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Role of the chemokines RANTES, monocyte chemotactic proteins-3 and -4, and eotaxins-1 and -2 in childhood asthma

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Role of the chemokines RANTES, monocyte chemotactic proteins-3 and -4, and eotaxins-1 and -2 in childhood asthma. E. Rojas-Ramos, A.F. Avalos, L. Pérez-Fernandez, F. Cuevas-Schacht, E. Valencia-Maqueda, L.M. Terán. ©ERS Journals Ltd 2003.

ABSTRACT: Eosinophil recruitment into the airways is a feature of asthma in children. However, the mechanisms by which these cells migrate into the airways are not fully understood. The present study investigated the presence of the eosinophil-activating chemokines regulated on activation, normal T-cell expressed and secreted (RANTES), monocyte chemotactic proteins (MCP)-3 and -4, and eotaxins-1 and -2 in the bronchoalveolar lavage (BAL) fluid obtained from both asthmatic (n=10, age 6–10 yrs) and normal children (n=10, age 5–10 yrs).

Measurements of chemokines in BAL fluid showed that levels of RANTES, MCPs-3 and -4, and eotaxins-1 and -2 were significantly increased in fluid obtained from asthmatic children when compared with normal children. Among the different chemokines, RANTES was the cytokine released in greatest quantities in BAL fluid from asthmatic children. There was a significant correlation between the concentrations of MCP-4 and eosinophil numbers in BAL fluid and a trend between both chemokines MCP-3 and eotaxin-2 and eosinophils.

Interestingly, the levels of most chemokines correlated with one another. These findings suggest that RANTES monocyte chemotactic proteins-3 and -4, and eotaxins-1 and -2 may regulate eosinophil trafficking into the airways of asthmatic children in a coordinated manner.

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Asthma is one of the commonest diseases in children and its prevalence has increased in many countries [1, 2]. The introduction of flexible fibreoptic bronchoscopy has allowed an improved understanding of the inflammatory changes that take place in the airways of adult asthmatic patients. This procedure has also become an important tool in the investigation of infants and children with airway disease [3, 4] and the main clinical indications are now well defined [5, 6]. Indeed, several studies have shown that inflammatory changes occur in the airways of children with mild asthma. These changes assist in the recruitment of leukocytes, including eosinophils, and neutrophils [7–12]. Neutrophil recruitment into the airways of asthmatic children has been associated with the release of the potent neutrophil attractant interleukin (IL)-8 [13, 14]. However, the chemoattractants involved in eosinophil trafficking remain to be shown. This is relevant as eosinophils are considered to cause tissue damage through the release of toxic proteases, lipid mediators, cytokines and oxygen free radicals.

Eosinophil recruitment from peripheral blood into the airways is controlled by adhesion molecules and chemokines. The chemokines are a group of chemotactic cytokines that have been subdivided into four subfamilies on the basis of the position of either one or two cysteine residues located near the amino terminus of the protein (CXCL, CCL, CL, and CX3CL) [15, 16]. Members of the CCL branch include regulated on activation, normal T-cell expressed and secreted (RANTES), monocyte chemotactic proteins (MCP)-2, -3 and -4, and eotaxins-1, -2 and -3. RANTES and MCPs-3 and -4 are chemotactic for eosinophils, monocytes and lymphocytes, while the eotaxins chemoattract eosinophils, basophils and lymphocytes of the T-helper cell type 2 (Th2) phenotype [16]. Because of their eosinophil chemotactic properties, these chemokines have attracted major attention in allergic inflammation. Studies conducted in adult asthmatics have shown that the chemokines RANTES, MCPs-3 and -4, and eotaxins-1 and -2 are implicated in the asthmatic reactions [17–21]. However, extrapolating from adult data into the young paediatric population may provide limited and potentially erroneous information. The National Heart, Lung, and Blood Institute (NHLBI) workshop summary on the "Effects of Growth and Development on Lung Functions Models for Study of Childhood Asthma" [22] offered several recommendations for evaluating paediatric asthma, including the use of research bronchoscopy to establish the features of airway disease. In the present study, the eosinophil-activating chemokines RANTES, MCPs-3 and -4, and eotaxins-1 and -2 have been systematically investigated in the bronchoalveolar lavage (BAL) fluid of asthmatic children.

Material and methods

Subjects

Asthma in children was defined according to the American Thoracic Society [23]. Twenty-three children determined by their attending physician to have a clinical indication for

bronchoscopy were eligible for participation. The children were divided in two groups.

Members of the first group included 13 wheeze children (aged 6–10 yrs, six male) previously treated as asthmatics, who, due to the persistence of respiratory symptoms and despite multiple interventions and evaluations, underwent bronchoscopy to identify potential anatomic and/or infectious aetiologies and to rule out aspiration by examining the BAL fluid for lipid-laden macrophages as well as for the presence of foreign bodies. Three of the 13 children who were using inhaled steroids at the time of the study were asked to stop taking this medication 3 weeks prior to bronchoscopy (two of these children suffered from severe asthma and one was a moderate asthmatic). Daily peak-flow measurements were performed to monitor asthma symptoms and β-agonists and cromolyn sodium given on a daily basis. The remaining 10 children had previously failed to improve with the use of inhaled steroids and were taking β-agonists. Children receiving antibiotic therapy, oral steroids, and/or antileukotriene drugs within 1 month of the bronchoscopy were excluded from the study.

The second group included 10 control children (aged 5–10 yrs, four female) selected from 30 children treated for gastro-oesophageal reflux disease, who, due to the persistence of respiratory symptoms and despite multiple interventions, underwent bronchoscopy to identify infectious aetiologies and to rule out aspiration by examining the BAL fluid for lipid-laden macrophages as well as for the presence of foreign bodies. The 10 control children were considered to be normal because no abnormalities were found during bronchoscopy and there were no lipid-laden macrophages or bacterial infection in the BAL fluid. In contrast, the remaining 20 children had lipid-laden macrophages (n=12) and/or bacterial infection in the BAL fluid (n=8). *Haemophilus influenzae* and/or *Streptococcus pneumoniae* were the most frequent bacteria to grow from BAL fluid. Written informed consent was obtained from the parents of the children and the study received the approval of the Ethics Committee of the Hospitals Instituto Nacional de Enfermedades Respiratorias and Instituto Nacional de Pediatria.

Atopy was defined as a positive skin-prick test (wheal at >3 mm in diameter) in the presence of positive histamine and negative diluent to one or more of the extracts of the common local aeroallergens, *i.e. Dermatophagoides pteronnisinus* and *D. farinae*, mixed grass, tree pollen, cat and dog dander and cockroach (Alk Bello, Round Rock, TX, USA).

Flexible bronchoscopy

All children were admitted to a day ward and transnasal fibreoptic bronchoscopy (Olympus model BFP10; Olympus America de Mexico Tokyo, Japan) was performed by monitoring with continuous oximetry and clinical evaluation by direct supervision/care provided by an anaesthesiologist. Premedication consisted of intramuscular atropine sulphate (0.01–0.02 $mg \cdot kg^{-1}$), midazolam (0.05 $mg \cdot kg^{-1}$), and salbutamol by nebuliser. Topical anaesthesia of the upper and lower airways consisted of lignocaine, 2 and 0.5%, respectively (maximum dose of lignocaine 5 $mg \cdot kg^{-1}$). The bronchoscope was passed through the nares and lignocaine was sprayed onto the larynx and then into the lower airways. If more sedation was required repeated doses of 0.05 $mg \cdot kg^{-1}$ midazolam (not to exceed 0.2 $mg \cdot kg^{-1}$) and 1.0 $\mu g \cdot kg^{-1}$ fentanyl (not to exceed 4.0 $\mu g \cdot kg^{-1}$) were given intravenously. Following direct visualisation of all primary segments of the right and left main stem bronchi, the bronchoscope was wedged into the right middle lobe bronchus. The BAL was performed with sterile saline solution in aliquots of 10 mL (total volume 3 $mL \cdot kg^{-1}$) [5]. Aspired fluid was collected into sterile plastic bottles and cooled gradually to 4°C. All children were given an additional 1.0 mg of nebulised salbutamol immediately after the procedure.

Bronchoalveolar lavage fluid processing

The recovered BAL fluid was pooled and centrifuged at 400×*g* for 15 min at 4°C. The cells were then separated and the supernatant was stored at -70°C prior to measurements of chemokines. The cell pellet was resuspended in phosphate-buffered saline and the cells counted using a Neubauer haemocytometer. To obtain differential cell counts, a 100-μL aliquot of cells was subjected to cytocentrifugation cytospin (Wescor Logan, UT, USA), air dried and stained with a Diff-Quick Stain kit (Dade Behring, Newark, DE, USA). A total of 400 cells per cytospin were counted.

Measurements of chemokines

Concentrations of the cytokines RANTES, MCPs-3 and -4, and eotaxins-1 and -2 in BAL fluid were measured using specific sandwich enzyme-linked immunosorbent assay (ELISA) (they did not have cross-reactivity). Briefly, a pair of specific antibodies for each chemokine was purchased and ELISA developed following the manufacturers' protocol (R&D Systems, Minneapolis, MN, USA). RANTES was measured in neat BAL fluid, while the remaining chemokine measurements were performed in two-times concentrated BAL fluid. The lower limit of detection for RANTES, eotaxins-1 and -2 and MCP-3 was 15 $pg \cdot mL^{-1}$. The corresponding value for MCP-4 was 7.8 $pg \cdot mL^{-1}$. Concentrations of the chemokines were corrected for the initial two-fold concentration.

Statistical analysis

Data for age are expressed as mean±SEM. Differential cell counts and concentrations of chemokines in BAL fluid were tested for significance with a Mann-Whitney U-test. Correlations between chemokine levels and eosinophil numbers were evaluated using the Spearman's rank correlation coefficient test. A p-value of <0.05 was considered statistically significant.

Results

Clinical findings

A total of 13 wheeze children and 10 normal children were evaluated using clinical history, bronchoscopy and BAL. Three of the 13 wheeze children were excluded from the study based on bronchoscopy and BAL findings. One patient (male) was found to suffer from stenosis in both the trachea and the right main bronchus (fig. 1), a second child (female) had lipid indices >75% (indicative of lipid-laden macrophages) in the airways consistent with aspiration and one male patient was found to have aspergillus in BAL fluid. The remaining 10 wheeze children had a mean age of 7.5±0.4 yrs, whereas control children had a mean age of 7.4±0.4 yrs. All wheeze children were atopic while control children were nonatopic. Based on this finding and clinical data, wheeze children were considered to be asthmatics. Lung function data show that wheeze children suffered from moderate-to-severe asthma with the exception of two children who were mild asthmatics. The forced expiratory volume in one second (FEV1) %

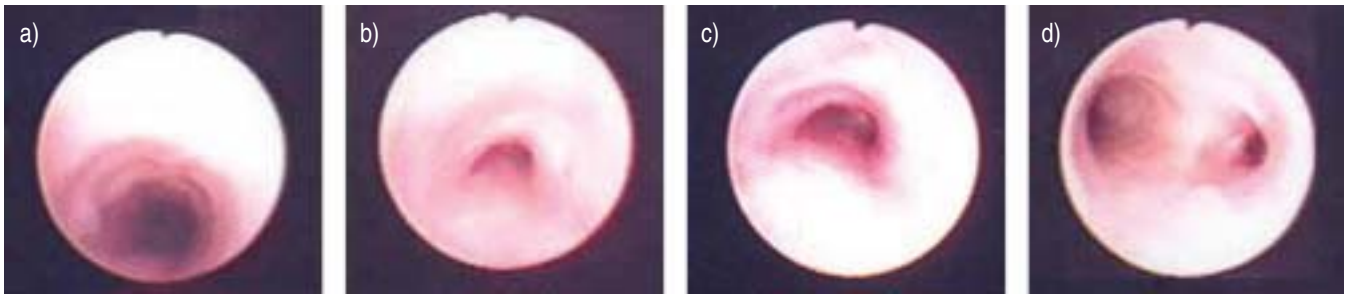

Fig. 1. – Series of photographs of a wheeze child who, during a bronchoscopy, was found to have stenosis of both the trachea and right main bronchus. a) View from proximal trachea, b) stenosis of the trachea (distal third of the trachea), c) immediately after the tracheal stenosis, d) stenosis of the right main bronchus.

predicted was lower in the asthmatics (median 78%, range 58–86%) than in the normal group (median 112%, range 95– 119%; $p<0.01$).

Total and differential bronchoalveolar lavage fluid cell counts

The mean recovery of BAL fluid was 48±13% and no significant difference was found between the two groups. Similarly, there was no significant difference in total cell counts between asthmatic and normal children. In contrast, eosinophil numbers in asthmatics were increased nine-fold when compared with normal controls ($p<0.001$) (table 1). Neutrophil numbers were high in three out of the 10 asthmatics. However, median values were not significantly different between normal and asthmatic children (3.0 *versus* 3.6 $\times 10^3 \cdot mL^{-1}$) (table 1). Similarly, no significant difference was found in macrophage, lymphocyte and epithelial cell numbers between the two groups of children.

Chemokines in bronchoalveolar lavage fluid

Measurements of the chemokines showed that BAL fluid from asthmatic children contained higher concentrations of RANTES immunoreactivity compared with normal children (medians 1,464 *versus* 108.5 $pg \cdot mL^{-1}$; $p<0.001$) (fig. 2). Interestingly, among the different eosinophil-activating chemokines studied, RANTES was the chemokine detected in greatest quantities in the BAL fluid obtained from asthmatic children. For example, concentrations of this cytokine were 4.2- and 7.7-times greater compared with eotaxin-1 and MCP-4, respectively. There was no significant correlation between the concentration of RANTES and eosinophil numbers in BAL fluid ($r=0.29$, $p=0.2$).

The concentration of eotaxins was higher in BAL fluid from asthmatic children than in those derived from control children (fig. 2). The median level of eotaxin-1 in asthmatic children was 347.5 $pg \cdot mL^{-1}$ while that in normals was 153.0 $pg \cdot mL^{-1}$ ($p<0.05$). Corresponding values for eotaxin-2 in asthmatic and normal children were 432.5 $pg \cdot mL^{-1}$ and 148.5 $pg \cdot mL^{-1}$ ($p<0.01$), respectively.

There was no significant correlation between the concentration of eotaxin-1 and the eosinophil number in BAL fluid ($r=0.30$, $p=0.15$) but there was a trend towards a significant correlation between these cells and eotaxin-2 ($r=0.52$, $p=0.06$) (fig. 3).

Like the eotaxins, MCP-3 and -4 levels were higher in BAL fluid from asthmatic children (medians 224.5 and 188.5 $pg \cdot mL^{-1}$, respectively) compared with controls (medians 168.0 and 135.0 $pg \cdot mL^{-1}$, respectively, $p<0.05$) (fig. 2). There was a correlation between the concentration of MCP-4 and eosinophil number in BAL fluid ($r=0.65$, $p=0.02$) and a trend towards a significant correlation between concentrations of MCP-3 and eosinophil numbers ($r=0.47$, $p=0.08$) (fig. 3).

Table 1. – Total and differential cell counts in bronchoalveolar lavage fluid

	Asthmatics	Normals
Total cells	77.2 (36.6–467.0)	61.8 (12–212)
Macrophages	49.6 (5.3–53.4)	33.0 (6.4–66.6)
Lymphocytes	8.0 (1.6–26.6)	9.0 (0.6–28.8)
Neutrophils	3.6 (0.7–25.9)	3.0 (0.4–11.5)
Eosinophils	1.8 (0.9–3.2)***	0.2 (0–2.0)
Epithelial cells	12 (8.5–43)	6.7 (0.4–10)

Cell counts are expressed as median (range). Cell counts are $\times 10^3 \cdot mL^{-1}$. ***: $p<0.001$.

Correlations among chemokine concentrations

To investigate whether there was any relationship among the eosinophil-activating chemokines, correlations in their concentrations were determined. Interestingly, concentrations of eotaxin-2 correlated with the levels of the other chemokines, including eotaxin-1 ($r=0.74$, $p=0.007$), RANTES ($r=0.62$, $p=0.027$), MCP-3 ($r=0.60$, $p=0.033$) and MCP-4 ($r=0.59$, $p<0.035$) (table 2). Concentrations of RANTES correlated with MCP-4 levels ($r=0.83$, $p=0.003$).

Discussion

Over the past few years flexible fibreoptic bronchoscopy has been used with increasing frequency and become an important tool in the investigation of infants and children with airway disease [5–10]. Using this technology, the present study has shown that the eosinophil-activating chemokines RANTES, MCPs-3 and -4, and eotaxins-1 and -2 are associated with the recruitment of eosinophils into the airways of asthmatic children.

Consistent with the report by MARGUET *et al.* [7], it was demonstrated that neutrophils are increased in a very small proportion of asthmatic children while eosinophils are consistently elevated in the BAL fluid. Neutrophils appear to play a more important role in both children with acute asthma and infantile wheezers [7, 11, 14, 24]. For example, in the case of infantile wheezers, three previous reports showed that neutrophils but not eosinophils are increased in their airways [7, 11, 24]. However, it must be taken into account that infantile wheeze is not a single condition because it occurs in infants with smaller airways during viral infections and others with immunoglobulin (Ig)E-mediated sensitisation. In a separate study, analysis of induced sputum from children with acute

asthma revealed that neutrophil inflammation is often associated with acute exacerbations while eosinophil infiltration is a reflection of the chronicity of the disease [14]. Although the mechanisms by which neutrophils are recruited are not fully understood, airway neutrophilia has been associated with the release of the potent neutrophil attractants IL-8 and leukotriene (LT)B_4 [9, 13, 14]. Similarly, it has previously been shown that IL-8 is associated with neutrophil activation in virus-induced exacerbations of asthma [25]. To date, however, the chemoattractants involved in eosinophil recruitment in childhood asthma remain to be shown. This is relevant as activated eosinophils may cause tissue damage through the release of reactive oxygen metabolites and cytotoxic granule-derived proteins, such as major basic protein [26, 27]. Moreover, these cells promote epithelial proliferation, matrix generation, and tissue remodelling through the release of cytokines, such as transforming growth factor (TGF)-α, TGF-β, and granulocyte-macrophage colony-stimulating factor.

To date, this is the first study to investigate whether the eosinophil-activating chemokines RANTES, MCPs-3 and -4, and eotaxins-1 and -2 are released into the airway epithelial lining fluid of asthmatic children. Previous reports in adult asthmatics have studied chemokines individually in BAL fluid [18–20], with the exception of that conducted by TILLIE-LEBLOND *et al*. [28] who reported elevated levels of the chemokines RANTES, MCP-3 and eotaxin-1 in BAL fluid derived from patients in status asthmaticus. In the latter study, however, concentrations of immunoreactive RANTES were lower than both MCP-3 and eotaxin-1. In a separate study, increased levels of the chemokines MCP-3 and -4 and eotaxin-1 were reported in BAL fluid from asthmatics. However, RANTES was not investigated [21]. The present study demonstrated that RANTES immunoreactivity is released in increased concentrations into the BAL fluid of asthmatic children; levels of this cytokine were 12-fold greater in asthmatics than in normal children. Moreover, compared with the other eosinophil-activating chemokines, RANTES was the chemokine released in the greatest concentrations.

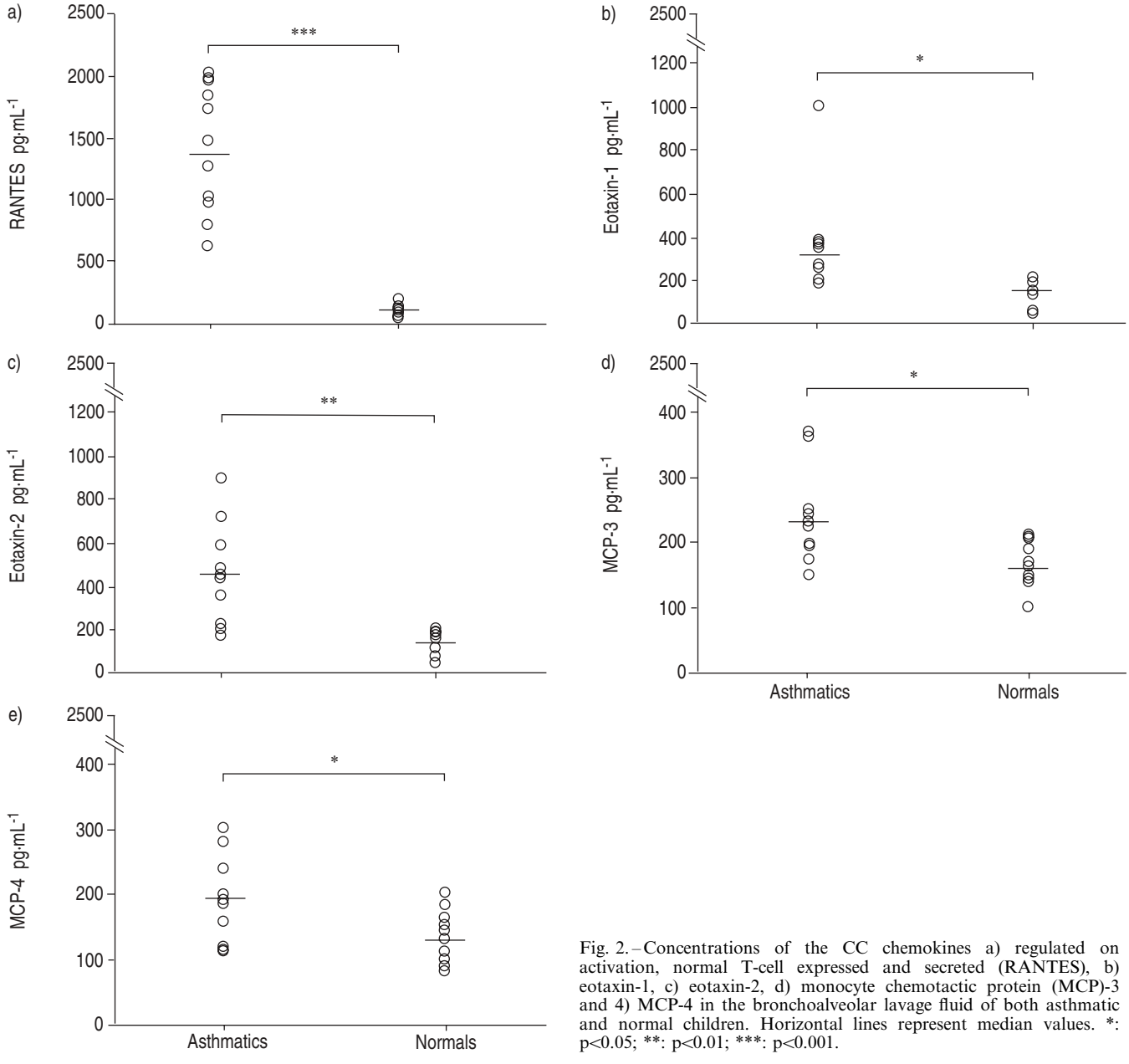

Fig. 2. – Concentrations of the CC chemokines a) regulated on activation, normal T-cell expressed and secreted (RANTES), b) eotaxin-1, c) eotaxin-2, d) monocyte chemotactic protein (MCP)-3 and 4) MCP-4 in the bronchoalveolar lavage fluid of both asthmatic and normal children. Horizontal lines represent median values. *: $p<0.05$; **: $p<0.01$; ***: $p<0.001$.

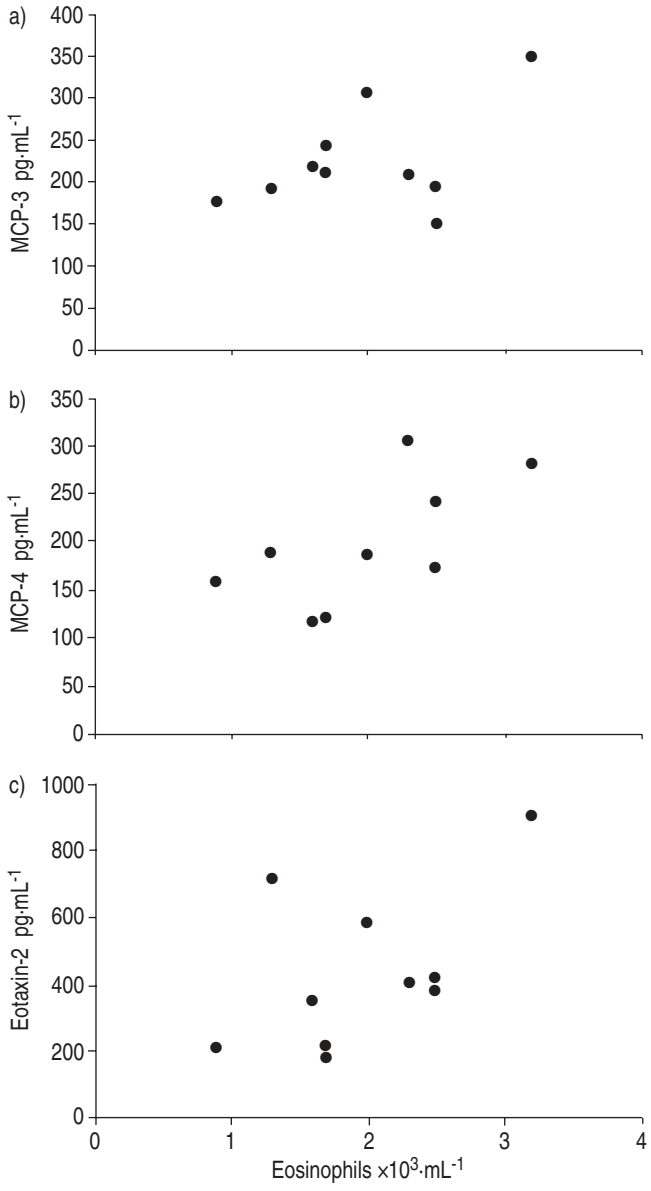

Fig. 3. – Correlations between eosinophil numbers and concentrations of the chemokines a) monocyte chemotactic protein (MCP)-3 (r=0.47, p=0.08), b) MCP-4 (r=0.65, p=0.02) and c) eotaxin-2 (r=0.52, p=0.06).

For example, concentrations of this cytokine were 4.2- and 7.7-fold greater compared with eotaxin-1 and MCP-4, respectively. These findings place RANTES as a major mediator in childhood asthma. Interestingly, the authors have previously shown that concentrations of BAL RANTES as low as 0.5 ng·mL^{-1} derived from asthmatic patients, are able to induce eosinophil migration in a chemotaxis assay [18].

Table 2. – Correlations between chemokine levels in asthmatic children

Chemokines	r	p-value
Eotaxin-2 *versus* eotaxin-1	0.74	0.007
Eotaxin-2 *versus* RANTES	0.62	0.027
Eotaxin-2 *versus* MCP-3	0.60	0.033
Eotaxin-2 *versus* MCP-4	0.59	0.035
Eotaxin-1 *versus* MCP-3	0.76	0.005
RANTES *versus* MCP-4	0.83	0.003

RANTES: regulated on activation, normal T-cell expressed and secreted; MCP: monocyte chemotactic protein.

The eotaxins are specific eosinophil attractants that activate eosinophils *via* the chemokine receptor CCR3 (RANTES, in addition to CCR3, uses CCR1 to activate eosinophils). Among the eotaxins, eotaxin-1 has been the most extensively studied in adult asthmatics [17, 19, 29–31]. However, the role of these cytokines in childhood asthma has not been previously investigated. The present study showed that there were increased levels of both eotaxins-1 and -2 in BAL fluid, suggesting that they may play a prominent role in children suffering from this disease. In a novel murine asthma model induced by house dust containing cockroach allergen, eotaxin-1 was found to represent the principal eosinophil attractant [32]. Indeed, in this latter study, neutralisation of eotaxin-1 with an anti-eotaxin-1 antibody inhibited airway eosinophilia following allergen challenge, suggesting that eotaxin-1 may be responsible for the pulmonary infiltration of eosinophils in response to cockroach allergen. Interestingly, cockroach is a major contributing factor to asthma exacerbations among inner-city children [33]. Studies conducted in adult asthmatics have shown increased eotaxin-1 messenger ribonucleic acid (mRNA) expression in bronchial biopsies, BAL fluid, sputum and serum [17, 19, 29]. Exposure to a relevant allergen increases eotaxin-1 mRNA expression further and protein release in either biopsies or BAL fluid of mild asthmatics [30, 31]. However, kinetic studies suggest that the production of eotaxins may function at distinct stages of allergic-disease progression. For instance, a study of late-phase allergic reactions in the skin of atopics showed that there is a temporal difference in the generation of eotaxins-1 and -2: upregulation of eotaxin-1 was observed 6 h after allergen skin challenge, whereas an increase in eotaxin-2 was not evident until 24 h [34]. Similarly, analysis of bronchial biopsies showed a selective upregulation of eotaxin-3 24 h after allergen challenge [35]. These observations suggest that the generation of the three eotaxins may occur at distinct times during the course of the allergic inflammatory reaction, hence they may be responsible for different phases of eosinophil recruitment. The elevated levels of eotaxins-1 and -2 detected in BAL fluid suggest that these cytokines are produced during the ongoing inflammatory process into the airways of asthmatic children. As there are currently no commercially available antibodies to measure eotaxin-3 in biological fluids it remains to be shown whether this cytokine is released in childhood asthma.

Both MCPs-3 and -4 also activate eosinophils *via* CCR3 but, in addition, activate other cell types through the receptors CCR1 and CCR2. Like the previous chemokines, concentrations of both MCPs were increased in the BAL fluid from asthmatic children, although there was a considerable overlap of these two chemokines in asthmatic children. Indeed, values of the concentration of the MCPs were evenly distributed (fig. 2) suggesting that they may be released by a similar mechanism during the ongoing inflammatory process from resident cells into the asthmatic airways. MCPs-3 and -4 have 58% identity at amino acid level and exhibit remarkable homology in their promoter regions, which contain deoxyribonucleic acid (DNA) sequences designed to control the rate of basal transcription, including TATA box, nuclear factor-κB binding sites and glucocorticoid regulatory elements [36, 37]. Tumour necrosis factor-α, a cytokine released during the allergic inflammatory process, has been shown to induce transcription factor-DNA complex formation and transcriptional activation of both MCPs-3 and -4 [37, 38]. Thus, it is likely that inflammatory cytokines produced during the inflammatory asthmatic process may induce the release of the eosinophil-activating chemokines by inducing transcription activating factors, which in turn may bind specific DNA sequences in the promoter region of the chemokines. To

investigate whether the MCPs and the other eosinophil-activating chemokines may be directly involved in eosinophil recruitment, correlations between the chemokines and eosinophil numbers in BAL fluid were sought. Interestingly, MCP-4 correlated with eosinophils and both MCP-3 and eotaxin-2 showed a trend correlation with these cells in BAL fluid. In a previous report, the authors showed that endobronchial allergen challenge to adult asthmatics induces RANTES release and that concentrations of this cytokine correlate with eosinophil numbers [18]. In the present study, no correlation between RANTES and eosinophils in BAL fluid from asthmatic children was found. However, it is not known whether allergen exposure leads asthmatic children to produce further RANTES and concentrations of this cytokine may then correlate with eosinophils. Similarly, levels of the eosinophil-activating chemokines did not correlate with either macrophages or lymphocytes. This was not surprising as these cell types were not increased in the BAL fluid from asthmatic children.

An interesting finding in the present study was the demonstration that there was a significant correlation among the concentrations of the chemokines themselves. For example, eotaxin-2 concentrations correlated with the concentrations of the other four chemokines. These findings suggest that the eosinophil-activating chemokines could be released in a coordinated fashion into the airways of asthmatic children. In a mouse model of asthma it has been shown that the use of neutralising antibodies against several chemokines reduces the inflammatory allergic inflammatory process by inhibiting different cellular and molecular pathways in a coordinated way [39].

In summary, the present study has demonstrated that asthmatic children release the CC chemokines RANTES, monocyte chemotactic proteins-3 and -4, and eotaxins-1 and -2 into the airway epithelial lining fluid, with regulated on activation, normal T-cell expressed and secreted, being the chemokine produced in greatest quantities. Interestingly, the concentration of some of these chemokines not only correlated or showed a trend correlation with eosinophil numbers but significant correlations were observed among them suggesting that they are released in a coordinated fashion. In combination, all these findings suggest that the chemokines RANTES, monocyte chemotactic proteins-3 and -4, and eotaxins-1 and -2 may regulate eosinophil recruitment into the airways of asthmatic children.

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