 were positive for Chlamydia compared with 8 (11%) of 70 matched nonrespiratory control subjects (p < 0.01); 17 (24%) of the positive blood cultures from the respiratory group were from patients with asthma. Elevation of total IgE was strongly associated with lavage culture positivity for Chlamydia. We therefore conclude that viable Chlamydia pneumoniae organisms are frequently present in the lung lavage fluid from this cohort of predominantly asthmatic pediatric patients. AM J RESPIR CRIT CARE MED 2005.

Haplotypes of G Protein–coupled Receptor 154 Are Associated with Childhood Allergy and Asthma 171:1089–1095

Rationale: Allergic diseases are influenced by both genes and environment. A 70-kb haplotype block in the G protein–coupled receptor for asthma susceptibility gene (GPR154; alias GRA) on chromosome 7p was recently identified to influence susceptibility to asthma and elevated total serum IgE levels in adults. Objectives: To assess the impact of GPR154 on childhood allergic disease, including allergic sensitization, asthma, and rhinoconjunctivitis, in study populations with diverse environmental backgrounds. Methods: We studied farm children, Steiner school children, and two reference groups from five Western European countries in the cross-sectional PARISFAL (Prevention of Allergy Risk factors for Sensitization In children related to Farming and Anthroposophic Lifestyle) study and a sample of children from the Swedish birth cohort study BAMS. DNA samples from 3,113 PARISFAL and 800 BAMS children were genotyped for 7 GPR154 polymorphisms and haplotypes were inferred. The proportions of alleles and haplotypes (H1–H7) were compared in affected children with their healthy counterparts. Results: Data indicate a global association of the haplotype block to sensitization (allergen-specific serum IgE > 0.35 kU/L, p = 0.022), with significant haplotype-specific associations for H1, H5, and H6. Haplotypes H1 and H5 were also significantly associated with childhood allergic asthma (p = 0.045 and p = 0.023, respectively), and H5 to asthma regardless of sensitization. A broader involvement of GPR154 in allergic diseases was further supported in allergic rhinoconjunctivitis (H3: p = 0.046). The associated haplotypes could be allocated into risk (H5/H6) and nonrisk (H1/H3) groups, a pattern supported by allelic association of single nucleotide polymorphisms (SNPs) rs324384 and rs324396. Conclusions: Our results indicate that polymorphisms and haplotypes in the haplotype block of GPR154 are associated with asthma, rhinoconjunctivitis, and sensitization in European children. AM J RESPIR CRIT CARE MED 2005.

Protective Effects of Tiotropium Bromide in the Progression of Airway Smooth Muscle Remodeling 171:1096–1102

Rationale: Recent findings have demonstrated that muscarinic M1 receptor stimulation enhances airway smooth muscle proliferation to peptide growth factors in vitro. Because both peptide growth factor expression and acetylcholine release are known to be augmented in allergic airway inflammation, it is possible that anticholinergics protect against allergen-induced airway smooth muscle remodeling in vivo. Objective: We investigated the effects of treatment with the long-acting muscarinic receptor antagonist tiotropium on airway smooth muscle changes in a guinea pig model of ongoing allergic asthma. Results: Twelve weekly repeated allergen challenges induced an increase in airway smooth muscle mass in the noncartilaginous airways. This increase was not accompanied by alterations in cell size, indicating that the allergen-induced changes were entirely from increased airway smooth muscle cell number. Morphometric analysis showed no allergen-induced changes in airway smooth muscle area in the cartilaginous airways. However, repeated ovalbumin challenge enhanced maximal contraction of open tracheal ring preparations ex vivo. This was associated with an increase in smooth muscle–specific myosin expression in the lung. Treatment with inhaled tiotropium considerably inhibited the increase in airway smooth muscle mass, myosin expression, and contractility. Conclusions: These results indicate a prominent role for acetylcholine in allergen-induced airway smooth muscle remodeling in vivo, a process that has been thus far considered to be primarily caused by growth factors and other mediators of inflammation. Therefore, muscarinic receptor antagonists, like the long-acting anticholinergic tiotropium bromide, could be beneficial in preventing chronic airway hyperresponsiveness and decline in lung function in allergic asthma. AM J RESPIR CRIT CARE MED 2005.