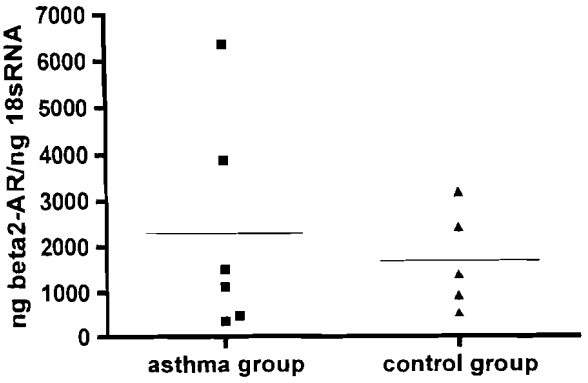


FIG 1. The $\beta_2$-adrenergic mRNA expression in freshly isolated PBMCs.

## Reply

*To the Editor:*

We thank the authors[1] for their thoughtful comments. To address their concerns that our results may be explained by $\beta_2$-adrenergic receptor downregulation in the asthmatic population, we measured $\beta_2$-adrenergic expression in PBMCs from the original asthmatic group and the healthy control group by real-time PCR. Total RNA was isolated from PBMCs according to the manufacturer's guidelines (Qiagen, Valencia, Calif), transcribed into cDNA, and analyzed by real-time PCR by using the dual-labeled fluorigenic probe method on an ABI Prism 7000 sequence detector (Applied Biosystems, Foster City, Calif). Primers and probes for the human $\beta_2$-adrenergic receptor and 18S RNA were purchased from Applied Biosystems. A standard curve was generated by using the fluorescent data from 10-fold serial dilutions of total RNA of the highest expression sample. Quantities of $\beta_2$-adrenergic receptor in test samples were normalized to the corresponding 18S RNA levels in each sample.

As shown in Fig 1, no differences were detected in the amount of $\beta_2$-adrenergic receptor expression: 2281 $\pm$ 966.0 ng/ng 18S RNA versus 1693 $\pm$ 486.8 ng/ng 18S RNA in the asthmatic and control groups, respectively. Therefore, it does not seem that the differences in PBMC responses to the low-dose fluticasone/salmeterol combination between the asthmatic and healthy control groups can be explained by $\beta_2$-adrenergic receptor downregulation in the asthmatic population.

We thank the authors for bringing the error in the figure legends for Fig E2 and Fig 3 to our attention. The correct figure legends are as follows:

**FIG E2.** Inhibition of PHA-induced IFN-$\gamma$ and TNF-$\alpha$ production by suboptimal concentration of fluticasone ($10^{-12}$ mol/L) in combination with salmeterol in PHA-stimulated supernatants from the healthy control group (**A** and **C**) or the allergic asthmatic group (**B** and **D**) (*$P < .05$ and **$P < .01$ as compared with PHA only).

**FIG 3.** Secretion of IL-13 (**A** and **B**) and IL-5 (**C** and **D**) in mitogen-stimulated cell cultures from patients with allergy and asthma in the presence of fluticasone or low-dose fluticasone/salmeterol combinations (*$P < .001$ and **$P < .05$ compared with PHA only).

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### REFERENCE

1. Friesen JHC, Ameredes BT, Calhoun WJ. Regarding "Differential control of $T_H1$ versus $T_H2$ cell responses by the combination of low-dose steroids with $\beta_2$-adrenergic agonists." J Allergy Clin Immunol 2005;115:424.

Available online November 12, 2004.
doi:10.1016/j.jaci.2004.09.040

## Origins of atopy in pediatric asthma

*To the Editor:*

In their interesting article, Heyman et al[1] describe an interaction of respiratory viral infection and atopy in exacerbations of pediatric asthma, resulting in hospitalization among children aged 3 years and older but not among younger children. I would like to comment on their discussion of potential factors influencing the development of atopy among older pediatric asthmatic patients. They discuss and dismiss the possible roles of previous respiratory syncytial virus infection, exposure to environmental tobacco smoke, and low socioeconomic status. They also briefly consider the potential contribution of mycoplasma and *Chlamydia* species, stating that the evaluation of these infections remains a challenge (I agree) and that these infections have been detected in only 5% of hospitalized asthmatic patients. I would like to expand on these points.

Although mycoplasma and *Chlamydia* species are rarely detected in the upper respiratory tract of asthmatic patients, recent studies on bronchoalveolar lavage fluid indicate that *Chlamydia pneumoniae* can be found in more than 50% of cases of pediatric asthma[2] and other pediatric obstructive lung diseases.[3] Furthermore, *C pneumoniae*–specific IgE has been associated with pediatric asthma,[4] and even more surprising is the emerging *in vitro* evidence that raises the possibility that pediatric *C pneumoniae* infection of human dendritic cells could even bias the developing immune response toward a $T_H2$ phenotype.[5,6]

In part because of the potential therapeutic applications of these and other related observations, I believe now is the opportune time to advocate for closer collaboration between chlamydiologists with expertise in microbiologic detection and generalist and specialist asthma researchers.

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**REFERENCES**

1. Heymann PW, Carper HT, Murphy DB, Platts-Mills TAE, Patrie J, McLaughlin AP, et al. Viral infections in relation to age, atopy, and season of admission among children hospitalized for wheezing. J Allergy Clin Immunol 2004;114:239-47.
2. Webley WC, Cirino F, West C, Andrzejwski Jr C, Stuart ES, Salva PS. Detection of viable *Chlamydia pneumoniae* organisms in the blood and bronchial lavages of pediatric patients with chronic bronchial asthma [abstract]. Am J Respir Crit Care Med 2004;169:A586.
3. Schmidt SM, Müller CE, Bruns R, Wiersbitzky SKW. Bronchial *Chlamydia pneumoniae* infection, markers of allergic inflammation and lung function in children. Pediatr Allergy Immunol 2001;12:257-65.
4. Emre U, Sokolovskaya N, Roblin P, Schacter J, Hammerschlag M. Detection of *Chlamydia pneumoniae*-IgE in children with reactive airway disease. J Infect Dis 1995;172:265-7.
5. Prebeck S, Kirschning CJ, Dürr S, da Costa C, Donath B, Brand K, et al. Predominant role of toll-like receptor 2 versus 4 in *Chlamydia pneumoniae*-induced activation of dendritic cells. J Immunol 2001;167: 3316-23.
6. Re F, Strominger JL. Toll-like receptor 2 (TLR2) and TLR4 differentially activate human dendritic cells. J Biol Chem 2001;276:37692-9.

Available online December 22, 2004.
doi:10.1016/j.jaci.2004.10.037

## Reply

*To the Editor:*

We appreciate the comments from Dr David Hahn,[1] who has previously described the positive association between *Chlamydia pneumoniae* infection and wheezing, asthmatic bronchitis, and the onset of asthma in adults.[2] As pointed out by Dr Hahn, the role of *C pneumoniae* in the cause of asthma and allergic inflammation in the lungs is not easy to evaluate. On the basis of current information, it is still difficult to determine whether *C pneumoniae* infection in the lower airways of children with symptomatic asthma is a common problem. Although the detection of viable *C pneumoniae* reported in bronchoalveolar lavage fluid from asthmatic children in the study by Webley et al[3] is of interest, bronchial lavage is rarely indicated or performed in children with asthma. For this reason, it would be important to know the clinical status and characteristics of the children who underwent this procedure in greater detail to judge the relevance of the results to the general population. In the study by Emre et al,[4] referenced by Dr Hahn, positive cultures for *C pneumoniae* from the nasopharynx (not bronchoalveolar lavage) were detected in 11% of children with wheezing. It is interesting that serum IgE antibody to *C pneumoniae* was detected by means of immunoblotting in a subset of these patients, but it would be important to confirm these results by using other methods for measuring *C pneumoniae*–specific IgE antibody.[5] By comparison, a much greater percentage of children with wheezing exacerbations are infected with rhinovirus, which almost all studies indicate is the dominant respiratory tract pathogen linked to acute asthma attacks in children.

In reference to our study, close to half of the children hospitalized for wheezing, ages 3 years and older, were admitted during the fall (September, October, and November).[6] This is a recurrent problem in Virginia. We suspect that this peak in hospitalizations is not significantly associated with acute *C pneumoniae* infections, which are not known to have an annual fall seasonal pattern. A role for *C pneumoniae* in the origins of atopy in pediatric asthma is an interesting hypothesis. In this regard, it would be helpful to know, for example, whether children with *C pneumoniae* induced–lung infections during infancy are more or less likely to become atopic and have asthma as they grow older. We agree with Dr Hahn that studies focused on *C pneumoniae* infections and the cause of asthma are of interest.

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**REFERENCES**

1. Hahn DL. Origins of atopy in pediatric asthma. J Allergy Clin Immunol 2005;115:425-6.
2. Hahn DL, Dodge RW, Golubjatnikov R. Association of *Chlamydia pneumoniae* (strain TWAR) infection with wheezing, asthmatic bronchitis, and adult-onset asthma. JAMA 1991;266:225-30.
3. Webley WC, Cirino F, West C, Andrzejwski Jr C, Stuart ES, Salva PS. Detection of viable Chlamydophila pneumoniae organisms in the blood and bronchial lavages of pediatric patients with chronic bronchial asthma [abstract]. Am J Respir Crit Care Med 2004;169:A586.
4. Emre U, Roblin PM, Gelling M, Dumornay W, Rao M, Hammerschlag MR, et al. The association of *Chlamydia pneumoniae* infection and reactive airway disease in children. Arch Pediatr Adolesc Med 1994;148:727-32.
5. Emre U, Sokolovskaya N, Roblin P, Schacter J, Hammerschlag M. Detection of *Chlamydia pneumoniae*-IgE in children with reactive airway disease. J Infect Dis 1995;172:265-7.
6. Heymann PW, Carper HT, Murphy DB, Platts-Mills TAE, Patrie J, McLaughlin AP, et al. Viral infections in relation to age, atopy, and season of admission among children hospitalized for wheezing. J Allergy Clin Immunol 2004;114:239-47.

Available online December 22, 2004.
doi:10.1016/j.jaci.2004.10.038

## Not enough spores?

*To the Editor:*

We wish to comment on a recent article by Dales et al[1] regarding the effect of outdoor aeroallergens on hospitalization for asthma.

The authors used Rotorod (rotational impaction) samplers and then enumerated the samples for aeroallergen content at 20 and 40 times magnification (we assume 200× and 400× magnification) by using 15 randomly chosen fields.

We reviewed a number of references cited in the 2002 edition of Multidata's operating instructions for their Rotorod sampler. We were able to find only one reference[2] for reducing the enumerated sample area by using a threshold level of 400 counted pollen grains.

Even though the 15 randomly chosen field enumeration method "had been previously tested," there is no citation