Chlamydia pneumoniae serological status is not associated with asthma in children or young adults

Graham D Mills, a Jennifer A Lindeman, b J Paul Fawcett, c G Peter Herbison d and Malcolm R Sears e

Background The factors that cause the allergic sensitization and inflammation in asthma still remain to be clarified. A role for Chlamydia pneumoniae has been suggested although serological studies have produced conflicting findings. This study aims to clarify the relationship between asthmatic variables and C. pneumoniae serological status.

Methods A case-control study was undertaken on an asthma-enriched subset from a longitudinal birth cohort. In all, 198 subjects (96 with self-reported asthma) had C. pneumoniae serology (microimmunofluorescence [MIF] IgG, IgA) undertaken at age 11 and age 21 and assessment made in relation to a number of asthma variables.

Results The only statistically significant finding was in subjects self-reporting asthma at age 21 who had evidence of lower IgG titres \( (P = 0.046) \), a finding in the opposite direction to that expected from the hypothesis. Subjects with high IgG titres \( (\geq 128) \) were less likely to have reported ever having asthma; odds ratio (OR) = 0.29, (95% CI : 0.10–0.87). No association existed between symptoms suggestive of asthma in the previous 12 months and either IgG \( (P = 0.127) \) or IgA \( (P = 0.189) \) antibody titres at age 21. Likewise, no association was found between symptoms suggestive of asthma in the previous two years and C. pneumoniae IgG antibody titre \( (P = 0.81) \) at age 11. There was no evidence of an association with any of the other variables examined at either age 11 or age 21. These included use of inhaled steroids, serum IgE levels, airway responsiveness, skin test evidence of atopy, or smoking status.

Conclusion The results of this study suggest that C. pneumoniae infection when diagnosed by MIF serology is not a major risk factor for the development of asthma in children and young adults. The study has not, however, addressed the role this organism may play in specific asthmatic subsets or asthma exacerbations.

Keywords Asthma, Chlamydia pneumoniae, serology, adolescence, child

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Asthma is a chronic inflammatory disorder of the airways manifested by recurrent episodes of wheezing, breathlessness, chest tightness and cough. Although childhood asthma is very strongly linked to allergy, 1 the factors that facilitate allergic sensitization and inflammation still remain to be clarified.

Epidemiological studies suggest the possible role of infection in the sensitization and inflammatory process. 2 Chlamydia pneumoniae, a respiratory pathogen with a role in at least 10% of community-acquired cases of adult pneumonia, has been suggested as playing a role in the development of wheezing in both children and adults. Hahn et al. 3 found a strong dose-response relationship between C. pneumoniae antibody levels, wheeze and the subsequent development of asthmatic bronchitis in adults with lower respiratory tract infections. Subsequent studies by the same group 4 showed that positive serological status was significantly more common among patients with asthma and asthmatic bronchitis than in patients with non-wheezing illnesses. Studies in children using culture 5 and the polymerase chain reaction (PCR) 6 have also suggested a possible role for C. pneumoniae. Other studies, however, have been less convincing. Björnsson et al. 7 found raised C. pneumoniae IgA

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levels in subjects with bronchial hyperreactivity (BHR) but no significant relationship between C. pneumoniae IgA titres and the diagnosis of asthma or wheezing. Furthermore, no significant relationship was found between C. pneumoniae IgE titres and the diagnosis of asthma or BHR.

To help clarify the relationship between C. pneumoniae serological status and the development of asthma in children and young adults, we undertook a prospective case-control study in an asthma-enriched cohort drawn from the longitudinal Dunedin Multidisciplinary Health and Development Study (DMHDS). Serum taken at ages 11 and 21 years was tested for IgG and IgA antibodies to C. pneumoniae and examined in relationship to the presence or absence of asthma, BHR, wheeze and allergy.

Material and Methods

Base population

The DMHDS is a comprehensive follow-up of a New Zealand birth cohort assembled from among the 1661 children born at Dunedin’s only maternity hospital between 1 April 1972 and 31 March 1973. The 1139 children who were still living in the province of Otago at the age of 3 years were invited to participate in a longitudinal study of child health with 1037 (91%) agreeing to take part. There were no significant differences in birth history or prenatal and postnatal characteristics between the children enrolled in the study and the full birth cohort of 1661 children. The birth cohort has now been assessed on a total of nine occasions (every 2 years until 15 years, then at 18 and 21 years). Follow-up of the Dunedin cohort has consistently been over 82% with 957 (93.8%) of the 1020 living subjects being evaluated at age 21 years. Questionnaires on respiratory health were parent completed at age 7–11 years and subject completed at age 13–21 years. Subjects have also undertaken spirometry (age 9, 11, 13, 15, 18, 21), methacholine challenge testing (9, 11, 13, 15, 21), and skin testing for atopy at age 13 and 21. Blood was taken for IgE estimations from 579 members (56%) of the cohort at age 11 and from 785 members of the cohort (77%) at age 21. The stored serum has been utilized in the present study. Of the 957 subjectsanalysed at age 21, 204 had asthma. All ‘ever asthma’ respondents with available serum.

Selection of Chlamydia study group

An asthma-enriched cohort of 198 subjects (subsequently called the Chlamydia study group) was selected from the birth cohort outlined above. Selection was undertaken as follows. In all, 204 birth cohort subjects responded in their age 21 review that they had ever had asthma. All ‘ever asthma’ respondents with availability of serum from ages 11 and 21 (n = 96) were selected. The other subjects included in the Chlamydia study group (n = 102) were randomly selected from the nearly 400 subjects who had stored serum from both ages 11 and 21 who also denied a previous diagnosis of asthma. Due to financial constraints, it was not possible to include all ‘non-asthma’ subjects with available serum.

Serological studies

The C. pneumoniae specific serum IgG and IgA antibodies were determined by the microimmunofluorescence (MIF) method using C. pneumoniae strain TW183 (MRL Diagnostics Chlamydia MIF Assay) elementary bodies as antigens. The IgG antibody titres were determined on sera from both age 11 and age 21, while IgA antibody titres were only determined on sera from age 21 because the sera from age 11 had undergone a number of freeze-thaw cycles. The IgG was neutralized (Gulssorb, Gull Laboratories, Salt Lake City, USA) before IgA titrations. Only even florescence of all the elementary bodies in the antigen dots were considered as positive. A C. pneumoniae IgG titre ≥16 was considered evidence of previous infection.

Definitions

Bronchial hyperreactivity was defined by PC20 ≤8 mg/ml in the methacholine challenge test, or a greater than 15% increase in FEV1 after administration of salbutamol in those subjects with baseline airflow obstruction (FEV1/VC <70%) who did not undergo a methacholine challenge test for safety reasons. Atopy was defined by a wheal ≥2 mm larger than the negative control to any of the following 11 allergens; house dust mite, cat fur, horse, dog, kapok, grass pollen, Penicillium mould, Alternaria mould, Cladosporium mould, Aspergillus fumigatus mould and wool. Initial selection of the asthma-enriched cohort was based on answers to the age 21 review question, ‘Have you ever had asthma?’ The presence of recurrent wheezing, or whistling in the chest, or nocturnal wakening with chest tightness were used to define symptoms suggestive of asthma. Smoking was defined as smoking at least one cigarette daily for at least one year.

Statistical analyses

The analysis of asthma risk in relation to serological evidence of C. pneumoniae infection is complicated by the lack of a uniform clinical definition of asthma, as well as changes in asthma status that occur over time. Various parameters reflecting asthmatic status were analysed using the Statistical Package for Social Sciences (SPSS). Tests of association were carried out using the Pearson χ² test with continuity correction. Due to the log normal distribution of C. pneumoniae titres, differences between the geometric mean titres (GTM) were tested using the Mann-Whitney U test. We used C. pneumoniae IgG titre groupings of <16, 16–64 and ≥128, while C. pneumoniae IgA titres were divided into <16 or ≥16.

Based on the number of subjects available in this longitudinal study, there was a power of 80% to detect an increase in the odds ratio (OR) to 2.3 assuming that there is a raised titre to C. pneumoniae in 50% of those without asthma, as suggested by previous epidemiological studies.

Results

The Chlamydia study group contained 198 individuals (99 males and 99 females) of whom 96 reported previous asthma, while 102 subjects denied ever having asthma (Table 1). Those reporting asthma had significantly higher levels of IgE and significantly higher prevalence of BHR, atopy, and the use of asthmatic medications. Inhaled steroids were used by 38 of the 96 with a history of asthma. Nine subjects in the study group
had ever been admitted to hospital for asthma, with only three
of these individuals using oral steroids in the previous 3 years.
Two subjects without a history of asthma occasionally used
beta agonist inhalers. There was no difference in prevalence of
smoking between subjects with asthma ever and those without
asthma.

A strong correlation existed within the Chlamydia study
cohort between *C. pneumoniae* IgG and IgA titres (r = 0.65
Spearman correlation coefficient), whereas no relationship
existed at all between *C. pneumoniae* titres and total IgE levels
(IgG r = 0.04, IgA r = 0.04).

Self-reported diagnosed asthma at age 21 was the only vari-
able examined in which any statistically significant association
was detected. Those subjects self-reporting diagnosed asthma had a
significantly lower IgG titre than subjects whom had never had
asthma (GMT 18.1 versus 24.9; *P* = 0.046 Mann-Whitney U).

When individuals were grouped by level of titre (Table 2), those
subjects with high IgG titres (>128) were less likely to have
reported ever having asthma; OR = 0.29 (95% CI: 0.10–0.87).

The findings were not reflected in IgA data at age 21 (*P* = 0.52).

Over the 10-year interval between antibody testing, IgG
antibody titres declined in 12 (6.1%), remained the same in 130
(65.7%) and increased in 56 (28.3%). These changes saw the
prevalence of *C. pneumoniae* titres >16 increase from 32.8% at
age 11 to 58.6% at age 21, a finding consistent with previous
sero-epidemiological studies. There was no association between
the development of symptoms suggestive of asthma in the decade
from age 11 to age 21 and change in *C. pneumoniae* IgG titre (*P* = 0.56).

Discussion

This study, based on a large cohort of children followed over
21 years has failed to detect any positive association between
features of asthma and serological markers of *C. pneumoniae*
infection. In particular, neither IgG nor IgA antibody levels
 correlated with a history of wheeze, diagnosis of asthma, treat-
ment of asthma, skin test evidence of atopy, serum IgE levels or
evidence of BHR as measured by methacholine challenge tests or
reversibility to beta agonist. In addition the new onset of

<table>
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<tr>
<th>Table 1</th>
<th>Characteristics of Chlamydia study group at age 21</th>
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<tr>
<td></td>
<td>Reported asthma</td>
</tr>
<tr>
<td>No. of subjects</td>
<td>96</td>
</tr>
<tr>
<td>Male (%)</td>
<td>54.2</td>
</tr>
<tr>
<td>BHRa (%)</td>
<td>22.9</td>
</tr>
<tr>
<td>Serum IgE (geometric mean)</td>
<td>116.1 IU/ml</td>
</tr>
<tr>
<td>Atopic (%)</td>
<td>72.9</td>
</tr>
<tr>
<td>Use of asthmatic medication in the 12 months (%)</td>
<td>62.5</td>
</tr>
<tr>
<td>Ever smoked (%)</td>
<td>43.8</td>
</tr>
</tbody>
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| a Bronchial hyperreactivity. |

No association was found between symptoms suggestive of
asthma in the previous 2 years and *C. pneumoniae* IgG anti-
body titre (*P* = 0.81) at age 11 (Table 3). Likewise there was no
association of symptoms suggestive of asthma in the previous 12
months with either IgG (*P* = 0.127) or IgA (*P* = 0.189) antibody
titres at age 21 (Table 4).

There was no association found between BHR and antibody
titres; IgG at age 11, *P* = 0.49; IgG at age 21, *P* = 0.72; IgA at age
21, *P* = 1.00. Both IgE and skin test evidence of atopy also failed
to reveal any association (data not shown). The use of any asthma
medication in the previous 12 months was also unrelated to
*C. pneumoniae* serological status: IgG age 11, *P* = 0.81; IgG age
21, *P* = 0.50; IgA age 21, *P* = 0.86 as was the use of inhaled
steroids (data not shown).

<table>
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<tr>
<th>Table 2</th>
<th>Chlamydia pneumoniae IgG and serology at age 21 versus self-reporting of asthma</th>
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<tbody>
<tr>
<td></td>
<td>Ever asthma</td>
</tr>
<tr>
<td>IgG titre &lt;16</td>
<td>44</td>
</tr>
<tr>
<td>IgG titre 16–64</td>
<td>47</td>
</tr>
<tr>
<td>IgG titre &gt;128</td>
<td>5</td>
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IgG *P* = 0.07 *χ*2.

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<tr>
<th>Table 3</th>
<th>Chlamydia pneumoniae IgG serology at age 11 and symptoms suggestive of asthma in the previous 2 years</th>
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<tr>
<td></td>
<td>Symptoms suggestive of asthma</td>
</tr>
<tr>
<td>Low titre &lt;16</td>
<td>44</td>
</tr>
<tr>
<td>Moderate titre 16–64</td>
<td>17</td>
</tr>
<tr>
<td>High titre &gt;128</td>
<td>5</td>
</tr>
</tbody>
</table>

*P* = 0.81 *χ*2.

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<tr>
<th>Table 4</th>
<th>Chlamydia pneumoniae IgG serology at age 21 and symptoms suggestive of asthma in the previous 12 months</th>
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<tbody>
<tr>
<td></td>
<td>Symptoms suggestive of asthma</td>
</tr>
<tr>
<td>Low titre &lt;16</td>
<td>55</td>
</tr>
<tr>
<td>Moderate titre 16–64</td>
<td>54</td>
</tr>
<tr>
<td>High titre &gt;128</td>
<td>9</td>
</tr>
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</table>

*P* = 0.127 *χ*2.
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symptoms of asthma between the ages of 11 and 21 was not correlated with seroconversion. The only statistically significant association observed was the lower C. pneumoniae IgG antibody titres in those subjects with a self-reported history of asthma, which is in the opposite direction to that expected from previous studies.

It is unlikely that these negative results are due to methodological issues. Selection of subjects from the DMHDS allowed for excellent matching of age, sex and place of birth. The symptom history, serum IgE level, skin testing for atopy and lung function data were collected prospectively over a number of years without reference to C. pneumoniae serological data. The serum collected and stored at age 11 and 21 was analysed for antibodies to C. pneumoniae blind to the clinical data. There is no evidence to suggest any confounding between C. pneumoniae serological status and other variables. The study had a power of 80% to detect an increase in the OR to 2.3. The results do not suggest a lack of power to explain the negative association.

Our findings are in agreement with a small study undertaken by Emre et al. They studied the serological status of 45 children (mean age 9.1 years)—25 asthmatic (14 culture positive for C. pneumoniae) and 20 non-asthmatic children (11 culture positive for C. pneumoniae) and failed to reveal any relationship of C. pneumoniae IgG positivity with asthma or culture status. Using an IgE immunoblot assay, they did, however, show that the culture positive asthmatic children were significantly more likely to have anti-C. pneumoniae IgE immunoblots (85.7%) compared with children with C. pneumoniae culture positive pneumonia (9.1%) or asthmatic children culture negative for C. pneumoniae (18.2%). Emre et al. questioned whether airway reactivity might be mediated through a specific IgE response to C. pneumoniae. No mention was made, however, of the relationship between total IgE levels and anti-C. pneumoniae IgE immunoblots. Our study did not undertake anti-C. pneumoniae IgE immunoblots, although we did find a complete lack of correlation between total IgE levels and either C. pneumoniae IgG or IgA titres. An explanation for the results of Emre et al. is not readily apparent.

Our findings differ from some of the serological studies undertaken in adults. Hahn et al. undertook a family practice case-control study of adult patients (mean age 34 years) presenting with lower respiratory tract illness. They found prior C. pneumoniae exposure was strongly associated (OR = 7.2, 95% CI : 2.2–23.4) with the development of a clinical diagnosis of asthmatic bronchitis in the 6 months following the respiratory illness. A subsequent retrospective small case-control study from the same group reviewed 25 adults (mean age 46 years) with recent onset mild asthma (i.e. symptoms >3 months but <2 years duration). The asthmatics had a significantly higher prevalence of IgA antibodies for C. pneumoniae (cases 72% versus controls 44%, P < 0.05). The IgG antibody titres did not differ between groups. Adult onset asthma may be a somewhat different condition to childhood asthma, with a significantly lower frequency of atopy. The study populations of Hahn et al. not only differ markedly in age from our cohort, but also address the relationship between C. pneumoniae serological status and the development of wheezing possibly limited in duration, rather than long-term asthma. The other differences are due to the birth cohort and longitudinal nature of this study. As a result, the Chlamydia study group contains milder asthmatics than either hospital- or clinic-based populations as well as considerable heterogeneity.

Bjornsson et al. undertook a well-matched case-control serological study on 197 adults (mean age 33 years) previously enrolled in the European Community Respiratory Health Survey. No significant relationship was found between the geometric mean titre of C. pneumoniae IgG and an asthma diagnosis, BHR or wheezing. Although raised C. pneumoniae IgA levels were more common in subjects with BHR than in subjects without BHR (22 versus 8%, OR = 3.3, 95% CI : 1.3–8.3), the prevalence of IgA antibodies was less than expected. This may have been due to the failure to neutralize IgG before undertaking IgA assays. This has been shown to lead to IgA titres that are both lower and more difficult to interpret which could account for the difference in IgA findings between studies. In all other respects the findings in this slightly older population are comparable with negative serological relationships for most asthmatic variables.

Does the lack of serological association from our study indicate C. pneumoniae is not a risk factor for the development of asthma in children and young adults? Unfortunately, recent studies have shown that seroconversion is not universally associated with C. pneumoniae infection. The problem appears to be due to the MIF test only detecting antibodies to surface-exposed determinants which do not appear to be immunodominant in C. pneumoniae as compared to C. trachomatis infection. We acknowledge that in an ideal world investigating the effect of a respiratory infection on a respiratory disease should not be undertaken by investigation of antibody levels in blood. However, the hypothesis that an association exists has been generated by poorly controlled serological studies. The current study does not have this limitation. The negative serological study has to therefore be interpreted cautiously and considered in relation to all other evidence from culture, PCR and treatment studies.

Cunningham et al. undertook a PCR-based study on 9–11-year-old children who were prone to wheezing episodes in an attempt to address the role of C. pneumoniae in asthma. The children were followed for one year with nasal aspirates obtained both during acute exacerbations and when well. Surprisingly, nasal lavage specimens revealed 43 of 96 (45%) children with PCR evidence of C. pneumoniae at some time during the year. They also found a relationship between the local production of secretory IgA antibodies for C. pneumoniae and the numbers of wheezy exacerbations. Serum antibody levels were not available in this study. A control group was not included in this study. Recent PCR studies by Falck et al. and Normann et al. have revealed colonization rates with C. pneumoniae in 45% of non-asthmatic children with upper respiratory tract infections and 12% in healthy children.

An alternative method of assessing the importance of C. pneumoniae in asthma is to assess the response to treatment. Emre et al. compared children (mean age 8.8 years) presenting with acute episodes of wheezing to an emergency department with age- and sex-matched healthy controls. In all, C. pneumoniae was isolated from 13 of the 118 wheezers (11%) versus 2 of 41 controls (5%). Three-quarters of the children demonstrated clinical and laboratory improvement of the reactive airway disease after effective anti-chlamydial treatment. A large multi-centre, placebo-controlled, randomized double-blind study of
6 weeks treatment with roxithromycin in adult subjects with asthma and either IgG antibodies to *C. pneumoniae* ≥1:64 and/or IgA antibodies ≥1:16 is currently in progress with results due within the next 12 months. The subjects are being followed for 6 months following treatment and any difference between the treatment groups should help in clarifying the role of *C. pneumoniae*.

*Chlamydia pneumoniae* infection appears to be common. The majority of adults have serological evidence of past infection and recent studies have found high rates of PCR positivity even in healthy children. The results of our study suggest that *C. pneumoniae* infection when diagnosed by MIF serology is not a major risk factor for the development of asthma in children. The results of our study suggest that *C. pneumoniae* may lead to an increase in the inflammatory response within the airways leading to asthma exacerbations as commonly occurs with viral infection. This study has not addressed this possibility. It is also possible that *C. pneumoniae* may play a role in selected population subsets. The Chlamydia study group did not allow us to test this further. Ongoing treatment studies should further clarify the role of this organism in asthma.

**Acknowledgements**

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**References**


