

Asthmatic Children Have Increased Specific Anti-*Mycoplasma pneumoniae* IgM but not IgG or IgE—Values Independent of History of Respiratory Tract Infection

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Background: Bronchial asthma is exacerbated by *Mycoplasma pneumoniae*-induced upper respiratory tract infections (URTIs) in children. Specific IgM and IgG isotypes are involved in the immune response to *M. pneumoniae*, but little is known about the role of specific IgE antibodies against *M. pneumoniae* in asthma.

Objective: To investigate the role of IgM-, IgG- and IgE-specific antibody responses to *M. pneumoniae* in children with persistent asthma in relationship to history of URTI within the past 6 months.

Methods: Total or specific anti-*M. pneumoniae* IgM, IgG and IgE antibody responses were studied in stable asthmatic pediatric patients (*M. pneumoniae* positive and negative) without current exacerbation and nonasthmatic controls (N = 23 and 13, respectively) (UniCAP total IgE Fluoroenzymeimmunoassay, enzyme-linked immunosorbent assay).

Results: Values of specific IgM correlated with specific IgG (Spearman correlation, $\rho = 0.61$, $P < 0.0001$) but not with specific IgE anti-*M. pneumoniae* antibodies (AMA) in asthmatic subjects compared with nonasthmatic controls. However, concentrations of specific IgG correlated with specific IgE AMA ($\rho = 0.49$, $P = 0.0017$). Asthmatic subjects had higher levels of specific IgM AMA levels compared with nonasthmatics (median [interquartile range]: 0.57 [1.00] versus 0.21 [0.19]; Kruskal–Wallis test, $P = 0.0008$). In addition, IgM positivity was significantly higher in asthmatic compared with nonasthmatic subjects (39.1% versus 0.0%; Fisher's exact test, $P = 0.01$). These results were independent of URTI history in the past 6 months, which was not associated with higher IgM, IgG or IgE AMA levels compared with no URTI history ($P = 0.25$ – 0.64).

Conclusions: Increased specific IgM anti-*M. pneumoniae* responses may indicate an important role for *M. pneumoniae* infection in asthma.

Key Words: asthma, immunoglobulin E, *Mycoplasma pneumoniae*, upper respiratory tract infection.

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Mycoplasma pneumoniae, an extracellular pathogen lacking a cell wall, causes acute respiratory infection in both adults and children,¹ and it has been implicated in the initiation and exacerbation of asthma.² Allergic asthma is characterized by airway hyperresponsiveness and bronchial inflammation, which is mediated by activated leukocytes, including eosinophils,³ mast cells³ and T and B lymphocytes.^{4,5} Asthma is also associated with increased secretion of T helper cell (Th) 2-type cytokines⁵ and altered IgE regulation.^{6–10}

Previous studies in our laboratory have demonstrated that IgE may play an important role in immunity to specific viruses, including parvovirus B19,¹¹ HIV-1,^{12,13} varicella zoster virus^{14,15} and influenza virus.¹⁶ Other studies in our laboratory also identified IgE antispirechete antibodies (*Borrelia burgdorferi*) and its persistence in serum of lyme-infected children,¹⁷ as well as detection of anti-*Chlamydia pneumoniae* (*C. pneumoniae*) IgE in children with reactive airway disease.¹⁸ Although associations between specific IgM and IgG antibodies to *M. pneumoniae* and exacerbation of asthma have been reported,^{19,20} the presence or antigenic specificity of IgE anti-*M. pneumoniae* antibodies in pediatric asthmatic patients with a past history of upper respiratory tract infection (URTI) is less well described. Given the high prevalence of *M. pneumoniae* infections in children,^{19–21} IgE responses to *M. pneumoniae* may play an important role in contributing to allergic inflammation in children with asthma.

In the current cross-sectional investigation, we characterized the prevalence of humoral immune responses by analyzing serum specific anti-*M. pneumoniae* IgM-, IgG- or IgE- antibody levels in asthmatic children without exacerbation. The results of this study suggest that increased specific anti-*M. pneumoniae* IgM responses may play an important role in asthma.

MATERIALS AND METHODS

Study Population

Subjects were recruited from the Pediatric Asthma and General Pediatric Clinics at Kings County Hospital Center (Brooklyn, NY). Subjects were eligible if they had a physician's diagnosis of asthma or current clinically defined persistent asthma symptoms,^{21,22} or both, and allergic rhinoconjunctivitis, with elevated serum IgE levels (>100 IU/mL). Subjects were nonsmokers and had no chest infections or antibiotic use in the previous 6 weeks. Exclusion criteria included history of autoimmune disorders or immunodeficiency, recent use of systemic corticosteroids (less than 30 days) or immunotherapy, personal or family history of cigarette smoking and incomplete follow-up. Control subjects were recruited from an outpatient pediatric practice (Brooklyn, NY) and defined by absence of asthma and/or atopy, based on clinical criteria.²³ *M. pneumoniae* status was confirmed by positive anti-*M. pneumoniae* IgM and IgG antibody levels (0.9–1.1 Ab index borderline; >1.1 Ab index, positive) (enzyme-linked immunosorbent assay [ELISA]). However, nasopharyngeal swabs were polymerase chain reaction

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negative for *M. pneumoniae* (see Results). Written informed consent was obtained from all study participants and/or their parents or guardians. The protocol was approved by the SUNY Downstate Medical Center Institutional Review Board, and the procedures followed were in accordance with institutional guidelines involving human subjects.

Peripheral blood (15 mL) was obtained from asthmatic patients (N = 23) and nonasthmatic controls (N = 13) (males/females; ages 1–20 years old), from Kings County Hospital Center (Brooklyn, NY) and from an outpatient pediatric practice (Brooklyn, NY). None of the subjects received allergen immunotherapy within the prior 6 months. Asthma treatment regimens included daily inhaled corticosteroids in 48% and a leukotriene modifier in 48%. A short course of oral corticosteroids had been given to 17% of patients with asthma within the past 6 months but not within 30 days before enrollment.

Immunoglobulin Determination

Total Serum IgE

Blood was collected and IgE levels were determined in serum using the UniCap Total IgE fluoroenzyme immunoassay (Pharmacia and Upjohn Diagnostics, Freiburg, Germany) performed according to the manufacturer's recommendations (reference range for healthy serum: 20–100 IU/mL). All tests were performed in the Clinical Diagnostic Laboratory at SUNY Downstate Medical Center (Brooklyn, NY).

M. pneumoniae Serum Antibody Detection: ELISA

1. IgG and IgM: Serum IgG and IgM antibodies to *M. pneumoniae* were determined by ELISA (Bio-Quant, San Diego, CA) according to manufacturer's recommendations. Data are reported as Ab index (ranges for *M. pneumoniae* Ab IgG and IgM: negative <0.9; borderline: 0.9–1.1; positive: >1.1).
2. IgE: The presence of IgE anti-*M. pneumoniae* bacteria antibodies was determined by a modification of ELISA using IgM *M. pneumoniae* ELISA kit (Bio-Quant). Briefly, samples were directly added (100 μ L) to the microwell plates precoated with *M. pneumoniae* antigen and incubated for 1 hour at room temperature. Goat polyclonal antihuman IgE (100 μ L) (ICN Biomedicals, Aurora, OH) diluted 1:100 in TBS (Tris-buffered saline) wash buffer (0.05% Tween) was added to each well and incubated for 1 hour. The wells were washed 3X in wash buffer. Rabbit anti-goat peroxidase-labeled antibody (ICN Biomedicals), diluted 1:1000 in washing/diluting buffer, was then added to each well and incubated for 1 hour. The wells were washed again 3X in washing/diluting buffer and developed in 3, 3', 5, 5'-tetramethylbenzidine substrate solution (100 μ L) (Bio-Quant) for 10 minutes. The reaction was stopped by adding 1N H₂SO₄ (100 μ L). Positive control used was *M. pneumoniae* IgG-positive control serum (MBL Bion, Des Plaines, IL), and negative control used was *M. pneumoniae* negative control serum (MBL Bion). 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 anti-*M. pneumoniae* IgE, data are reported as O.D. values (range: >0.8 O.D. Value, positive). Final O.D. value reported was subtracted from chromagen blank O.D. value (background).

Statistical Methods

Immunoglobulin levels are presented as median [interquartile range] because they were not normally distributed. Spearman

tests were used to nonparametrically correlate levels of anti-*M. pneumoniae* IgM, IgG and IgE in all subjects. Kruskal–Wallis tests were used to nonparametric compare immunoglobulin levels between asthmatics and nonasthmatics as well as URTI and no URTI. Anti-*M. pneumoniae* antibodies (AMA) were dichotomized using predefined assay thresholds (0.9, 0.9 and 0.8, respectively), and differences of positivity were assessed in asthmatics compared with nonasthmatics using Fisher's exact tests. All data and statistical analyses were performed in SAS version 9.2 (SAS Institute, Cary, NC). A 2-sided *P* value <0.05 was taken to indicate statistical significance for all tests.

RESULTS

Subjects

Thirty-six subjects were recruited into the study; 23 were asthmatic patients and 13 were nonasthmatic controls. There were no significant differences of age (14.5 ± 7.7 , 11.3 ± 6.3 ; *P* = 0.21, respectively) or gender (female gender: 11(47.8%), 5(38.5%); *P* = 0.59, respectively) between asthmatic and nonasthmatic subjects. Asthmatic subjects had significantly higher total serum IgE antibody levels than nonasthmatic subjects (median [interquartile range]: 157.19 [191.02] versus 25.35 [80.80], *P* = 0.017). All subjects were *M. pneumoniae* polymerase chain reaction negative (nasopharyngeal swabs). At the time of enrollment, 57% and 0% of subjects with asthma were taking inhaled and oral corticosteroids, respectively.

Correlation Between AMA Isotypes

There were moderate correlations between IgM and IgG AMA levels (Spearman correlation, $\rho = 0.61$, *P* < 0.0001) (Fig 1) and between IgG and IgE AMA levels ($\rho = 0.49$, *P* = 0.0017) (Fig 1). However, the correlation between IgM and IgE AMA levels was only marginally significant ($\rho = 0.31$, *P* = 0.06) (Fig 1; Fig., Supplemental Digital Content 1, <http://links.lww.com/INF/B450>).

Association Between Asthma and AMA Levels

Asthmatic subjects had significantly higher IgM AMA levels compared with nonasthmatic subjects (median [interquartile range]: 0.57 [1.00] versus 0.21 [0.19], respectively; Kruskal–Wallis test, *P* = 0.0008) (Fig 2; Fig., Supplemental Digital Content 2, <http://links.lww.com/INF/B451>). However, there were no significant differences of IgG or IgE AMA levels between asthmatics and nonasthmatic subjects (IgG: 0.78 [1.07] versus 0.45 [0.63], *P* = 0.19; IgE: 0.78 [0.21] versus 0.81 [0.26], *P* = 0.83) (Fig 2; Supplemental Digital Content 2, <http://links.lww.com/INF/B451>).

Anti-*M. pneumoniae* antibody levels were dichotomized using assay thresholds for positivity. IgM positivity was significantly higher in asthmatic compared with nonasthmatic subjects (39.1% versus 0.0%; Fisher's exact test, *P* = 0.01) (Table 1). However, there were no significant differences of specific IgG and specific IgE levels between asthmatic and nonasthmatic subjects (*P* = 0.29 and 0.50, respectively) (Table 1).

No Association Between URTI and AMA Levels

Subjects with asthma reporting history of URTI in the past 6 months (42%) did not have significantly higher IgM, IgG or IgE AMA levels compared with those with no URTI history (58%) (Kruskal–Wallis test, *P* = 0.25–0.64) (Fig 3; Fig., Supplemental Digital Content 3, <http://links.lww.com/INF/B452>). Moreover, there were no differences of total serum IgE antibody levels in subjects with asthma reporting a history of URTI compared with no URTI (*P* = 0.97) (Fig 3; Fig., Supplemental Digital Content 3, <http://links.lww.com/INF/B452>).

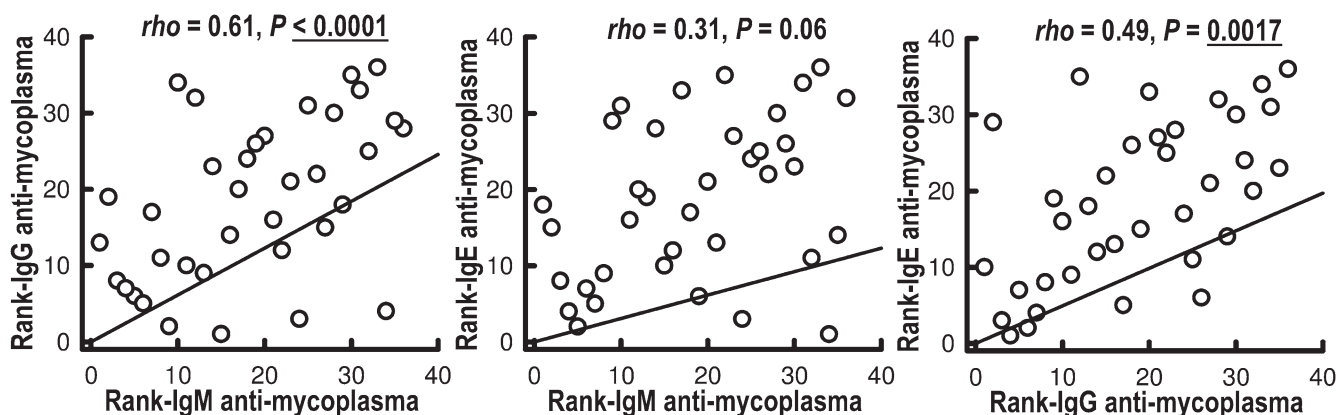


FIGURE 1. Correlation between anti-*M. pneumoniae* isotypes (IgG, IgM and IgE). Asthmatic patients (N = 23) and nonasthmatic subjects (N = 13). Spearman correlation.

DISCUSSION

The present study demonstrates that asthmatic subjects have increased IgM AMA levels even without history of URTI. We found that (1) there were moderate correlations between levels of specific IgM and IgG and specific IgG and IgE AMA; (2) asthmatic subjects had higher IgM AMA levels compared with nonasthmatic subjects; (3) however, history of URTI in the past 6 months was not associated with higher IgM, IgG or IgE AMA levels compared with no URTI history.

These results are consistent and in agreement with findings from prior literature that reported that *M. pneumoniae* infection is associated with significant specific IgE responses, in addition to specific IgG and IgM responses.²⁰ The authors concluded that since IgE is involved in allergic reactions, the production of *M. pneumoniae*-specific IgE may have a role in exacerbation of asthma²⁰; however, their patients did not have asthma. Earlier studies of Tipirneni et al¹⁹ were the first to report the presence of IgE antibodies to *M. pneumoniae*, using a radioallergosorbent test assay, in 5 patients with asthma and/or atopic dermatitis¹⁹; however, the pathogenesis of IgE AMA was not addressed nor were total Ig/IgE levels $\pm$ URTI investigated. Studies of Seggev et al²⁴ demonstrated the presence of high levels of IgM AMA (ELISA, Western blot) in adult patients (21%) infected with *M. pneumoniae* during asthma exacerbation. Furthermore, a case report by Yano et al²⁵ described a patient in whom a previous acute *M. pneumoniae* respiratory infection led to an initial onset of asthma.²⁵ Similar results were observed by Mok et al,²⁴ who reported that in children infected with *M. pneumoniae* 10% developed first-time clinical signs of asthma; however, those patients had a family history of atopic disease.²⁶ Thus, it could be, development of allergy in certain children might be associated with *M. pneumoniae* infection.²⁷ However in those studies *M. pneumoniae* infection was diagnosed either by culture or serology because polymerase chain reaction technology was not yet readily available.

The mechanisms of how *M. pneumoniae* affects the airway (eg, wheezing) are not fully described. Possible explanations include the production of *M. pneumoniae*-specific IgE antibodies,²⁵ in addition to direct effects on airway epithelium,^{25,28,29} inflammatory reaction on airways^{25,28} and alteration in the autonomic nervous system.^{25,30} In the current study, the lack of IgE-specific AMA antibodies may be due to differences in study design, heterogeneity of methods or patient characteristics compared with the aforementioned studies.

Although there are many clinical studies related to IgE and IgM levels in patients following acute infection, there are very few articles in which *M. pneumoniae* serology has been performed on asthmatic children who were not in an exacerbation compared with

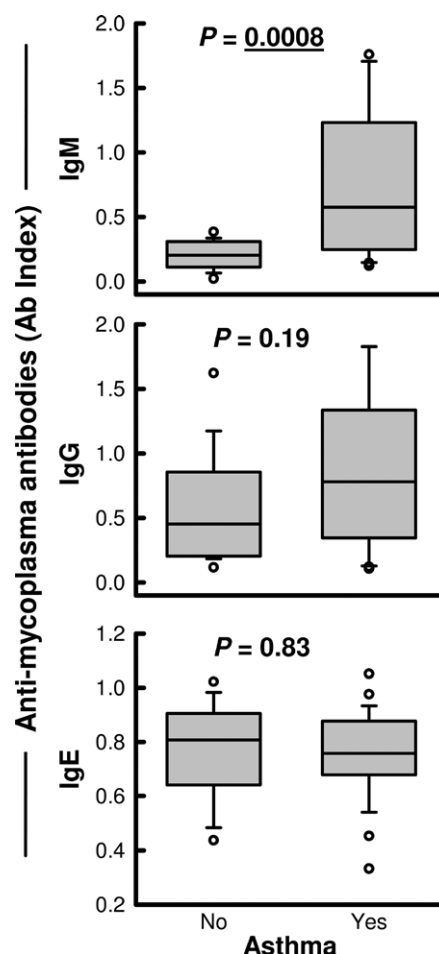


FIGURE 2. Association between asthma and anti-*M. pneumoniae* antibody levels. Asthmatic patients compared with nonasthmatic subjects. Kruskal–Wallis test.

controls.³¹ In the present studies, our finding of higher levels of IgM-anti-*M. pneumoniae* antibodies suggests either (1) more recent infection, (2) stronger specific immune responses to *M. pneumoniae* infection in our patients with asthma or (3) persistence of antigen. However, to our knowledge, there are no reports in the literature

TABLE 1. Antimycoplasma Antibodies in Asthmatics and Nonasthmatic Controls

Antimycoplasma Immunoglobulin	Asthma, no. (%)		P*
	Yes	No	
IgM–	14 (60.9)	13 (100.0)	0.01
IgM+	9 (39.1)	0 (0.0)	
IgG–	13 (56.5)	10 (76.9)	0.29
IgG+	10 (43.5)	3 (23.1)	
IgE–	11 (47.8)	8 (61.5)	0.50
IgE+	12 (52.2)	5 (38.5)	

Cutoff for IgM and IgG positivity is an antibody index >0.9. Cutoff for IgE positivity is O.D. value >0.8.

*Fisher's exact test was used because at least 1 cell count was ≤5.

demonstrating that children with asthma produce higher levels of IgM to infection. In the current study, it is also interesting to note that history of URTI in the past 6 months was not associated with higher IgM, IgG or IgE AMA levels compared with no URTI history.

In other studies, Fernald et al³² and Biberfeld³³ demonstrated large variations in the duration of IgM responses, but asthma status was not reported. However, persistence of IgM could indicate persistence of antigen.³³ In our patients, this suggests that *M. pneumoniae* might contribute to asthma inflammation without recent documented clinical infection perhaps due to persistent or subclinical infection, or via mechanisms that have not been fully identified (eg, non-IgE-mediated immunity, Th17 responses). It should be mentioned that patients and control subjects were enrolled during the same time period and from the same geographic region; therefore, differences in epidemiology and exposure rates are unlikely to explain differences in infection rates.

We propose that impaired immune responses might be responsible for *M. pneumoniae*'s potential relationship between asthma inflammation and health. A deficient immune response characterized by persistent IgM responses with relatively little IgG production has been previously described by Atkinson et al.³¹ However, in our asthma patients, lower IgG responses were not detected, perhaps suggesting no impairment in function in regards to IgG production. Thus, the increased IgM AMA responses may be a reflection of increased susceptibility to the infection in children with asthma. The immunologic mechanisms that mediate deficient host defense responses against *M. pneumoniae* in asthma patients are poorly understood, but may include impaired Toll-like receptor 2 function in asthma patients.³⁴

Total serum IgE levels, but not specific IgE levels, were increased in the asthmatic subjects, compared with nonasthmatic subjects, thus indicating another unknown component in the total IgE fraction that may be responsible in asthma inflammation, as well as *M. pneumoniae* infection. The lack of synchrony between total antigen levels and antigen-specific antigen antibody levels of select antibody isoforms has been described in other infections.³⁵ Because we did not identify a significant association in IgE AMA between subjects with and without asthma, other mechanisms responsible for asthma airway pathology related to *M. pneumoniae* mentioned above may be of more relevance.

Other bacterial pathogens that have been studied in reactive airway disease in children include *C. pneumoniae*^{36–38} and *Helicobacter pylori*.³⁶ It has been demonstrated that *M. pneumoniae* and *C. pneumoniae* may play a role in development of asthma exacerbations in childhood, whereas no relationship between *H. pylori* and asthma was observed.³⁶ Earlier studies in our laboratory reported that the presence of anti-*C. pneumoniae* IgE (immunoblotting) was associated with wheezing.¹⁸ Although production

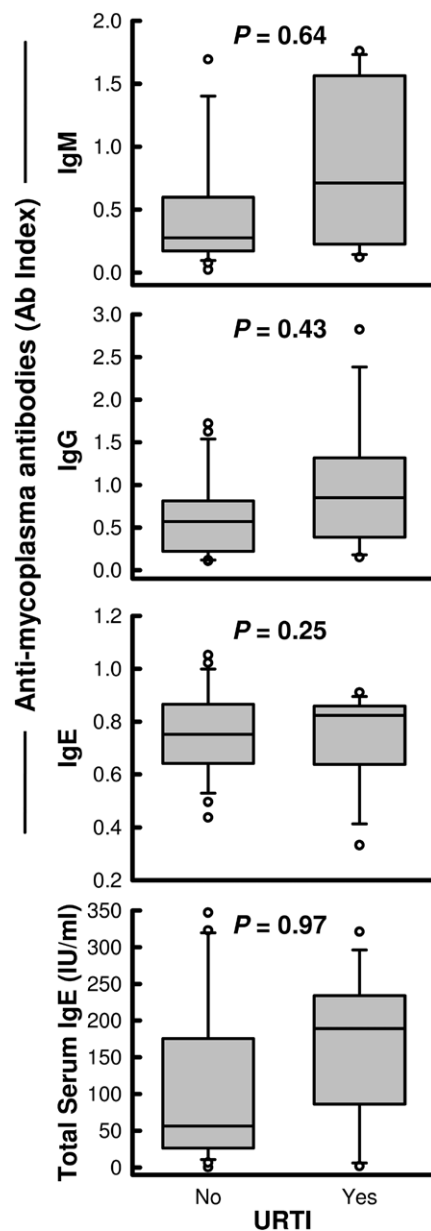


FIGURE 3. Comparison of subjects with asthma and anti-*M. pneumoniae* IgM, IgG and IgE antibody levels by history of URTI within past 6 months. Asthmatic patients with URTI (42%) compared with asthmatic patients without URTI (58%). Kruskal-Wallis test.

of *C. pneumoniae*-specific IgE may be an underlying mechanism leading to reactive airway disease in some patients with *C. pneumoniae* infection,¹⁸ there may exist other mechanisms causing asthma symptoms in patients with *M. pneumoniae* infection.

There are several limitations to our study, including: (1) small study/sample size, which is associated with decreased power and increased type II error. Future larger scale studies are needed to confirm the findings of this pilot study. (2) Reported URTI is likely to be complicated by potential recall bias. Children possibly had more respiratory tract diseases unknown or unreported by parents to the physician, thus creating a problem for the researchers to devise correct generalizations; however, all subjects have close

follow-up with their asthma providers and are regularly instructed to inform them of any acute respiratory illness immediately as well as at each visit. (3) Only 1 blood sample was obtained from each pediatric subject, thus the immunological/serological response might not reveal an earlier response. However, the strengths of this study are (1) that our results are highly relevant to addressing a possible relationship between *M. pneumoniae*, IgE and asthma and (2) these studies can be the framework for further exploration of this topic in children infected with *M. pneumoniae*. However, these studies would include prospective testing because our study suggests that infections may not be recognized easily on clinical grounds.

In conclusion, the current study suggests that in this cross-sectional study, asthmatic children have increased specific IgM anti-*M. pneumoniae* responses, whereas production of specific IgE AMA may play a less important role in asthma. Deficient host defense mechanisms in children with asthma may contribute to a higher prevalence of IgM AMA, which likely reflects an increased susceptibility to the infection.^{31,34}

Our results suggest that infection with *M. pneumoniae* may be common, yet underdiagnosed in children with asthma compared with nonasthmatic children. Additional prospective studies using both direct and indirect diagnostic tests are necessary to define criteria to identify patients at risk for *M. pneumoniae* infection who would benefit from testing and treatment.

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