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Original article

Eosinophilia, interleukin-5, and tumour necrosis factor-alpha in asthmatic children

Background: There are few paediatric studies of the interrelationships between inflammatory markers and asthma severity. We therefore assessed the relationships between eosinophil-associated markers, cytokines, and asthma severity in asthmatic children aged 8–12 years.
Methods: Forty-five children were tested twice, 2 weeks apart. Asthma severity was measured in terms of symptoms, lung function, medication needs, and histamine responsiveness. Peripheral inflammatory markers measured included eosinophil numbers, serum ECP, IL-5, and TNF-α and mononuclear cell IL-5, and TNF-α production.
Results: Histamine responsiveness was correlated with circulating eosinophils ($r=0.56$, $P=0.0001$) and serum ECP ($r=0.54$, $P=0.003$). Eosinophilia was increased in children with severe as opposed to mild airway hyperresponsiveness ($P=0.02$) and those who lost days at school as opposed to those who did not ($P=0.01$). There were no other associations between markers of asthma severity and inflammation. Children taking inhaled corticosteroids had lower serum IL-5 levels than those on β-agonists ± cromolyn (mean and 95% CI: 20.5 [11.7–35.7] pg/ml *vs* 64.3 [26.6–155.4] pg/ml; $P=0.04$). Cellular IL-5 production correlated with serum TNF-α ($r=0.63$, $P=0.0062$) and IL-5 ($r=-0.59$, $P=0.005$).
Conclusions: Serum levels of TNF-α and IL-5 were not related to peripheral eosinophilia and asthma severity in these children but were related to their own cellular production *ex vivo*. This study confirms that eosinophilia is the index of inflammation that is most closely related to the clinical severity of childhood asthma.

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Eosinophilic inflammation is a characteristic feature of asthma. The products released by human eosinophils following activation can cause human airway constriction (1) and also induce human airway hyperresponsiveness (AHR) *in vitro* (2). However, eosinophilia occurs in other lung diseases, but not necessarily in association with AHR (3). Activated T cells, mast cells, and eosinophils themselves release mediators which are implicated in eosinophil recruitment to, and activation in, the airways. Studies in transgenic mice have revealed that T-helper lymphocytes as well as proeosinophilic mediators are required to induce eosinophil degranulation and AHR following allergen exposure (4). Cytokines such as interleukin (IL)-5, in particular, are thought to play a central role in this process (2, 4).

The cytokine IL-5 specifically affects human eosinophils and is produced by T cells, mast cells, and eosinophils. IL-5 promotes not only maturation of eosinophils in the bone marrow but also, together with other mediators, their migration from the vasculature into the surrounding tissues and their activation and survival at the inflammatory site (reviewed in Ref. 5). Elevated levels of IL-5 have been found in the serum and airway fluid of patients with asthma (3, 6). In asthmatic subjects, IL-5 inhalation increased AHR, as well as the recruitment of activated eosinophils to the airways (7).

Another cytokine likely to play a central role in promoting eosinophilia in asthma is the proinflammatory cytokine tumour necrosis factor-alpha (TNF-α). It is also present in increased amounts in asthmatic airways (3). It is produced by a variety of inflammatory cells in response to various stimuli, including infectious agents and allergen. TNF-α regulates recruitment of inflammatory cells, such as eosinophils, by increasing the expression of adhesion molecules on vascular endothelium (reviewed in Ref. 5). It is also a potent activator of eosinophils (8).

Few studies have explored the relationships between inflammatory markers and asthma in asthmatic children because of the invasive nature of the current techniques of sample collection. The use of circulating

eosinophil numbers and levels of eosinophil granule protein in serum has been suggested for monitoring asthma in children (9–11). The question of whether other markers of inflammation, such as levels of proinflammatory cytokines, would be better to use in children is only just being explored.

The aims of the present study in asthmatic children were, firstly, to quantify serum levels of the cytokines IL-5 and TNF-α and determine whether or not the cytokine levels are associated with both eosinophilia and asthma severity, and, secondly, to quantify TNF-α and IL-5 production by peripheral blood mononuclear cells (PBMC) activated *ex vivo*, and to explore the relationships between cellular production and serum levels of these cytokines and eosinophilia.

Material and methods

Subjects

In this study, we extended and analysed baseline data collected from children enrolled for a study of the effects of dietary fatty acids on the clinical severity of asthma. The results of the latter study have been published elsewhere (12). Forty-five 8–12-year-old asthmatic children with histories of episodic wheeze in the previous 12 months and airway hyperresponsiveness (AHR) to histamine were recruited over a 16-month period through asthma outpatient clinics and by newspaper advertisements. Children were excluded from the study if they had known dietary salicylate or aspirin sensitivity, were taking regular oral corticosteroids, or had other significant diseases. The study was approved by the Ethics Review Committee of the Central Area Health Service and by the Human Ethics Committee of the University of Sydney.

Study design

There were two baseline visits separated by a 2-week run-in period to obtain diary-card data of asthma severity prior to randomization to dietary treatment. Data from these two baseline visits are reported in this paper. Responses to allergen skin prick tests and the child's history of respiratory symptoms, medication use, and the number of days lost from school due to asthma in the last 12 months were documented at the initial visit. At both visits, AHR was recorded and venous blood collected, not only to determine eosinophil numbers and TNF-α production by PBMC, as reported previously (12), but also IL-5 production by PBMC and serum levels of ECP, IL-5, and TNF-α.

Lung function and AHR

Lung function and AHR were measured at each visit. Subjects were asked not to use short-acting aerosol bronchodilators for 6 h and long-acting bronchodilators for 24 h prior to each visit. Forced vital capacity (FVC) and forced expiratory volume in 1 s (FEV_1) were measured with a Vitalograph wedge bellows spirometer (Vitalograph, Buckinghamshire, UK). The percentage of predicted values was calculated from the highest FEV_1 value recorded that was repeatable within 100 ml (13).

Airway responsiveness was assessed with histamine inhalation tests by the method of Yan et al. (14). Briefly, histamine was administered with hand-held De Vilbiss no. 45 plastic nebulizers (De Vilbiss Health Care, Inc, Somerset, PA, USA) in doubling doses from 0.03 to 7.8 μmol until the FEV_1 fell by at least 20%. The dose of histamine which caused a 20% fall in FEV_1 ($PD_{20}FEV_1$) was determined by linear interpolation of the dose-response curve. In addition, we calculated the dose-response ratio (DRR) (15) as the percent fall in FEV_1 at the final dose divided by the maximum cumulative dose of histamine administered. Subjects with $PD_{20}FEV_1 < 3.9$ μmol histamine (equivalent to DRR > 8.1% fall/μmol histamine) were classified as having mild AHR, while subjects with $PD_{20}FEV_1 < 0.6$ μmol histamine (equivalent to DRR > 36% fall/μmol histamine) were classified as having moderately severe AHR.

Atopy

The atopic status of each subject was determined by individually applying 13 allergens (Hollister Stier, Bayer Corporation, Spokane, WA, USA) by the skin prick method. Atopy was defined as a mean wheal size of ≥3 mm to at least one of the following allergens: house dust, *Dermatophagoides pteronyssinus*, *D. farinae*, cat hair and dander, dog hair and dander, horse, mixed feather, ragweed, plantain, timothy grass, ryegrass, *Aspergillus fumigatus*, and *Alternaria tenuis*.

Asthma severity

Asthma severity was assessed over 1 week, immediately before the second laboratory visit, as described previously (12). A daily diary was used to record peak expiratory flow rate (PEF) (percentage of predicted value), day and night symptoms (wheeze, cough, and shortness of breath), and medication use (frequency and type). Asthma severity was measured by a composite score (maximum 16) made up of the scores from these four components. PEF was measured on waking, before bronchodilator use, by an Assess peak flowmeter (HealthScan Products, NJ, USA) or a Mini-Wright peak flowmeter (Clement Clarke International Ltd, Essex, UK).

Blood collection and assays

Venous blood (≤20 ml) was collected at each visit, and plasma and serum fractions were prepared where possible. Full blood counts were performed by automated full blood count analyser (Bayer Technicon H2, Tarrytown, CT, USA). All appropriate fraction samples from a subject were assayed simultaneously for a particular mediator. Assay procedures for the various mediators are described below.

Serum levels of ECP. Enough blood was collected from 28 of the children for the preparation of serum samples. Blood was collected into Vacutainer^{R} SST^{R} tubes, clotted at 20–24°C for 1 h exactly after collection, and the serum was removed after centrifugation and stored at −20°C. Serum ECP levels were determined by a radioimmunoassay kit (Kabi Pharmacia Diagnostics, Uppsala, Sweden). The lower limit of detection of the kit was 2 ng/ml.

IL-5 and TNF-α. Levels of both cytokines were determined in 26 of the above serum samples by ELISA. For IL-5, a rat anti-mouse/human IL-5 monoclonal antibody (TRFK5) was used as capture antibody and a biotinylated rat anti-human IL-5 monoclonal antibody (JES1-5A10) used as detection antibody in a standard ELISA protocol supplied by the manufacturer (Pharmingen, San Diego, CA, USA). For TNF-α, the human TNF-α Duoset^{TM} system (Genzyme Diagnostics, Cambridge, MA, USA) was used. The lower limit of detection was 5 pg/ml for both cytokines.

TNF-α and IL-5 production capacity. Peripheral blood mononuclear cells (PBMC) were purified from anticoagulated blood by discontinuous density gradient centrifugation with monopoly resolving medium (ICN Biomedicals, Costa Mesa, CA,

USA) (16) and cultured in RPMI-1640 supplemented with Monomed A (CSL Ltd, Victoria, Australia) at 2×10^6 cells/ml in a 5% CO_2 in air atmosphere at 37°C. PBMC cultures were left untreated or activated with lipopolysaccharide (LPS) (Sigma), 10 ng/ml, or purified phytohaemagglutinin (PHA) (Murex Biotech, Dartford, UK), 1 μg/ml, for the determination of TNF-α and IL-5 production, respectively; harvested after 24 h incubation; and stored at −80°C. Total PBMC synthesis of TNF-α and IL-5 was assessed by ELISA (see above) of the appropriate cell cultures after several cycles of freeze-thawing.

Statistical analysis

Since there were no significant differences between the values from the first and second visits for any variable, values were calculated as the mean of the two values. Analyses were performed with Analyse-it V1.27 for Microsoft Excel (Analyse-it Software Ltd, UK). Shapiro-Wilk tests of normality showed that the DRR; circulating eosinophil numbers; and ECP, TNF-α, and IL-5 values were log-normally distributed; therefore, the data were log-transformed prior to analysis. Undetectable levels of the cytokines were set at 1 pg/ml. Data are reported as geometric mean and 95% confidence intervals. Correlations between variables were calculated by Pearson's correlation coefficient, and differences were calculated by Student's *t*-tests or paired *t*-tests as appropriate. A value of $P < 0.05$ was considered to be statistically significant.

Results

The characteristics of the children, their lung function, medication use, morbidity, and peripheral blood eosinophil numbers are shown in Table 1. There was a wide range of asthma severity in the group. More than half of the children were taking inhaled corticosteroids, 24 had moderately severe airway AHR ($PD_{20}FEV_1$ <0.6 μmol histamine), and more than two-thirds had missed time from school in the last 12 months due to asthma. During the week prior to the second assessment, 25 children experienced asthma symptoms severe enough to interfere with their normal daily activity or to cause them to wake at night.

Eosinophilic inflammation was measured as the number of circulating eosinophils and as serum ECP levels. Serum samples were prepared from the peripheral blood of 28 of the 45 children. Children who had serum collected did not differ from those who did not with respect to FEV_1, as % predicted ($P = 0.66$), $PD_{20}FEV_1$ ($P = 0.62$), asthma severity score ($P = 0.68$), or circulating eosinophil numbers ($P = 0.23$). Detectable levels of ECP (geometric mean and 95% confidence intervals: 37.7 [29.3–48.4] μg/ml) were present in all serum samples from the children and were elevated in all but three subjects (normal range 0–15 μg/ml). Subjects taking inhaled corticosteroids did not differ significantly from those on β_2-agonists ± cromolyn with respect to circulating eosinophil numbers (mean and 95% CI: 0.74 [0.62–0.85] cells $\times 10^9$ /l *vs* 0.71 [0.55–0.91] cells $\times 10^9$/l; $P = 0.79$) or serum ECP levels (mean and 95% CI: 40.9 [29.6–55.5] μg/ml *vs* 31.7 [21.5–46.5] μg/ml; $P = 0.36$).

The histamine DRR was significantly correlated with circulating eosinophil numbers ($r = 0.56$, $P < 0.0001$, $n = 43$) (Fig. 1a) and with serum ECP levels ($r = 0.54$, $P = 0.003$, $n = 28$) (Fig. 1b). Circulating eosinophil numbers were closely related to serum ECP levels ($r = 0.71$, $P < 0.0001$, $n = 28$). Lung function, as FEV_1, percent predicted, correlated significantly with circulating eosinophil numbers ($r = -0.38$, $P = 0.012$, $n = 42$), and approached a significant association with serum ECP levels ($r = -0.37$, $P = 0.06$, $n = 26$). The asthma

Table 1. Characteristics of subjects

$n = 45$	
M/F	25/20
Age (years)	10.3 (9.91–10.69)
Atopic	41.0 (91%)
Eosinophils $\times 10^9$/l	0.73 (0.63–0.84)
FEV_1 (% predicted)	85.9 (81.7–90.1)
$PD_{20}FEV_1$ (μmol histamine)	0.64 (0.41–1.01)
Dose-response ratio (% fall FEV_1/μmol histamine)	32.2 (20.7–49.9)
Medication use	
β_2 agonist only	8 (18%)
Cromolyn	10 (22%)
Inhaled corticosteroids	27 (60%)
Morbidity	
(time lost from school due to asthma in the last 12 months)	
None	14 (31%)
1–6 days	21 (47%)
>1 week	10 (22%)

Values are means (95% confidence intervals), or number (% of group).

Table 2. Circulating eosinophil numbers and serum ECP levels in subjects classified according to three markers of asthma severity: severe AHR ($PD_{20}FEV_1$ <0.6 μmol histamine) *vs* mild AHR; subjects with severe symptoms (causing night waking or limiting daily activity) during 2-week study period *vs* those with mild symptoms in same period; and subjects who reported losing 1 or more days from school due to asthma over 12 months prior to study, *vs* those who did not miss any school days

	Eosinophils ($\times 10^9$/l)			Serum ECP (μg/ml)		
	n	Mean	*P*	*n*	Mean	*P*
Severe AHR	23	0.86 (0.73–1.01)		13	46.6 (32.7–66.2)	
Mild AHR	21	0.61 (0.45–0.76)	0.02	15	31.3 (22.3–44.0)	0.13
Recent severe symptoms	25	0.78 (0.66–0.92)		17	42.7 (32.1–56.7)	
Mild symptoms	19	0.66 (0.51–0.86)	0.31	11	31.1 (19.6-49.2)	0.23
Had days off school	30	0.83 (0.70–0.98)		21	41.7 (31.2-55.6)	
No days off school	14	0.55 (0.43–0.70)	0.01	7	27.8 (17.2-44.9)	0.18

Values are geometric means (95% confidence intervals).

Table 3. Serum cytokine levels in subjects classified according to three markers of asthma severity. Severity classifications are the same as in Table 2

	Serum IL-5 (pg/ml)			Serum TNF-α (pg/ml)		
	n	Mean	*P*	*n*	Mean	*P*
Severe AHR	11	34.0 (18.9–61.4)		9	32.7 (19.1–56.0)	
Mild AHR	11	25.5 (10.8–60.4)	0.60	9	15.7 (6.0–41.2)	0.21
Recent severe symptoms	13	26.3 (13.9–49.8)		11	28.6 (15.2–53.7)	
Mild symptoms	9	34.7 (14.3–84.2)	0.62	7	15.7 (5.4–45.5)	0.32
Had days off school	17	23.5 (13.7–40.1)		12	22.6 (9.9–51.4)	
No days off school	5	63.8 (19.2–212)	0.11	6	22.8 (13.7–38.0)	0.99

Values are geometric means (95% confidence intervals).

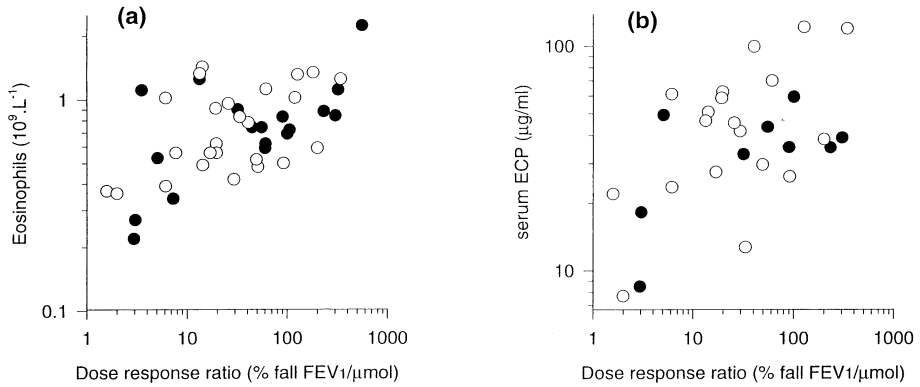


Figure 1. In 8–12-year-old asthmatic children, DRR to histamine, a measure of AHR, was significantly related to a) circulating eosinophil numbers ($r=0.56$, $P=0.0001$, $n=43$) and b) serum levels of eosinophil cationic protein (sECP) ($r=0.54$, $P=0.003$, $n=28$). Values are the mean of two readings 2 weeks apart for individual subjects taking inhaled corticosteroids (open circles) or taking β_2-agonists ± cromolyn (closed circles).

severity score did not correlate significantly with either eosinophil numbers or serum ECP levels. To further examine the relationship between eosinophilic inflammation and asthma severity, we compared eosinophil numbers and serum ECP levels in subjects classified according to three different markers of asthma severity (Table 2). There were no significant differences in circulating eosinophils or serum ECP levels between subjects with or without recent severe symptoms. However, eosinophil numbers were higher in those with severe AHR than in those with mild AHR, and in those who had lost days from school due to asthma in the last 12 months than in those whose attendance was not affected.

Cytokine levels were measured in serum samples from 26 children. TNF-α was detectable in serum from 18 children (mean 22.7 [12.9–39.8] pg/ml), and IL-5 was detectable in serum from 22 children (29.5 [17.6–49.2] pg/ml) (Fig. 2). Children taking inhaled corticosteroids had similar serum TNF-α levels to those on β-agonists ± cromolyn (mean and 95% CI: 21.1 [10.1–48.5] pg/ml *vs* 23.7 [11.5–48.9] pg/ml; $P=0.91$), but lower serum IL-5 levels (20.5 [11.7–35.7] pg/ml *vs* 64.3 [26.6–155.4] pg/ml; $P=0.04$). Neither serum TNF-α nor serum IL-5 was significantly correlated with AHR, as DRR to histamine, with FEV_1 percent predicted, or with the asthma severity score. Table 3 shows geometric mean values for serum TNF-α and serum IL-5 in children classified according to markers of asthma severity. There were no differences in either TNF-α or IL-5 between children with or without recent severe symptoms, those with severe or mild AHR, or those who had or had not lost days from school due to asthma.

Sufficient peripheral blood was available in 42 of the 45 children to isolate PBMC and measure both TNF-α and, in 37 children, also IL-5 production. TNF-α was detectable in all LPS-stimulated cultures (1224 [1060–1419] pg/ml) and in unstimulated PBMC cultures (443 [332–592]pg/ml) (Fig. 3). IL-5 was detected in all PHA-stimulated PBMC cultures (82.5 [65.3–104.4] pg/ml) and in unstimulated PBMC cultures (16.9 [13.3–21.6] pg/ml) from 15 subjects only (Fig. 3). TNF-α and IL-5 production by unstimulated PBMC or PBMC activated *ex vivo* was not correlated with histamine DRR, FEV_1 (% predicted), individual markers of asthma severity, or the composite asthma score.

Finally, the relationships between eosinophil markers and the levels and production of the cytokines TNF-α and IL-5 were examined. Neither TNF-α nor IL-5 production by unstimulated PBMC or PBMC activated *ex vivo* was correlated with eosinophil numbers or

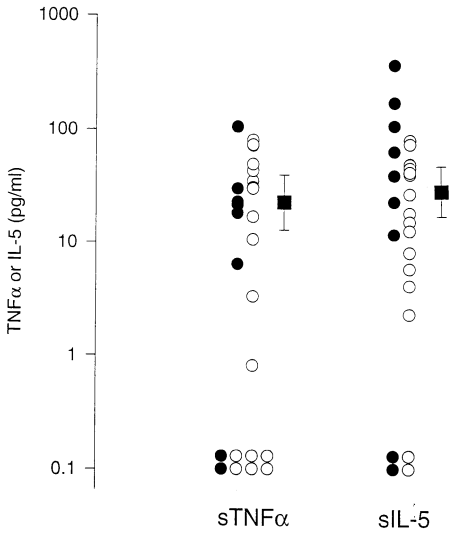


Figure 2. Serum levels of tumour necrosis factor-alpha (sTNF-α), detectable in 18 of 26 subjects, and interleukin-5 (sIL-5), detectable in 22 of 26 subjects, in asthmatic children aged 8–12 years. Values are the mean of two readings 2 weeks apart for individual subjects taking inhaled corticosteroids (open circles) or taking β_2-agonists ± cromolyn (closed circles). Values shown as closed squares are group geometric mean values and 95% confidence intervals.

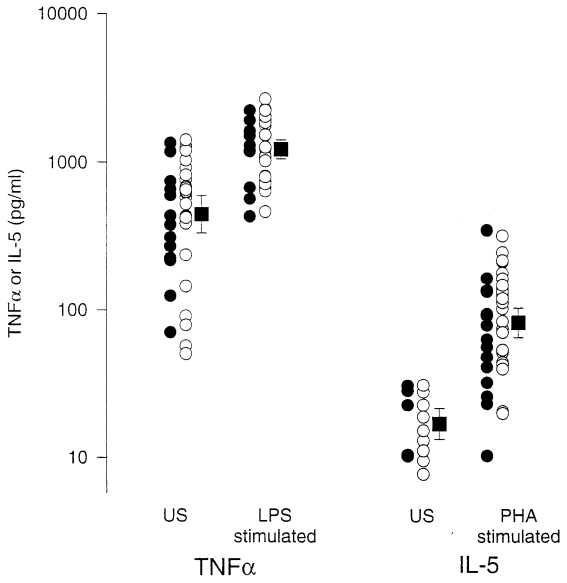


Figure 3. Tumour necrosis factor-alpha (TNF-α) ($n=42$) and interleukin-5 (IL-5) ($n=37$) production by peripheral blood mononuclear cells (PBMC) activated *ex vivo* with either lipopolysaccharide (LPS) or phytohaemagglutinin (PHA) for 24 h or left unstimulated (US). Values are the mean of two readings 2 weeks apart for individual subjects taking inhaled corticosteroids (open circles) or taking β_2-agonists ± cromolyn (closed circles). Values shown as closed squares are group geometric mean values and 95% confidence intervals.

serum ECP levels. Unstimulated and stimulated TNF-α was also not correlated with serum TNF-α levels. However, stimulated IL-5 production was significantly correlated with serum levels of TNF-α ($r=0.63$, $P=0.006$, $n=17$) and negatively correlated with serum IL-5 levels ($r=-0.59$, $P=0.005$, $n=21$).

Discussion

This study of a group of 8–12-year-old children with well-defined asthma has shown that there is a significant correlation between peripheral blood eosinophilia and asthma severity. Eosinophilia was significantly increased in children with severe AHR compared to those with mild AHR and also in children that had lost days at school due to asthma in the last year compared with those who had not. However, there was very little association between asthma severity and either serum levels or cellular production of inflammatory cytokines. Children taking inhaled corticosteroids had significantly lower levels of serum IL-5 than those on β_2-agonists ± cromolyn. There were significant associations between serum levels of TNF-α and IL-5 and production of IL-5 by circulating mononuclear cells following their isolation and activation *ex vivo*. This study provides new information on inflammatory markers and their interrelationships in asthmatic children.

The findings of this study are likely to be applicable to other populations of children with asthma. The subjects were volunteers, recruited by advertisements in newspapers and clinics for a study of a dietary intervention (12), and thus may represent a group who are willing to consider alternative, nonpharmacologic interventions. As a group they include a wide range of severity, with more than half taking inhaled corticosteroids. The group on inhaled corticosteroids did not differ from the noninhaled corticosteroids group with respect to lung function, AHR, morbidity, or asthma severity scores, although the severity score tended to be slightly higher in the group on inhaled corticosteroids (8.31.0 *vs* 6.81.3; $P=0.07$), suggesting that the amount of inhaled corticosteroids taken was insufficient to achieve complete control of the asthma. We do not have data for the degree of adherence to prescribed therapy in this population, but anecdotal evidence suggests that it may be poor.

Eosinophilia, measured both in the peripheral circulation and in the airways, is a characteristic feature of asthma. Furthermore, the severity of the eosinophilia, estimated from the number of circulating eosinophils, is correlated with objective markers of the severity of asthma such as lung function (9, 17). Our data showing correlations between circulating eosinophil numbers and lung function and AHR support these findings. In addition, we have shown that circulating eosinophil numbers are related to less direct markers of severity, such as the number of days lost from school due to asthma. These findings support the view that eosinophil markers may have a role in monitoring asthma severity, particularly in young children where lung-function tests are not always available.

The study also shows that ECP, a marker of eosinophil activation, is related to airway function and provides some evidence that levels of eosinophil-activation markers, such as serum ECP, are no more closely related to objective markers of severity than are the eosinophil numbers. However, in this study, serum ECP levels were determined in fewer subjects; therefore, the study may not have had sufficient power to detect clinically relevant differences in serum ECP levels.

TNF-α is thought to play an important role in eosinophil recruitment from the vasculature into sites of inflammation (reviewed in Ref. 5). The number of TNF-α-positive cells is greater in bronchial biopsies from symptomatic than asymptomatic asthmatic subjects (18), suggesting that levels of this cytokine in serum and the airways might be expected to be related to eosinophil numbers, lung function, and asthma severity. However, while studies *in vitro* have shown that TNF-α-treated eosinophils have increased survival and are activated (8, 19), reports of its effects on eosinophil degranulation vary (8, 20). Moreover, the effect of TNF-α on eosinophil behaviour in asthma will be modulated by the many other inflammatory mediators and cells also present. Therefore, the lack of association between serum TNF-α levels and eosinophilia or serum

ECP levels in this study is perhaps not surprising. Their levels and relationships in the airways may not be accurately reflected by those in the periphery.

IL-5 specifically regulates eosinophil maturation and function (reviewed in Ref. 2); therefore, a relationship between circulating eosinophil numbers, their activation status, and serum IL-5 levels was expected. Serum IL-5 levels did not correlate with circulating eosinophil numbers or serum ECP levels in the 21 children where it could be studied. Although this observation is in contrast to some studies in adult asthmatics (5, 17), it is not without precedent, since ten Hacken et al. (21), in a study of 17 asthmatic adults, found no relationship between serum IL-5 and eosinophil number. In our study, children taking regular inhaled corticosteroids had significantly reduced serum IL-5 levels. Despite this, they did not have reduced eosinophil numbers. It would appear that in these children peripheral blood eosinophilia, at least, must be strongly regulated by proeosinophilic mediators other than, or in addition to, IL-5.

In the present study, the IL-5 production by PHA-stimulated PBMC from 37 asthmatic children was also not related to circulating eosinophil numbers, in agreement with the findings of Kang et al. (22), in 21 adolescent asthmatics. However, a positive relationship between eosinophil numbers and concanavalin A-stimulated IL-5 production in PBMC from 22 children aged up to 14 years has been observed (23). Whether or not the difference in observations is due to the stimulus used is still to be determined.

Interestingly, there was a negative correlation between IL-5 production by activated PBMC and serum levels of IL-5. It is unlikely that there is a negative feedback loop between serum IL-5 levels and the cells producing IL-5 in the periphery, as there is no evidence of an IL-5 receptor on either memory T cells or natural killer cells, the likely IL-5 producers in that compartment (24, 25). IL-5-producing cells in tissues, as well as in the vasculature, would contribute to the IL-5 present in serum. Nearly all T cells in the lung are memory cells (26), and, arguably, they are the major source of IL-5 in the lungs of asthmatics (27). The negative correlation between the children's serum IL-5 levels and IL-5- production by their PBMC is consistent with the hypothesis that the IL-5-producing cells have moved out of the circulation and are sequestered in the lung, where they continue to contribute to serum IL-5 levels, but not to PBMC IL-5 production.

IL-5 production by PBMC might be regulated by TNF-α, as we observed a positive relationship between IL-5 production by PBMC stimulated with PHA *ex vivo* and serum levels of TNF-α. This observation requires confirmation by further *in vitro* studies of IL-5 production by stimulated PBMC in the presence and absence of exogenous TNF-α. There is already considerable evidence that TNF-α does induce lymphoid cells to produce cytokines (28), and that signalling pathways affected by TNF-α (29) are also involved in IL-5 production (30).

In this study of asthmatic children, we have quantified serum levels of the proinflammatory cytokines IL-5 and TNF-α and found them to be unrelated to either eosinophilia or asthma severity. However, eosinophilia was related to lung function, the degree of airway hyperresponsiveness, and morbidity. We have also established for the first time the relationships between serum levels of both cytokines and their production by peripheral blood-derived cells following activation *ex vivo*. There was a positive association between IL-5 production and serum TNF-α levels and a negative association with serum IL-5 levels. Our findings are consistent with the hypotheses that levels of TNF-α and IL-5 in the blood control the numbers and/or responsiveness of IL-5-producing cells remaining in the circulation, and that peripheral blood eosinophilia is regulated by proeosinophilic mediators other than, or in addition to, TNF-α and IL-5. This study has confirmed that, of the peripheral markers assessed so far, the number of circulating eosinophils remains the index of inflammation which is most closely related to the clinical severity of childhood asthma.

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