

Are hypodense eosinophils in children activated or immature?

Moens MM, Mertens AV, De Clerck LS, Van Bever HP, Bridts CH, Stevens WJ. Are hypodense eosinophils in children activated or immature?

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In patients with allergic asthma and rhinitis high numbers of hypodense eosinophils (HE) have been demonstrated. In a previous study we reported that asthmatic and healthy children had more HE than their adult counterparts. We assumed that this might, in part, be due to the presence of immature eosinophils in children. To distinguish between immature and activated eosinophils, determination of eosinophil cationic protein (ECP) might be interesting as it is known that high serum levels of ECP are associated with increased activation of eosinophils. In this study we determined the levels of ECP in serum in asthmatic and healthy children and adults trying to distinguish activated from immature eosinophils. We found that ECP levels were not increased in children (healthy and asthmatic) compared to adults (healthy and asthmatic). This supports the hypothesis that increased numbers of HE in childhood are, at least in part, immature eosinophils. Nevertheless, we could confirm that inflammation was present in children because soluble interleukin-2-receptor (sIL-2R), a marker of lymphocyte activation, was higher in asthmatic children as compared to healthy children. IL-6, a marker of macrophage/monocyte activation, was not different in the different patient groups. We conclude that although signs of inflammation are present in childhood asthma, the increased numbers of HE in children are in part due to the presence of immature eosinophils.

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Different cell types, including eosinophils, are involved in the pathogenesis of bronchial asthma (1, 2). Eosinophils are heterogeneous in their density as demonstrated by density gradient centrifugation (3–5). Hypodense eosinophils (HE) seem to be the activated eosinophils, as shown *in vivo* in patients with hypereosinophilia and *in vitro*, after activation of normodense eosinophils (6, 7, 8). In a previous study we determined the density distribution profiles of eosinophils in asthmatic children and adults and in healthy controls. Children (healthy and asthmatic) had significantly higher numbers of HE as compared to their adult counterparts (9). Although there is no clear explanation for these findings, we assume that this might be due, apart from the presence of activated eosinophils, to the presence of immature eosinophils, being also hypodense (10). Since eosinophil cationic protein (ECP) is considered to be a specific marker of increased activation of eosinophils, the

determination of ECP could be helpful to distinguish between these two possibilities (2).

In addition to eosinophils, lymphocytes and macrophages are also involved in airway inflammation in asthma (11–15). Soluble interleukin-2-receptor (sIL-2R) is considered to be a marker of lymphocyte activation (16–20). Interleukin-6 (IL-6) is a marker of macrophage/monocyte activation (21–24). In this study we determined ECP, sIL-2R and IL-6 in healthy and asthmatic subjects (children and adults), in order to evaluate the degree of activation of eosinophils, lymphocytes and macrophages.

Material and methods

Venous blood was taken from healthy controls (adults: $n = 14$ and children: $n = 18$) and house dust mite sensitive asthmatic patients (adults:

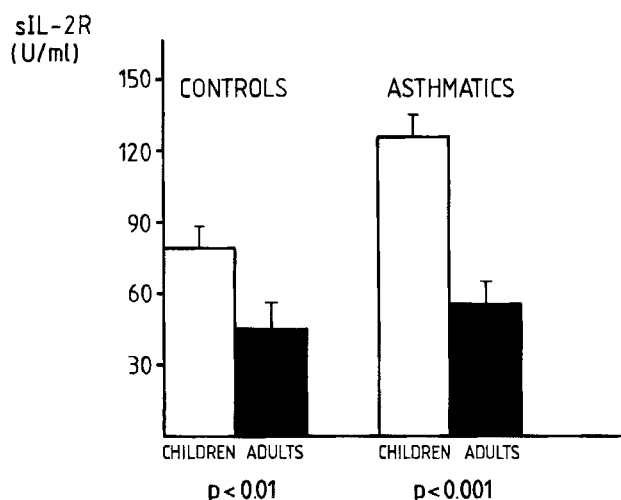


Fig. 1. Mean $\pm$ SEM of sIL-2R in children compared to adults (healthy and asthmatic).

n = 12 and children: n = 18) as demonstrated by the presence of a positive skin prick test (*Dermaphagoides pteronyssinus*, HAL, Haarlem Allergen Laboratories, Haarlem, the Netherlands) and positive specific IgE (CAP-test, Pharmacia, Brussels). Patients had mild to moderate asthma and were stable at the moment of blood sampling. All patients used inhaled β_2 -mimetics and most of them also inhaled corticosteroids (8 adults and 12 children). β_2 -mimetics were stopped 24 h and inhaled corticosteroids 48 h before blood sampling.

Determination of activation markers

ECP in serum was determined by a double antibody radioimmunoassay (Pharmacia ECP RIA). Briefly, ECP in the sample competes with a fixed amount of ^{125}I -labelled ECP for the binding sites of a polyclonal rabbit anti-ECP. Bound and free ECP were separated by addition of sheep anti-rabbit IgG bound to sepharose. The radioactivity in the pellet was measured in a gamma counter (Packard Instruments). The concentration of ECP in serum was expressed in microgram per liter.

The sIL-2R was determined by a sandwich enzyme-linked immunosorbent assay with two monoclonal antibodies recognizing two different epitopes of the IL-2R (Eurogenetics). Briefly, microtiterplates coated with an anti-IL-2R monoclonal antibody were incubated with standard sera and patient samples. The sIL-2R bound to this first antibody was detected with a peroxidase conjugated anti-sIL-2R antibody directed against a second epitope on the IL-2R. After washing, tetramethylbenzidine/ H_2O_2 as a substrate was added. The enzymatic reaction was stopped with 2N H_2SO_4 , the absorbance was measured at 460 nm.

The concentration sIL-2R was expressed in units per milliliter.

IL-6 was also determined by a sandwich enzyme-linked immunoabsorbent assay with two monoclonal antibodies (Eurogenetics). Microtiter-plated, coated with monoclonal mouse anti-IL-6 were incubated with standard sera and patient samples. After washing, the cups were first incubated with an anti-IL-6 biotinylated conjugate and subsequently with a streptavidin-peroxidase conjugate. After washing, tetramethylbenzidine/ H_2O_2 was added. The enzymatic reaction was stopped and the absorbance was measured at 450 nm. The concentration of IL-6 was expressed in picogram per milliliter.

Statistical analysis – The Kruskal and Wallis H-test and the Mann Whitney U-test were used where appropriate. A $p < 0.05$ was considered as statistically significant.

Results

Detailed results are shown in the table and in the figure. ECP was significantly higher in asthmatic patients (children and adults) than in controls ($p < 0.0005$ and $p < 0.05$ respectively). However, ECP was not different between children and adults (healthy children versus healthy adults and asthmatic children versus adult asthmatics). The sIL-2R level in serum was not different between adult asthmatics and adult controls. In contrast, asthmatic children had significantly higher levels of sIL-2R than healthy children ($p < 0.005$). Children had higher levels of sIL-2R as compared to adults, both in the control group and in the asthmatic group ($p < 0.01$ and $p < 0.001$ respectively). IL-6 levels were comparable in all groups.

Discussion

In a previous study higher numbers of HE were found not only in asthmatic children but also in healthy children, as compared to their adult counterparts (9). Because of these findings, the ques-

Table 1. ECP, sIL-2R and IL-6 in asthmatics and controls (results expressed as median and range).

	Adults		Children	
	controls (n = 14)	asthmatics (n = 12)	controls (n = 18)	asthmatics (n = 18)
ECP ($\mu\text{g/l}$)	8 (4-62)	24 (5-62)*	11 (4-29)	21 (6-65)***
sIL-2R (U/ml)	41 (1-127)	44 (30-135)	74 (44-159)	116 (55-238)**
IL-6 (pg/ml)	8 (6-10)	10 (6-26)	8 (6-55)	9 (7-23)

* $p < 0.05$, ** $p < 0.005$, *** $p < 0.0005$, asthmatic patients compared to controls

tion raised if these HE were activated or immature (5–7, 10, 25). Trying to distinguish between immature and activated eosinophils, we determined ECP, being a marker of activated eosinophils and not of immature eosinophils (26–34). Increased levels of ECP were found in asthmatics, both adults and children. However, the ECP levels of healthy adults were not different from those of healthy children, suggesting that immature eosinophils could, at least in part, be responsible for the higher numbers of HE found in children.

Apart from eosinophils, activation of lymphocytes has also been examined in the present study in order to document ongoing inflammation in the asthmatic subjects (11–18). Asthmatic children were found to have higher sIL-2R levels than healthy children, confirming activation of lymphocytes in asthmatic children. This was not found in adults which might be due to the stability of their asthmatic symptoms at the moment of blood sampling. Furthermore, higher sIL-2R levels were found in children as compared to adults. This is in agreement with previous studies reporting an age-related decrease of sIL-2R (16, 18).

It has been demonstrated *in vitro* that alveolar macrophages of broncho-alveolar lavages produce more IL-6 when stimulated, compared to resting macrophages (21, 22). In our study the similar in IL-6 serum levels in the different groups is probably because the IL-6 concentration was measured in the peripheral blood and not at the site of inflammation, although in other inflammatory diseases such as rheumatoid arthritis, the IL-6 concentration in peripheral blood is associated with active disease (23, 24). In conclusion, these data support the hypothesis that immaturity of eosinophils accounts at least in part to the HE in children, whereas increased levels of ECP and sIL-2R in asthmatic children, also confirm ongoing activation of these cells. The IL-6 concentration in serum is not suitable as a marker of inflammation in asthma.

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