

# Polyclonal and allergen-induced cytokine responses in adults with asthma: Resolution of asthma is associated with normalization of IFN- $\gamma$ responses

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**Background:** Atopic disease is associated with skewing of immune responses away from a T<sub>H</sub>1 toward a T<sub>H</sub>2 profile. Previous studies have implicated this cytokine imbalance in the development of disease. However, it is not known whether normalization of this imbalance is conversely associated with disease resolution.

**Objective:** To further delineate the role of reduced T<sub>H</sub>1 and increased T<sub>H</sub>2 cytokine production in the pathogenesis of atopic disease and to determine whether disease resolution is associated with alteration of cytokine profiles, we investigated cytokine responses in a cohort of adult patients with asthma followed from childhood.

**Methods:** A cohort of wheezy children and control subjects aged 7 to 10 years were recruited from 1964 to 1967. Subjects were reevaluated every 7 years to monitor the outcome of childhood asthma. At the 42-year follow-up, 89 subjects from this cohort were evaluated for mitogen and house dust mite (HDM)-induced T<sub>H</sub>1 (IFN- $\gamma$ ) and T<sub>H</sub>2 (IL-4, IL-5, and IL-13) cytokine responses. Cytokine responses were compared in patients with ongoing asthma, patients with resolved asthma, and control subjects.

**Results:** Patients with severe ongoing asthma had significantly reduced HDM-induced IFN- $\gamma$  production compared with that of control subjects and patients with resolved asthma. In contrast, HDM-induced IFN- $\gamma$  production in patients with resolved asthma was equivalent to that seen in control subjects. Patients with ongoing and resolved asthma produced significantly higher levels of IL-5 in response to HDM compared with that seen in control subjects, with levels being equivalent in patients with active and resolved asthma. HDM-induced IL-13 production was significantly increased in the patients with resolved asthma when compared with that seen in the control subjects. PHA-induced cytokine responses did not parallel HDM-induced responses.

**Conclusion:** Patients with persistent and severe atopic asthma have a reduced HDM-induced T<sub>H</sub>1 response, whereas those with resolved asthma do not. This suggests that reduced HDM-induced IFN- $\gamma$  production might be an important factor con-

tributing to ongoing severe asthma and that normalization of allergen-induced T<sub>H</sub>1 responses might be important for disease resolution. The finding that all subjects with a history of asthma displayed increased HDM-induced T<sub>H</sub>2 (IL-5 and IL-13) cytokine responses, irrespective of the presence or absence of asthma, suggests that increased T<sub>H</sub>2 responses reflect the presence of the atopic state *per se* rather than being specifically linked to asthma. (J Allergy Clin Immunol 2002;110:450-6.)

**Key words:** Cytokines, adult, asthma, IFN- $\gamma$ , IL-4, IL-5, IL-13, allergens, mitogen, house dust mite

Asthma and atopy have increased in prevalence worldwide over the past 5 decades.<sup>1</sup> Asthma is one of the most common chronic disorders in childhood, affecting up to 30% of children in Australia.<sup>2</sup> Epidemiologic studies have shown a reduction in asthma symptoms with age<sup>3-5</sup>; however, asthma persists in 30% to 80% of adult patients.<sup>4,6</sup> The factors leading to disease persistence or resolution are poorly understood. Atopic disease is associated with skewing of immune responses away from a T<sub>H</sub>1 (IFN- $\gamma$ ) toward a T<sub>H</sub>2 (IL-4, IL-5, and IL-13) cytokine profile.<sup>7-9</sup> We and others have previously implicated defective IFN- $\gamma$  production as a predisposing factor to the development of atopic disease.<sup>10,11</sup> Infants who had atopic disease in the first year of life had reduced IFN- $\gamma$  production at birth, before the onset of atopic disease. In addition, persistence of T<sub>H</sub>2-skewed allergen-induced responses beyond the first year of life was associated with the subsequent development of atopic disease.<sup>12</sup> Hence T lymphocytes from atopic subjects exhibit immunologic differences that precede exposure to allergens and the development of clinical atopic disease. However, it is not known whether converse changes in T cell–cytokine responses are associated with disease remission. Because deficient T<sub>H</sub>1 and increased T<sub>H</sub>2 responses might contribute to the onset of asthma, it is possible that correction of this imbalance might contribute to disease remission.

There have been few studies examining immunologic parameters when asthma resolves. Children with asthma have been shown to have reduced IFN- $\gamma$  production and increased T<sub>H</sub>2 cytokine responses,<sup>13</sup> and persistence of asthma in adolescents was associated with reduced T<sub>H</sub>1 and increased T<sub>H</sub>2 immune responses to allergen.<sup>14-16</sup> These observations suggest that strong T<sub>H</sub>2 and reduced T<sub>H</sub>1 responses are associated with ongoing disease. However, it is not known whether suppression of T<sub>H</sub>2

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*Abbreviation used*

HDM: House dust mite

and an increase of  $T_H1$  responses are associated with disease remission.

Cytokine responses were evaluated in a group of asthmatic patients who had ongoing asthma and were compared with responses in patients who had asthma resolution to further examine this question. Subjects were part of the Melbourne Epidemiological Study of Childhood Asthma that was commenced in 1964 with the aim of determining the prevalence and natural history of asthma and wheezing bronchitis in children. Three hundred seventy-eight wheezy children and 106 control subjects aged 7 to 10 years were recruited and followed prospectively at 7-year intervals (14, 21, 28, 35, and 42 years) for clinical and atopic status and respiratory function. Eighty-nine subjects from this cohort were reviewed at 42 years for nonspecific (PHA) and allergen-induced (house dust mite [HDM])  $T_H1$  (IFN- $\gamma$ ) and  $T_H2$  (IL-4, IL-5, and IL-13) cytokine responses. Cytokine production was then compared in patients with ongoing asthma, patients with resolved asthma, and control subjects. We hypothesized that the  $T_H1/T_H2$  cytokine imbalance previously reported in asthmatic patients would resolve with resolution of asthma.

## METHODS

### Patients

From 1964 to 1967, a community-based study of a group of 378 children aged 7 to 10 years with a history of wheezing and asthma before 7 years of age and 106 age-matched control subjects was commenced to determine the prevalence and natural history of asthma and wheezy bronchitis in children.<sup>17</sup> The subjects were restudied at 14, 21, 28, 35,<sup>18</sup> and currently 42 years of age with respect to clinical and atopic status and respiratory function. At the 42-year follow-up, 87% of the original cohort agreed to participate in the study. Eighty-nine of these subjects were available for and consented to having blood drawn for additional immunologic testing. These 89 subjects were evaluated for nonspecific (PHA) and allergen-specific (HDM)  $T_H1$  (IFN- $\gamma$ ) and  $T_H2$  (IL-4, IL-5, and IL-13) cytokine responses. Patients were categorized according to the current severity of their asthma into the following groups: (1) control subjects, subjects who were nonatopic and had never wheezed ( $n = 19$ ); (2) patients with resolved asthma at 42 years, subjects who had not wheezed for 3 years ( $n = 25$ ); (3) patients with moderate asthma at 42 years, subjects who had wheeze occurring less frequently than weekly ( $n = 26$ ); and (4) patients with severe asthma at 42 years, subjects with daily wheeze ( $n = 19$ ). The demographic data for these patients are displayed in Table I. All asthmatic patients were atopic at the age of 10 years and remained atopic at 42 years defined on the basis of skin prick test response positivity to one or more of a panel of 5 allergens (HDM, rye grass, cat, dog, and egg white). HDM sensitivity was observed in 76% of patients with resolved asthma, 85% of patients with moderate asthma, and 86% of patients with daily asthma.

None of the asthmatic patients were experiencing an acute exacerbation of their disease at the time of the evaluation, and none were taking oral corticosteroids on the day of investigation. Of the patients with severe asthma evaluated in this study, all but one were

taking inhaled corticosteroids (beclomethasone dipropionate,  $n = 11$ ; budesonide,  $n = 5$ ; and fluticasone propionate,  $n = 2$ ). Of the 26 patients with moderate asthma, 8 were taking inhaled corticosteroids, and 4 had been treated with inhaled corticosteroids intermittently in the preceding 12 months (beclomethasone dipropionate,  $n = 10$ ; budesonide,  $n = 3$ ; and fluticasone propionate,  $n = 1$ ). None of the patients with resolved asthma had received either inhaled or oral corticosteroids in the preceding 7 years. Histamine challenge was performed according to the method of Yan et al,<sup>19</sup> and results were negative in 25 of the 26 patients with resolved asthma who were tested.

Informed consent was obtained from all subjects, and the study was approved by the ethics committee of the Royal Children's Hospital, Melbourne.

### Reagents

Reagents used were as follows: AIM-V medium (GIBCO);  $\beta$ -mercaptoethanol (ICN); RPMI-1640 medium (GIBCO); FCS (GIBCO); L-glutamine, penicillin, and streptomycin (Flow Laboratories); PHA (Wellcome Diagnostics); staphylococcal enterotoxin B (Sigma Chemical Co); avidin-peroxidase (Sigma Chemical Co); Ficoll-Hypaque (Pharmacia); and anti-IFN- $\gamma$ , biotinylated anti-IFN- $\gamma$ , anti-IL-4, biotinylated anti-IL-4, anti-IL-5, biotinylated anti-IL-5, anti-IL-13, biotinylated anti-IL-13, rh-IFN- $\gamma$ , rh-IL-4, rh-IL-5, and rh-IL-13 mAbs (Pharmingen).

### Allergen preparation

HDM (*Dermatophagoides pteronyssinus*, CSL Australia) was prepared according to the method of Stewart and Thomas.<sup>20</sup> Briefly, crude dried HDM was homogenized in PBS, clarified by means of bench centrifugation, dialyzed in 0.05 mol/L ammonium bicarbonate overnight, freeze-dried, and stored in aliquots at  $-70^{\circ}\text{C}$ . Aliquots were reconstituted in PBS, filter sterilized (pore size, 2  $\mu\text{m}$ ), and stored at  $-70^{\circ}\text{C}$  until use in cell culture.

### Plasma IgE measurement and RASTs

Total IgE and allergen-specific IgE levels were determined by means of UniCAP total IgE and specific IgE fluoroenzyme immunoassays (Pharmacia & Upjohn Diagnostics). Levels of total IgE are expressed in kilounits per liter (normal range for adults, 0–200 kU/L<sup>21</sup>). Allergen-specific IgE levels also are expressed as kilounits per liter.

### Cell cultures and proliferation

PBMCs were separated from heparinized blood by means of Ficoll-Hypaque density gradient centrifugation. The PBMC layer was washed 3 times with sterile PBS. PBMCs were cultured in triplicate at a concentration of  $1 \times 10^6$  cells/mL in AIMV supplemented with B-mercaptoethanol ( $5 \times 10^{-6}$  mol/L) for allergen-induced responses or RPMI-1640 supplemented with 10% FCS, 2 mmol/L L-glutamine, 100 U/mL penicillin, and 100  $\mu\text{g/mL}$  streptomycin for mitogen-induced responses at  $37^{\circ}\text{C}$  in a humidified atmosphere with 5%  $\text{CO}_2$ . PBMCs were stimulated with PHA (1  $\mu\text{g/mL}$ ), purified allergen (HDM, 10  $\mu\text{g/mL}$ ), or media alone. Proliferative responses were assessed at 48 hours (PHA) or 7 days (allergen). Sixteen hours before harvesting, cultures were pulsed with 0.5  $\mu\text{Ci}$  of tritiated thymidine per  $1 \times 10^5$  cells. Radioactivity was measured by means of liquid scintillation ( $\beta$  counter), and proliferation was expressed as a stimulation index calculated as the ratio of mean counts per minute of stimulated to unstimulated sample.

### Cytokine production

Time-course experiments revealed PHA-induced cytokine production was maximal at 24 hours for IL-4 and at 72 hours for IL-5,

TABLE I. Patient demographic and clinical data

|  | Control subjects<br>(n = 19) | Patients with resolved<br>asthma (n = 25)         | Patients with moderate<br>asthma (n = 26) | Patients with severe<br>asthma (n = 19) |
|--|------------------------------|---|---|---|
| Sex (n)  |                              |   |   |   |
| Male   | 12                           | 22  | 15  | 17                                      |
| Female   | 7                            | 3   | 11  | 2                                       |
| Age/time when last wheezed                         |                              | 56% age <14 y,<br>64% age <21 y,<br>68% age <28 y | 81% wheezed within<br>last 1-3 mo         | 100% wheezed daily<br>within last 3 mo  |
| Average wheezing frequency per year<br>in last 3 y | 0                            | 0   | 6-10 (almost monthly)                     | Almost daily                            |
| Skin prick test (mean [SD] wheal size, mm)         |                              |   |   |   |
| Histamine  | 6.9 (1.8)                    | 6.9 (1.8)   | 6.8 (1.4)                                 | 6.6 (1.4)                               |
| Rye grass  | 0.6 (2.1)                    | 5.7 (4.5)   | 7.0 (5.4)                                 | 9.3 (3.5)                               |
| Dust mite  | 1.4 (2.1)                    | 7.8 (4.5)   | 9.4 (3.7)                                 | 9.0 (4.9)                               |
| Cat  | 0.0 (0.0)                    | 2.9 (4.0)   | 3.4 (3.5)                                 | 5.1 (4.4)                               |
| Dog  | 0.0 (0.0)                    | 0.4 (1.5)   | 0.8 (1.7)                                 | 1.6 (2.3)                               |
| Egg  | 0.0 (0.0)                    | 0.2 (1.0)   | 0.6 (1.6)                                 | 1.0 (2.0)                               |
| FEV <sub>1</sub> % predicted (mean [SD])           | 106 (13.5)                   | 104 (13.7)  | 94 (16.5)                                 | 79 (17.4)                               |

IL-13, and IFN- $\gamma$ , whereas allergen-induced cytokine production was maximal at 48 hours for IL-4 and at 6 days for IL-5, IL-13, and IFN- $\gamma$ . Cell cultures were centrifuged, and cell-free supernatants were collected at these time points and stored at  $-70^{\circ}\text{C}$ . INF- $\gamma$ , IL-4, IL-5, and IL-13 were assayed by using a sandwich ELISA, as previously described.<sup>22</sup> Briefly, high-binding ELISA plates were coated with purified capture mAb (anti-IFN- $\gamma$ , anti-IL-4, anti-IL-5, or anti-IL-13) at a concentration of 1  $\mu\text{g/mL}$  in carbonate buffer (pH 9.6) and incubated overnight at  $4^{\circ}\text{C}$ . Plates were blocked with PBS-Tween-10% FCS for 2 hours. Samples were incubated at room temperature for 4 hours. After washing, biotinylated mAb (anti-IFN- $\gamma$ , anti-IL-4, anti-IL-5, or anti-IL-13; 0.5  $\mu\text{g/mL}$ ) was added and incubated at room temperature for 45 minutes, followed by avidin-peroxidase (1:400 of 1 mg/mL) for 30 minutes. After a final washing, TMB substrate (KPL) was added, and the reaction was stopped by the addition of  $\text{H}_2\text{SO}_4$ . Plates were read at 450 nm on a spectrophotometer. All standards and samples were performed in duplicate, and data were analyzed with Biomek 1000 data reduction software (Beckman). Minimum detection levels were calculated with Biomek 1000 software and were as follows: IFN- $\gamma$ , 10 pg/mL; IL-4, 2 pg/mL; IL-5, 10 pg/mL; and IL-13, 5 pg/mL.

### Statistical analysis

For the purpose of displaying the raw data, if the level of cytokine measured was less than detectable limits, a value of half of that of the lower detectable limit of that assay was allocated (ie, 5 pg/mL, 1 pg/mL, 5 pg/mL, and 2 pg/mL for the IFN- $\gamma$ , IL-4, IL-5, and IL-13 assays, respectively). The significance of the difference in total and allergen-specific IgE and PHA- and HDM-induced IFN- $\gamma$ , IL-4, IL-5, and IL-13 production between groups was examined by using the Mann-Whitney nonparametric  $U$  test.  $P$  values were not formally adjusted for multiple comparisons but are interpreted conservatively. All statistical analysis was performed with Stata statistical software, release 5 (StataCorp 1997).

## RESULTS

### Total and HDM-specific IgE levels

Total and HDM-specific IgE levels correlated with asthma activity, with levels being highest in those with severe asthma and lowest in those with resolved asthma (Table II and Fig 1). All 3 asthmatic groups had total and

HDM-specific IgE levels that were significantly higher than those of the control subjects.

### T<sub>H</sub>1 and T<sub>H</sub>2 cytokine responses

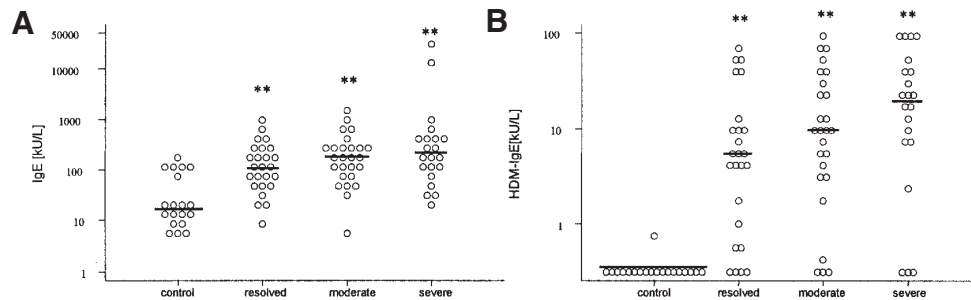
HDM- and PHA-induced T<sub>H</sub>1 and T<sub>H</sub>2 cytokine production was determined in asthmatic patients with ongoing disease, in those who had resolution of disease, and in control subjects to examine whether resolution of asthma is associated with an alteration in cytokine balance (Table II).

**HDM-induced cytokine responses.** HDM-induced T<sub>H</sub>1 and T<sub>H</sub>2 cytokine production was examined in asthmatic patients with severe, moderate, and resolved disease and in control subjects.

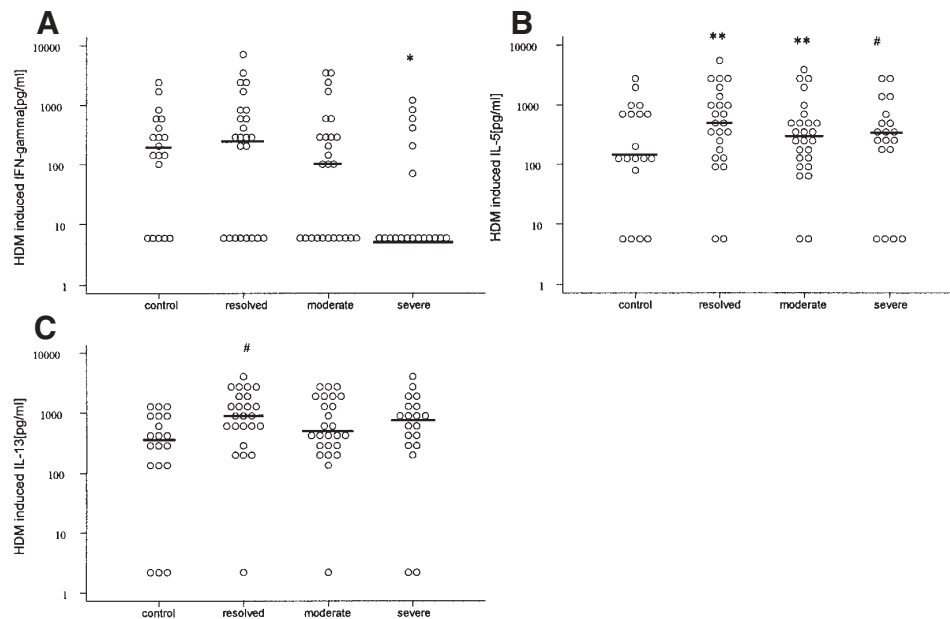
Patients with severe asthma had significantly reduced HDM-induced IFN- $\gamma$  production compared with that seen in control subjects ( $P = .02$ ; Fig 2, A). Patients with resolved asthma had similar IFN- $\gamma$  production compared with that seen in control subjects ( $P = .4$ ; Fig 2, A). HDM-induced IFN- $\gamma$  production was significantly lower in patients with severe asthma compared with that seen in patients with resolved asthma ( $P = .01$ ). No correlation between mite-specific serum IgE and HDM-induced IFN- $\gamma$  secretion was observed (data not shown). All 3 categories of patients with asthma produced significantly higher levels of IL-5 in response to HDM compared with those seen in control subjects (resolved asthma,  $P < .001$ ; moderate asthma,  $P < .001$ ; and severe asthma,  $P = .001$ ; Fig 2, B). Levels of HDM-induced IL-5 production were similar in patients with active and resolved asthma.

HDM-induced IL-13 production was significantly increased in the group with resolved asthma when compared with that in the control group ( $P < .01$ ; Fig 2, C). HDM-induced IL-4 levels were generally very low or below the lower limit of detection.

These findings show that adult asthmatic patients produced increased levels of IL-5 in response to HDM, irrespective of disease activity or severity. In contrast, only patients with severe asthma and not patients with resolved



**FIG 1.** Total and HDM-specific IgE (in kilounits per liter). Levels of total (A) and HDM-specific (B) IgE in patients with resolved asthma, moderate asthma, and severe asthma and control subjects. Data are represented on a log scale, with the horizontal line indicating the median,  $**P < .001$ .



**FIG 2.** HDM-induced cytokine responses. HDM-induced production of IFN- $\gamma$  (A), IL-5 (B), and IL-13 (C) was measured in patients with resolved asthma, moderate asthma, and severe asthma and control subjects. Data are represented on a log scale, with the horizontal line indicating the median.  $*P < .05$ ;  $\#P < .01$ ;  $**P < .001$ .

asthma had significantly reduced HDM-induced IFN- $\gamma$  production compared with that seen in control subjects.

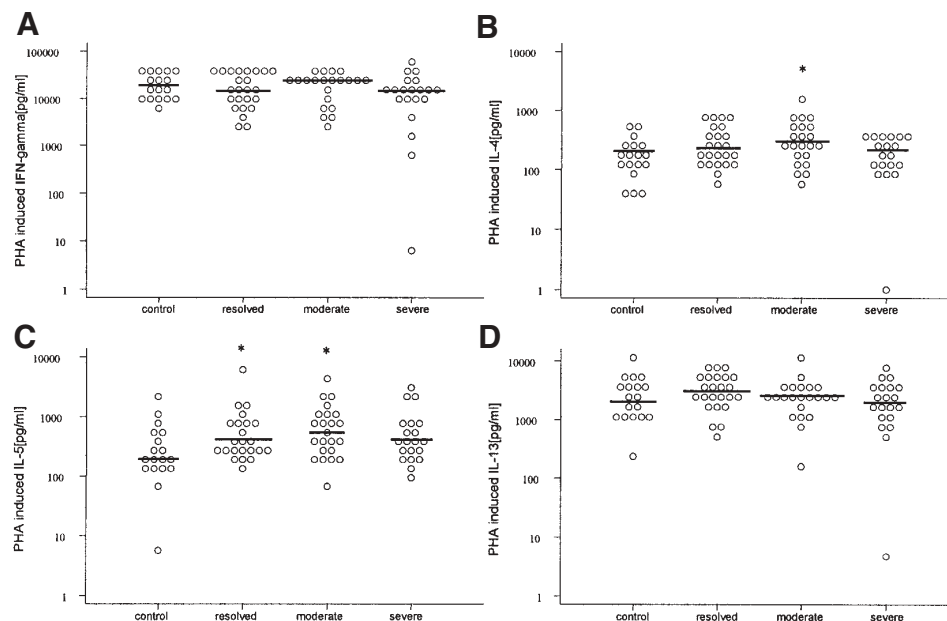
**PHA-induced cytokine responses.** PHA-induced INF- $\gamma$  production in patients with symptomatic and resolved asthma was not different from that seen in control subjects (Fig 3, A). No differences in PHA-induced IFN- $\gamma$  production were observed between the different categories of active asthma and the patients with resolved asthma (Fig 3, A).

PHA-induced IL-5 production was increased in all asthmatic groups compared with that seen in control subjects and similar between the 3 categories of asthmatic patients (resolved asthma,  $P = .02$ ; moderate asthma,  $P = .01$ ; severe asthma,  $P = .09$ ; Fig 3, C). PHA-induced IL-4 and IL-13 production were not significantly different in any of the asthmatic groups compared with that seen in control subjects (Fig 3, B and D). These findings show

that adult asthmatic patients produced increased levels of IL-5 in response to PHA, irrespective of disease activity or severity; however, they did not have increased PHA-induced IL-4 and IL-13 production or reduced PHA-induced IFN- $\gamma$  production compared with that seen in control subjects.

### Proliferative responses

HDM-induced proliferation was not different between control subjects and the 3 asthmatic groups. PHA-induced proliferation was significantly increased in patients with severe asthma ( $P = .04$ ) when compared with that seen in control subjects (data not shown). No difference in mitogen-induced proliferation was apparent in patients with moderate or resolved asthma when compared with that seen in control subjects (data not shown).



**FIG 3.** PHA-induced cytokine responses. PHA-induced production of IFN- $\gamma$  (**A**), IL-4 (**B**), IL-5 (**C**), and IL-13 (**D**) was measured in patients with resolved asthma, moderate asthma, and severe asthma and control subjects. Data are represented on a log scale, with the horizontal line indicating the median, \* $P < .05$ .

**TABLE II.** Median total and HDM-specific IgE levels and PHA- and HDM-induced cytokine levels

|                           | Control subjects<br>(n = 19) | Patients with resolved<br>asthma (n = 25) | Patients with moderate<br>asthma (n = 26) | Patients with severe<br>asthma (n = 19) |
|---------------------------|------------------------------|---|---|---|
| Median total IgE (kU/L)   | 18                           | 107 ( $P < .001$ )                        | 190 ( $P < .001$ )                        | 211 ( $P < .001$ )                      |
| HDM-specific IgE          | ND                           | 5.5 ( $P < .001$ )                        | 9.5 ( $P < .001$ )                        | 20 ( $P < .001$ )                       |
| HDM-induced IFN- $\gamma$ | 191                          | 270 (NS, $P = .4$ )                       | 101 (NS, $P = .4$ )                       | 5 ( $P = .02$ )                         |
| HDM-induced IL-5          | 114                          | 497 ( $P < .001$ )                        | 451 ( $P < .001$ )                        | 517 ( $P = .001$ )                      |
| HDM-induced IL-13         | 348                          | 889 ( $P < .01$ )                         | 470 (NS, $P = .08$ )                      | 734 (NS, $P = .07$ )                    |
| PHA-induced IFN- $\gamma$ | 18,189                       | 14,689 (NS, $P = .2$ )                    | 23,825 (NS, $P = .6$ )                    | 14,346 (NS, $P = .2$ )                  |
| PHA-induced IL-4          | 191                          | 223 (NS, $P = .1$ )                       | 279 ( $P = .03$ )                         | 208 (NS, $P = .6$ )                     |
| PHA-induced IL-5          | 196                          | 393 ( $P = .02$ )                         | 548 ( $P = .01$ )                         | 374 (NS, $P = .09$ )                    |
| PHA-induced IL-13         | 2052                         | 3014 (NS, $P = .2$ )                      | 2540 (NS, $P = .8$ )                      | 2004 (NS, $P = .5$ )                    |

ND, Not detected; NS, not significant.

## DISCUSSION

We have studied cytokine responses in a carefully characterized and prospectively followed group of patients with atopic asthma from The Melbourne Epidemiological Study of Childhood Asthma cohort. This cohort provided a unique opportunity to correlate cytokine responses with the natural history of atopic asthma. We evaluated the relationship between clinical disease and cytokine responses in these patients to determine whether asthma resolution was associated with a change in cytokine profile. Mitogen- and allergen-induced cytokine responses were examined in parallel because the T-cell cytokine responses to these stimuli are not necessarily congruent.<sup>23</sup> Previous studies have suggested that increased  $T_H2$  and reduced  $T_H1$  cytokine responses might contribute to the development of atopic disease. It is not known whether reversal or correction of this imbalance is in turn important for disease resolution.

If this were the case, one would expect to see a normalization of cytokine responses on resolution of asthma. There have been few studies to evaluate cytokine responses in asthmatic patients with ongoing disease compared with in those with resolved disease. One study comparing adolescent asthmatic patients with active versus remitted disease found concanavalin A-induced IL-4 production was not different between groups.<sup>14</sup> There have been no studies examining more extensive cytokine profiles in these groups.

In this study asthma resolution was not associated with a reduction in either polyclonal or allergen-induced  $T_H2$  cytokine production that one might predict if it was considered that increased  $T_H2$  cytokine responses were a critical determinant of ongoing disease. All asthmatic patients, irrespective of disease activity, had increased PHA-induced IL-5 (but not IL-4 or IL-13) and increased HDM-induced IL-5 levels compared with those seen in



control subjects. This suggests that an increased IL-5 cytokine response is not necessarily a reflection of asthma activity but rather of the atopic state per se. Our findings further suggest that IL-5 is not the principal cytokine relevant to production of asthmatic symptoms. This is supported by the fact that treatment with a humanized anti-IL-5 mAb was not successful in improving adult atopic asthma symptoms, despite a marked reduction of circulating and airway eosinophilia.<sup>24</sup> It could be argued that the persistently increased T<sub>H</sub>2 responses in patients with resolved asthma might reflect ongoing airway inflammation in the absence of symptoms because increased bronchial responsiveness has been reported in asymptomatic asthmatic patients.<sup>25-27</sup> However, respiratory function data on the group with resolved asthma was entirely normal, with no underlying bronchial hyperresponsiveness on histamine challenge. This suggests that the persistently increased T<sub>H</sub>2 responses in our patients with resolved asthma does not relate to asthma but rather to the underlying atopic state per se. Our finding that production of PHA-induced IL-4 and IL-13 was not increased along with the increased IL-5 production in asthmatic patients is consistent with previous studies of mitogen-induced cytokine responses in adult atopic disease or asthma that have reported increased PHA-induced IL-5 production<sup>28,29</sup> but no difference in concanavalin A–induced IL-4 production<sup>14</sup> or PHA-induced IL-13 production compared with that seen in control subjects.<sup>28,30</sup>

An important observation in this study was that ongoing asthma was associated with reduced allergen-induced T<sub>H</sub>1 cytokine responses, whereas resolved asthma was not. Reduced HDM-induced IFN- $\gamma$  production was associated with increased severity of asthma. This suggests that correction of the reduced HDM-induced IFN- $\gamma$  response might be associated with resolution of the asthmatic state. This is supported by the finding that HDM-induced IFN- $\gamma$  production was reduced in adolescent asthmatic patients with active disease but not in those with asthma remission.<sup>14</sup> In addition, an inverse correlation between HDM-specific IFN- $\gamma$  production and disease activity score has been reported in adult patients with atopic asthma.<sup>15</sup> These findings suggest that enhancement of T<sub>H</sub>1 responses might induce remission of asthma. Indeed, CpG oligonucleotides that switch immune responses toward a T<sub>H</sub>1-dominated cytokine pattern have been shown to be effective in reducing airway inflammation and airway hyperresponsiveness in an animal model of asthma.<sup>31,32</sup>

Interestingly, however, mitogen-induced T<sub>H</sub>1 cytokine responses in asthmatic patients did not parallel antigen-induced T<sub>H</sub>1 responses and were not decreased when compared with those of control subjects. Although we have reported reduced mitogen-induced IFN- $\gamma$  production in atopic asthmatic children,<sup>13</sup> a similar reduction has not been observed in adults. Mitogen-induced IFN- $\gamma$  responses in adults with atopic disease were not significantly different from those of control subjects.<sup>33</sup> Furthermore, mitogen-induced IFN- $\gamma$  production was not different in adolescent asthmatic patients with active versus

remitted disease.<sup>14</sup> This suggests that a defect of polyclonal IFN- $\gamma$  production is present in childhood but resolves with increasing age and that this holds true for both atopic and nonatopic subjects. Consistent with this, we have recently reported age-related changes in polyclonally induced IFN- $\gamma$  responses in atopic subjects.<sup>22</sup> Atopic children have reduced staphylococcal enterotoxin B–induced IFN- $\gamma$  production compared with that seen in nonatopic children in early childhood that resolves by 10 years of age.<sup>22</sup> The presence of reduced IFN- $\gamma$  levels after stimulation with allergen but not mitogen suggests that allergen-induced cytokine responses might provide a more accurate marker of inflammation in asthma. It would be of interest to examine airway levels of IFN- $\gamma$  after HDM challenge to determine whether similar findings to those for PBMCs are observed.

The use of inhaled corticosteroids in our patients is unlikely to have influenced cytokine production. Hydrocortisone, mometasone, and dexamethasone have been shown to inhibit production of IFN- $\gamma$ ,<sup>34-36</sup> IL-4,<sup>35,37,38</sup> IL-5,<sup>38,39</sup> and IL-13<sup>35,40</sup> in vitro at concentrations of greater than 390  $\mu$ g/L. The systemic concentration after a 1000- $\mu$ g dose of inhaled fluticasone propionate is 0.34  $\mu$ g/L,<sup>41</sup> and hence it is unlikely that the systemic levels of corticosteroids reached with use of inhaled therapy in our patients would influence cytokine production. Indeed, in vivo administration of oral glucocorticoid in allergen-challenged asthmatic subjects did not influence the expression of IL-4 or IFN- $\gamma$ .<sup>42-44</sup> Moreover, if the reduced IFN- $\gamma$  level in patients with severe asthma was related to steroid therapy, one would also have expected a reduction in IL-5 production.

In summary, we have found that atopic asthmatic patients with persistent, severe disease have a reduced allergen-induced T<sub>H</sub>1 response, whereas those with resolved asthma do not. This suggests that reduced HDM-induced IFN- $\gamma$  production might be an important factor contributing to ongoing severe asthma and supports the future development of therapies aimed at enhancing the T<sub>H</sub>1 response. Another important finding in this study was that subjects with a history of asthma all displayed increased HDM-induced T<sub>H</sub>2 (IL-5 and IL-13) cytokine responses, irrespective of the presence or absence of ongoing asthma. This suggests that increased T<sub>H</sub>2 responses might reflect the presence of the atopic state per se rather than being specifically linked to ongoing asthma and that inhibition of T<sub>H</sub>2 cytokine responses might not be required or sufficient for disease resolution.

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