Differential regulation of allergen-specific T_{H2}- but not T_{H1}-type responses by alveolar macrophages in atopic asthma

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Background and Objective: Previous studies have suggested that quantitative differences in T_{H2}-type cytokine responses in the airways are of particular importance in the pathogenesis of asthma. In this study we investigated whether alveolar macrophages (AMs) and peripheral blood monocytes (PMNs) are able to significantly influence the profiles of allergen-induced T_{H1} (IFN-γ) and T_{H2} (IL-4 and IL-5) cytokine production by CD4+ T cells in atopic asthmatic subjects versus atopic nonasthmatic subjects and nonatopic normal subjects.

Methods: Peripheral blood CD4+ T cells were cultured alone or cocultured with either PMNs or AMs with allergen stimulation in the 3 groups.

Results: Although allergen stimulation did not change T_{H1} or T_{H2} cytokine responses in cultures of CD4+ T cells alone, the addition of PMNs to the cultures induced a significant increase in production of IL-4, IL-5, and IFN-γ ($P < .01$ or $P < .001$) in atopic asthmatic subjects and atopic nonasthmatic subjects. However, PMNs induced a significant increase for IFN-γ ($P < .05$) only in normal subjects. AMs from atopic asthmatic subjects significantly enhanced production of all 3 cytokines ($P < .01$ or $P < .001$), whereas the AMs from atopic nonasthmatic subjects significantly increased only production of IL-4 ($P < .01$) and IFN-γ ($P < .05$) but not IL-5. Furthermore, IL-4 ($P = .066$) and IL-5 ($P < .01$) production in allergen-stimulated AM-CD4+ cell cocultures was higher in atopic asthmatic subjects but significantly lower in atopic nonasthmatic subjects ($P < .05$) as compared with the PMN-cocultures. For IFN-γ, no difference was found between the AM and PMN cocultures in either atopic group. Allergen-stimulated IL-5 production in coculture with both AMs and PMNs inversely correlated with both baseline FEV_1 percent predicted and PD_{20} methacholine in atopic asthmatic subjects ($P < .05$, $P < .01$, or $P < .001$).

Conclusion: These data suggest that AMs from atopic asthmatic subjects but not atopic nonasthmatic subjects, play a significant role in airway pathogenic immunity through enhancing T_{H2}-type cytokine production. (J Allergy Clin Immunol 1998;102:368-75.)

Key words: *T_{H1},T_{H2}, alveolar macrophage, CD4+ T cell, asthma*

Allergen exposure initiating the activation of specific CD4+ T cells is believed to be a major factor in asthma in atopic individuals. It has been shown that allergen-driven T_{H2} cytokine production by CD4+ T-helper cells plays a crucial role in the induction and maintenance of allergic inflammation in the bronchial mucosa in atopic asthmatic subjects.[1-5]

An important question for understanding asthma is why not all atopic individuals who are sensitized to inhaled allergens develop asthma. There is some evidence that allergen exposure, either naturally or experimentally, can result in a substantial difference in IL-5 mRNA expression in bronchial biopsy specimens and IL-5 production by cells from broncheoalveoar lavage (BAL) fluid in atopic asthmatic subjects compared with atopic nonasthmatic subjects. Furthermore, these differences are related to the differential density and activation of eosinophils in the airways in these atopic individuals.[6-8] Airway eosinophilia is thought to be the major cause of the bronchial mucosa damage resulting in the symptoms of asthma.[9,10] Taken together, these findings suggest that quantitative differences in allergen-specific T_{H2}-type responses in the airways have a potentially important influence in the development of atopic asthma.

The mechanisms by which allergen exposure triggers quantitatively different IL-5 responses in the airways with contrasting clinical outcomes in atopic individuals is poorly understood. To date, there has been no convincing evidence for a difference in the intrinsic potential of T cells to produce T_{H2}-type cytokines in response to allergen between atopic asthmatic subjects and atopic nonasthmatic subjects. However, many recent studies have shown that the cytokine secretion pattern of CD4+ T cells can be modulated by antigen presenting cell (APC)–related factors present in the microenvironment of the T cells during their activation.[11-18] Although these in vitro studies have clarified the polarizing effects of particular APC-related factors that favor T_{H1}- or T_{H2}-

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Supported by Glaxo Wellcome Australia, The Alfred Foundation and the NH and MRC of Australia.

Received for publication Jan 29, 1998; revised April 28, 1998; accepted for publication May 21, 1998.

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0091-6749/98 $5.00 + 0 **1/1/91951**

TABLE I. Characteristics of patients and control subjects

Groups	Age (y)	Sex (F/M)	FEV_1 (% pred)	PD_{20} meth (mg)*
AA (n = 16)	34 (20-70)	6/10	87 (70-102)†‡	0.019 (0.002-1.006)
AN (n = 12)	42 (21-61)	1/11	120 (88-132)	>2
N (n = 10)	26 (19-46)	3/7	110 (99-120)	>2

All data are shown as medians and ranges.
FEV_1 performed as baseline for PD20 measurement having abstained from short acting bronchodilator for at least 6 hours.
AA, Atopic asthmatic subjects; *AN*, atopic nonasthmatic subjects; *N*, nonatopic normal subjects.
*Provocative dose of methacholine producing 20% fall in FEV_1.
†Compared with AN, $P = .002$.
‡Compared with N, $P < .001$.

Abbreviations used
AM: Alveolar macrophage
APC: Antigen presenting cell
BAL: Bronchoalveolar lavage
HDM: House dust mite
PMN: Peripheral blood monocyte

type responses, the role of distinct cell populations in the lungs in influencing the pattern of allergen-specific CD4+ cell cytokine responses in atopic subjects remains to be identified.

Alveolar macrophages (AMs) are likely candidates to play a role in this respect, although dendritic cells and not AMs are considered as the most important "professional" APCs in human lungs. An important role for AMs in T-cell–mediated pathogenic immunity in asthma has been suggested by previous studies showing the differential effects of AMs on T-cell blastogenic responses to antigens or mitogens in vitro in asthmatic subjects versus nonasthmatic subjects.[19-24] Recent work from our laboratory has revealed that AMs from atopic asthmatic subjects, but not from atopic nonasthmatic subjects, enhance IL-5 production by CD4+ T cells on stimulation with allergen or mitogen, further indicating a critical role of AMs in the development of local "asthmagenic" cytokine responses.[25] However, in view of the observations that mRNA expression and protein production of IL-5 rather than IL-4 and IFN-γ by airway T cells most discriminate atopic asthmatic subjects from atopic nonasthmatic subjects,[4-7] confirmation of a specific regulatory role for AMs requires that the effects of AMs on IL-5 response be compared with their effects on IL-4 or IFN-γ responses.

Therefore in this study we compared the effects of AMs versus those of peripheral blood monocytes (PMNs) on allergen-induced production of IL-4, IL-5, and IFN-γ by peripheral blood CD4+ T cells in atopic asthmatic subjects, atopic nonasthmatic subjects, and nonatopic normal subjects. We hypothesized that AMs from atopic asthmatic subjects exert different effects on the pattern of CD4+ T-cell cytokine production compared with those from atopic nonasthmatic subjects and that the differences influence the development of asthma in the atopic population.

METHODS

Subjects

Sixteen atopic asthmatic subjects, 12 atopic nonasthmatic subjects and 10 nonatopic normal subjects were studied (Table I). Atopic asthmatic subjects had a history of periodic wheeze and were using β_2-agonist when required for relief of symptoms, but none had received inhaled or oral corticosteroid therapy in the month before the study. All asthmatic subjects demonstrated a positive PD20 (ie, 20% fall in $FEV_{1.0}$ from baseline) of less than 2 mg methacholine and had documented reversible airflow obstruction (at least 15% improvement in FEV_1 either spontaneously or in response to inhaled β_2-agonist). All atopic subjects had 1 or more positive skin test responses to a panel of common environmental allergens, demonstrating a positive skin test reaction to rye grass pollen or house dust mite (HDM) with at least 3-mm wheal diameter. These allergens were used in culture experiments.

Atopic nonasthmatic subjects had no past or present asthmatic symptoms and had never received anti-asthma treatments. Nonatopic normal subjects had no history of asthma, bronchitis, or allergic disease and had negative skin prick test responses to the same panel of allergens. None of the subjects were current smokers (or exsmokers of more than 10 pack-years).

This study was approved by The Alfred Hospital Ethics Committee and informed consent was given by all patients and control subjects.

Bronchoscopy

Fiberoptic bronchoscopy was undertaken according to the guidelines of the American Thoracic Society.[26] Subjects were premedicated with nebulized albuterol and received intravenous atropine (0.6 mg) and intravenous midazolam for sedation. They received supplemental oxygen at 4 L/min throughout and were monitored by continuous pulse oximetry. Lidocaine (4% and 2% solutions) was used for local anesthesia as required. BAL was performed with 3 aliquots of 60 mL of PBS at 37°C introduced by gentle hand pressure into a subsegment of the right middle lobe and recovered by gentle suction at a pressure of –80 mm Hg. The BAL fluid was collected into polypropylene tubes and immediately placed on ice. Forty milliliters of venous blood was drawn at the same time into a heparinized container for later separation of monocytes and CD4+ T cells.

Physiology

Airway function and responsiveness to methacholine were measured at least 2 days preceding the bronchoscopy and within 28 days for all subjects. Airflow measurements, including FEV_1 and FVC, were performed according to American Thoracic Society guidelines.[27] Airway responsiveness to methacholine was assessed with a standard methacholine challenge protocol as described before[28]: doubling doses of methacholine, generated by a compressed air

TABLE II. Cytokine production (pg/mL) in cultures of CD4+ T cells alone

Groups	IL-4		IL-5		IFN-γ	
	Medium	Allergen*	Medium	Allergen	Medium	Allergen
AA (n = 16)	400 (<31-1415)	400 (<31-1092)	398† (<31-1042)	383† (<31-1331)	438 (<50-2123)	516 (<50-2123)
AN (n = 12)	258 (<31-735)	347 (<31-845)	120 (<31-593)	158† (<31-810)	571 (<50-1530)	888 (83-1993)
N (n = 10)	79 (<31-382)	77 (<31-539)	59 (<31-296)	34 (<31-268)	377 (95-1302)	392 (184-1307)

All data are shown as medians and ranges.
AA, Atopic asthmatic subjects; *AN*, atopic nonasthmatic subjects; *N*, nonatopic normal subjects.
*Allergen stimulation: rye grass pollen 20 μg/mL or HDM 10 μg/mL.
†Compared with N, $P < .05$.

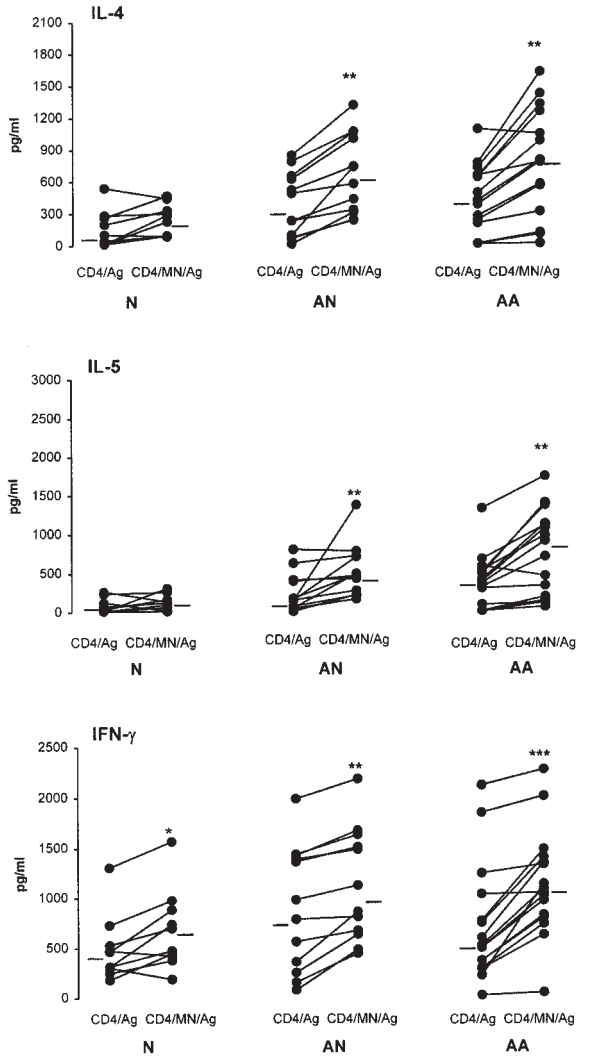

FIG 1. PMN-induced changes in production of IL-4, IL-5, and IFN-γ by allergen-stimulated CD4+ T cells in 16 atopic asthmatic subjects *(AA)*, 12 atopic nonasthmatic subjects (*AN*), and 10 nonatopic normal subjects (*N*). Differences between cultures with and without PMN: *$P < .05$, **$P < .01$, ***$P < .001$. *Bars* represent median values.

driven jet nebulizer, were inhaled at 5-minute intervals. Doses ranged from 3.12 μg to a possible maximum of 6400 μg. The test was stopped when there had been a greater than 20% decrement in FEV_1. The dose producing a 20% fall in FEV_1 was calculated by linear interpolation from a graph of FEV_1 against log cumulative dose. All physiologic measurements were made at the same time of day and at least 8 hours after any inhaled β_2-agonist medication.

Isolation of peripheral blood CD4+ T cells, PMNs, and AMs

CD4+ T cells, PMNs, and AMs were prepared by using the same protocols as previously described.[25] Briefly, isolated PBMCs were incubated with magnetic beads coated with mAb against CD4 (Dynal A.S., Oslo, Norway) at a ratio of 4:1 (beads to CD4+ T cells) at 4°C for 1 hour with gentle tilting and rotation. The rosetted CD4+ T cells were isolated by placing the test tube on a magnet for 2 to 3 minutes, washing with PBS containing 2% FCS, and using DETACHaBEADS (Dynal A.S., Oslo, Norway) to remove the bound beads from CD4+ T cells. This procedure resulted in 99% or greater CD4+ T cells by FACS analysis with 99% or more viability by trypan blue dye exclusion. There was less than 1% CD14 positive cells (ie, monocytes) in this fraction.

PMNs were prepared by adherence of the CD4+ T cell–free fraction of PBMCs on plastic dishes for 1 hour at 37°C in a 5% CO_2 humidified atmosphere. The nonadherent cells were discarded, and the adherent cells were removed by gently scraping with a plastic scraper and then resuspended in RPMI 1640 medium supplemented with 5% heat-inactivated FCS, 2 mmol/L L-glutamine, penicillin 100 U/mL, and gentamicin 125 μg/mL. By 2 rounds of the protocol, the adherent cells were more than 95% PMNs, with a viability of 95% or more as judged by Quick Dip and trypan blue staining. AMs were isolated directly from BAL cell suspensions by the same adherence procedure, the resulting population containing more than 95% AMs, with a viability of more than 90%.

Cell cultures

Purified peripheral blood CD4+ T cells were cultured (1×10^6/mL per well) alone with and without allergen, or cocultured at a 2:1 ratio with autologous PMNs or AMs with allergen in RPMI 1640 medium, with the same supplements as described in above. The ratio of 2:1 T cells to accessory cells was chosen following preliminary ratio-changing studies. The cultures were performed for 5 days. Rye grass pollen (20 μg/mL) or HDM (10 μg/mL) was used for atopic subjects according to their skin test sensitivity and for normal control subjects at random. The same batch of rye grass pollen and HDM were used for all the experiments (Greer Laboratories, Lenoir, NC).

ELISA

Quantification of IL-4, IL-5, and IFN-γ concentrations in cell culture supernatants were performed by ELISA with paired antibodies (PharMingen, San Diego, Calif) according to the procedure recommended by the manufacturer. Recombinant human IL-4, IL-5, and IFN-γ proteins were used as standards. The limits of these detections were 31 pg/mL of IL-4 and IL-5 and 50 pg/mL of IFN-γ.

Statistics

The Mann Whitney U test and the Wilcoxon signed rank test were used for intergroup and intragroup comparisons, respectively. The relationships were analyzed by Spearman's rank correlation. In all statistical analyses, 50% of the limits of detectability was used for the undetectable cytokine levels in the supernatants of cell cultures.

RESULTS

Allergen-induced changes in cytokine production by CD4+ T cells

A comparison of cytokine production in cultures of CD4+ T cells alone is shown in Table II. In unstimulated CD4+ cell cultures, a nonsignificant trend for an increased IL-4 production was shown by both the atopic groups when compared with that for nonatopic normal subjects ($P = .07$ and $P = .09$ compared with atopic asthmatic and atopic nonasthmatic subjects, respectively). For IL-5 production, atopic asthmatic subjects demonstrated a significantly higher value compared with nonatopic normal subjects ($P < .05$). No significant difference was seen between the two nonasthmatic groups ($P = .23$). For IFN-γ production, the 3 groups behaved similarly.

Allergen stimulation in this culture did not result in a significant change in production of IL-4, IL-5, and IFN-γ in the 3 subject groups. However, a significant difference developed for IL-5 production between atopic nonasthmatic subjects and nonatopic normal subjects after allergen stimulation ($P < .05$).

PMN-induced changes in cytokine production by allergen-stimulated CD4+ T cells

Comparable increases in cytokine production occurred with the addition of PMNs to allergen-stimulated CD4+ T-cell cultures in both the atopic groups (Fig 1). The median levels of IL-4 were significantly enhanced to 779 pg/mL (<31 to 1625 pg/mL) in atopic asthmatic subjects and to 659 pg/mL (241 to 1320 pg/mL) in atopic nonasthmatic subjects ($P < .01$). This was also the case for the median levels of IL-5 (to 812 pg/mL [67 to 1742 pg/mL] for atopic asthmatic subjects and to 475 pg/mL [174 to 1380 pg/mL] for atopic nonasthmatic subjects, $P < .01$) and for IFN-γ (to 1070 pg/mL [53 to 2278 pg/mL] for atopic asthmatic subjects, $P < .001$ and to 998 pg/mL [450 to 2188 pg/mL] for atopic nonasthmatic subjects, $P < .01$). In nonatopic normal subjects, the increase induced by the addition of PMNs was significant only for the median level of IFN-γ (to 590 pg/mL [193 to 1572 pg/mL], $P < .05$) but not for IL-4 and IL-5.

In allergen-stimulated PMN-CD4+ cell cocultures, both the atopic groups showed significantly higher production of IL-4 ($P < .05$ and $P < .01$, respectively), IL-5 ($P < .001$ and $P < .01$, respectively), and IFN-γ ($P < .05$) compared with nonatopic normal subjects. No significant difference was seen between the 2 atopic groups.

AM-induced changes in cytokine production by allergen-stimulated CD4+ T cells

The addition of AMs to allergen-stimulated CD4+ cell cultures caused a further increase in the median levels of IL-4 (to 896 pg/mL [110 to 1775 pg/mL], $P < .001$) and IL-5 (to 959 pg/mL [177 to 2431 pg/mL], $P < .001$), as well as an increase in IFN-γ (to 1152 pg/mL [407 to 2233 pg/mL], $P < .01$) in atopic asthmatic subjects (Fig 2). In atopic nonasthmatic subjects, the addition of AMs resulted in elevated median levels of IL-4 (to 489 pg/mL [157 to 1205 pg/mL], $P < .01$) and IFN-γ (to 1230 pg/mL [400 to 2303 pg/mL], $P < .05$) but not in IL-5 (233 pg/mL [80 to 930 pg/mL]). In nonatopic normal subjects, an increase was found only in the production of IFN-γ (to 666 pg/mL [245 to 1639 pg/mL], $P < .05$).

In allergen-stimulated AM-CD4+ cell cocultures, there was a significant increase in production of IL-4 (*P*

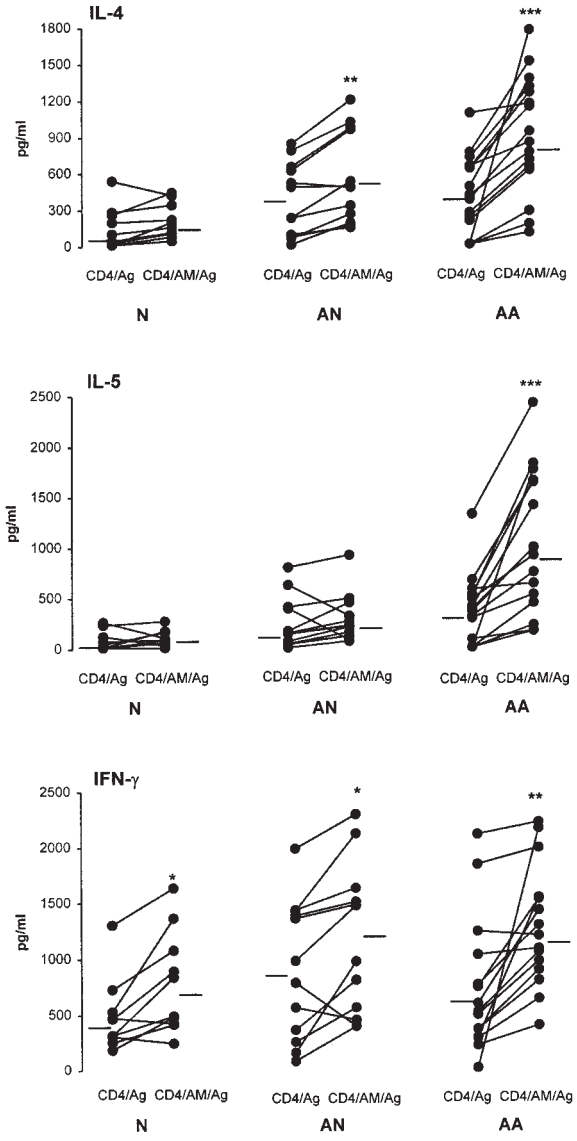

FIG 2. AM-induced changes in production of IL-4, IL-5, and IFN-γ by allergen-stimulated CD4+ T cells in 16 atopic asthmatic subjects *(AA)*, 12 atopic nonasthmatic subjects *(AN)*, and 10 nonatopic normal subjects (*N*). Differences between cultures with and without AM: **P* < .05, ***P* < .01, ****P* < .001. *Bars* represent the median values.

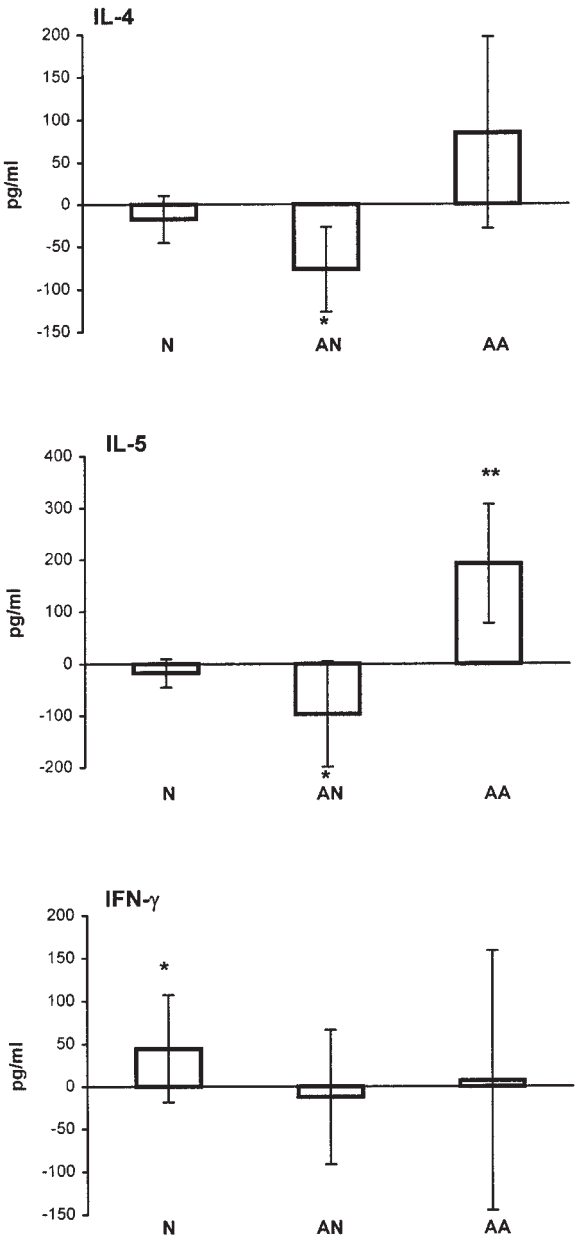

FIG 3. Differences in IL-4, IL-5, and IFN-γ production between allergen-stimulated AM- and PMN-CD4+ T cell cocultures in 16 atopic asthmatic subjects *(AA)*, 12 atopic nonasthmatic subjects *(AN)*, and 10 nonatopic normal subjects *(N)*. Data are shown as medians with SEM. **P* < .05, ***P* < .01.

< .001 and $P < .05$, respectively) and IL-5 ($P < .001$ and $P < .01$, respectively) in both the atopic groups compared with that found in nonatopic normal subjects. However, no significant difference was found in IFN-γ production among the 3 subject groups. Of particular interest, there was a significant difference in IL-5 production between atopic asthmatic subjects and atopic nonasthmatic subjects ($P < .01$).

Differences in cytokine production between allergen-stimulated AM- and PMN-CD4+ cell cocultures

There were quantitative differences in IL-4, IL-5, and IFN-γ production between AM- and PMN-CD4+ cell cocultures upon allergen stimulation in the 3 subject groups. As shown in Fig 3, in atopic asthmatic subjects, there was a trend for increased production of IL-4 (median 84 pg/mL [–175 to 1775 pg/mL], $P = .066$) and a markedly higher secretion of IL-5 (median, 192 pg/mL [–480 to 1622 pg/mL], $P < .01$) by allergen-stimulated CD4+ T cells with the addition of AMs compared with the addition of PMNs. In contrast, atopic nonasthmatic subjects showed significantly lower median levels of IL-4 (–77 pg/mL [–582 to 100 pg/mL], $P < .05$) and IL-5 (–97 pg/mL [–1150 to 140 pg/mL], $P < .05$) in AM-CD4+ cell cocultures compared with that in the PMN cocultures. Neither atopic group showed a significant difference in IFN-γ production between the AM- and PMN-CD4+ cell cocultures. However, in nonatopic normal subjects, there was a significant increase in the median level of IFN-γ (47 pg/mL [<50 to 165 pg/mL], $P < .05$) in the AM-CD4+ cell cocultures compared with the PMN cocultures.

Relation between physiological indexes and cytokine production in atopic asthmatic subjects

There was a trend for a negative association between FEV_1 percent predicted in atopic asthmatic subjects and spontaneous IL-5 production by their CD4+ cells alone ($r = –0.432$, $P = .08$). This relation became significant in allergen-stimulated PMN-CD4+ cell cocultures ($r = –0.497$, $P < .05$) and was even more marked in the AM-cocultures ($r = –0.754$, $P < .001$). There was also an inverse relation in this group between log PD_{20} methacholine and IL-5 production in unstimulated cultures of CD4+ cells alone ($r = –0.492$, $P < .05$), allergen-stimulated PMN-CD4+ cell cocultures ($r = –0.657$, $P < .01$), and in the AM- cocultures ($r = –0.523$, $P < .05$). These physiological indexes did not correlate with either IL-4 or IFN-γ levels (Fig 4).

DISCUSSION

The findings of this study demonstrated a differential regulation of allergen-specific T_{H2} (IL-4 and IL-5) cytokine responses by AMs in atopic asthmatic subjects compared with atopic nonasthmatic subjects. This differential regulation was most striking for the enhancement of IL-5 production.

Whereas allergen stimulation per se did not change IL-4, IL-5, or IFN-γ production in cultures of CD4+ T cells alone, the addition of PMNs or AMs to the cultures induced differential increases in the capacity of CD4+ T cells to produce cytokines in response to allergen in the 3 subject groups. PMNs from both atopic groups showed comparable effects in significantly promoting IL-4, IL-5, and IFN-γ production by allergen-stimulated CD4+ T cells. In nonatopic normal subjects, however, the PMN-

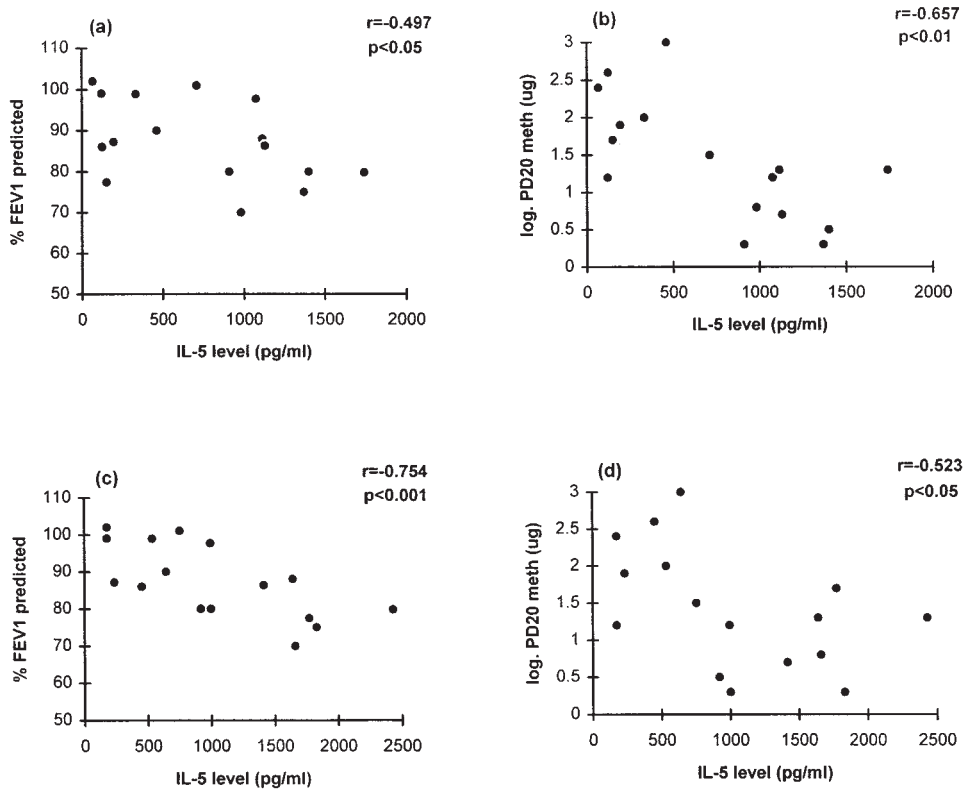

FIG 4. Relation between IL-5 production in allergen-stimulated PMN- or AM-CD4+ T cell cocultures and FEV_1 percent predicted (**a** and **c**), log PD_{20} methacholine (**b** and **d**) in 16 atopic asthmatic subjects.

induced increase was significant only for IFN-γ production. AMs from atopic asthmatic subjects also significantly enhanced production of the 3 cytokines in cocultures with allergen-stimulated CD4+ T cells, whereas the AMs from atopic nonasthmatic subjects only increased IL-4 and IFN-γ secretion significantly. Furthermore, IL-5 production in allergen-stimulated AM-CD4+ cell cocultures was markedly higher in atopic asthmatic subjects but significantly lower in atopic nonasthmatic subjects when compared with their respective PMN cocultures. For IFN-γ production, no difference was found between the AM and PMN cocultures in the atopic groups. In nonatopic normal subjects, however, there was a significantly higher production of IFN-γ induced by the AMs than that stimulated by the PMNs.

In this study the intrinsic ability of allergen-specific CD4+ T cells to respond to a relevant allergen appeared to be identical in atopic asthmatic subjects and atopic nonasthmatic subjects, because the cells from both the atopic groups responded to antigen presented by PMNs with similar increases in IL-4, IL-5, and IFN-γ production. In nonatopic normal subjects, in contrast, there was a significant elevation in production of IFN-γ but neither IL-4 nor IL-5 by allergen-stimulated CD4+ T cells under the same conditions. Compatible with our observations are studies showing that allergen-specific responses are characterized by both T_{H1}- and T_{H2}-type cytokine production in atopic individuals but only T_{H1} type cytokine secretion in nonatopic individuals.[1,29-31] It seems likely, therefore, that the essential immunologic difference between atopic and nonatopic individuals is based on the ability of their allergen-specific CD4+ T cells to produce T_{H2} cytokines on allergen stimulation rather than lack of the ability to recognize the antigen.[32,33] Indeed, a recent study has shown a proliferative response in the lymphocyte population in both atopic and nonatopic individuals on allergen exposure,[34] although we have not studied that index of cell activation in this interaction model.

On the other hand, the difference in allergen-induced immune responses in atopic subjects with and without asthma is characterized by different magnitudes of IL-5 responses. Previous studies have shown a significant difference in IL-5 production by allergen-stimulated PBMCs, but especially by BAL cells, between atopic asthmatic subjects and atopic nonasthmatic subjects.[8,10,35] Compatible with this was a recent study demonstrating increased numbers of cells expressing IL-5 mRNA and protein in the bronchial mucosa of atopic asthmatic subjects as compared with atopic nonasthmatic subjects.[9] These findings give further support to the hypothesis that quantitative differences in T_{H2} cytokine responses, particularly in IL-5 production, are of vital importance in the pathogenesis of asthma.[36] Furthermore, our present data on the correlation of in vitro IL-5

production with both baseline lung function and airway responsiveness in atopic asthmatic subjects provide supportive evidence for the clinical relevance of these findings.

An important observation made here is that the magnitude of allergen-specific T_{H2} cytokine responses in atopic asthmatic subjects and atopic nonasthmatic subjects was markedly modulated by AMs. The increased levels of IL-4 and IL-5 production by allergen-stimulated CD4+ T cells were very similar in the 2 atopic groups when conditioned by PMN but significantly different when mediated by AMs. It could be argued, therefore, that AMs from atopic asthmatic subjects may play a significant role in pathogenic immunity in the airway through enhancing differentially T_{H2}-type cytokine production. Moreover, the fact that in atopic nonasthmatic subjects there was a significant decrease in production of both IL-4 and IL-5 in allergen-stimulated AM-CD4+ T cell cocultures, compared with the parallel PMN cocultures, may reflect a protective mechanism offered by AMs from these subjects. Previous findings consistent with this have shown that AMs inhibit T cell proliferative responses in normal but not asthmatic subjects, and these immunosuppressive properties are particular for AMs compared with autologous blood PMNs.[19,20,23,24] Thus there appears to be a special role for AMs in determining the local immune responses provoked by allergen exposure in atopic individuals.

The mechanism for the differential effect of AMs in atopic asthma was not examined in this study. It will be important to investigate whether this is related to different patterns of cytokine production or phenotype differences in AMs in atopic asthmatic subjects versus atopic nonasthmatic subjects. Recent studies have suggested the importance of APC-derived cytokines in affecting the development of T_{H2}-like cells in allergic airway reactions. Among such modulating cytokines, IL-12 appears to be the most important cytokine in promoting the induction of the T_{H1} phenotype and thereby inhibiting the allergic/asthmatic reaction.[11,37,38] On the other hand, IL-1β, IL-6, and prostaglandin E_2 have been shown to encourage a T_{H2} response.[11,18,39] The relative levels of these modulating cytokines in the T-cell micromilieu may influence the differentiation of naive or memory T_{H0} cells into either T_{H1} or T_{H2} effectors, or even elicit different patterns of cytokine production from the same effector population.[17,37,38,40] We presume that "asthmatic" T_{H2}-type development in our 5-day culture model would involve T cells mainly at the stage of memory cells, influenced by an imbalance of production of T_{H2}- versus T_{H1}-favoring modulators by AMs during allergen-specific activation of CD4+ T cells. Indeed, our recent work has suggested that the effects of AMs from atopic asthmatic subjects in enhancing IL-5 production by allergen-specific CD4+ T cells were significantly inhibited by using monoclonal antibodies against IL-1β and IL-6.[25] The relation between the balance of IL-12 and IL-1β and IL-6 production by AMs and IL-5 production by CD4+ T cells in atopic subjects with and without asthma is under current investigation in our laboratory.

Compatible with other descriptions of heterogeneity in cytokine production among T_{H2} cells under different circumstances,[40,41] our data show that CD4+ T-cell cytokine responses vary for individual cytokines in CD4+ T cell–AM cocultures and do not completely fit a T_{H1}/T_{H2} paradigm. There was a significant difference in IL-5, but not IL-4, production in the cocultures between atopic asthmatic subjects and atopic nonasthmatic subjects. Although an increased level of IL-5 seemed already to exist in vivo in atopic asthmatic subjects, as reflected in unstimulated CD4+ cell IL-5 production, the effect of AMs in amplifying the difference in IL-5 production by peripheral blood CD4+ T cells between the 2 atopic groups in vitro was striking. This effect needs to be further confirmed on airway T cells. However, the excessive IL-5 response in this model, although with comparable IL-4 production, in atopic asthmatic subjects versus atopic nonasthmatic subjects appears to be clinically highly relevant because the present data, together with previous studies, have consistently demonstrated that IL-5, but not IL-4, is closely related to asthma phenotype expression.[9,30,35,36]

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