

Peripheral blood eosinophils and IL-4 in infancy in relation to the appearance of allergic disease during the first 6 years of life

Borres MP, Björkstén B. Peripheral blood eosinophils and IL-4 in infancy in relation to the appearance of allergic disease during the first 6 years of life.

Pediatr Allergy Immunol 2004; 15: 216–220. © 2004 Blackwell Munksgaard

Eosinophils in peripheral blood and serum levels of interleukin (IL)-4 were analysed at 3 and 18 months and at 6 years in a prospective study comprising 67 children, of whom the majority had atopic heredity. The children were monitored from birth up to 6 years of age and the findings were related to family history of allergy and development of allergic disease. Twenty-nine children (43%) of the original group of 67 children were diagnosed as allergic at the age of six. Fifteen of 23 children with eosinophilia ($> 4 \times 10^8$ eosinophils/l) at 3 months of age were regarded as allergic at 6 years, when compared with 12 out of 38 children with normal eosinophil counts at 3 months ($\chi^2 = 6.7$, $p < 0.01$). Children with detectable IL-4 in serum at 18 months were more often allergic at 6 years, when compared with those children with no detectable IL-4 ($\chi^2 = 8.6$, $p < 0.01$). Furthermore, the allergic children had a mean IL-4 value of $0.35 \mu\text{g/l}$ (95% CI: 0.10–1.48) at 18 months, when compared with $0.17 \mu\text{g/l}$ (95% CI: 0.10–0.72, $p < 0.001$) in the non-allergic children. At 6 years of age, only nine children had detectable levels of IL-4 and five of them were classified as allergic. Eosinophilia at this age was also associated with allergic disease. We conclude that eosinophilia during infancy and increased levels of IL-4 at 18 month of age are associated with allergic disease during the first 6 years of life. This might indicate that the first 2 years of life are particularly important for the development of allergy.

M. P. Borres¹ and B. Björkstén²

¹Department of Paediatrics, Faculty of Medicine, Sahlgrenska Academy at Göteborg University and Pharmacia Diagnostics, Uppsala, ²Center for Allergy Research and Institute of Environmental Medicine, Karolinska Institute, Stockholm, Sweden

Key words: infancy; allergy; prediction; eosinophils; interleukin 4

Magnus P. Borres, Pharmacia Diagnostics, S-751 82 Uppsala, Sweden

Tel.: +46 70 527 2543

Fax: +46 18 16 63 90

E-mail: magnus.borres@pharmacia.com

Accepted 23 November 2003

Elevated eosinophil counts are associated with current allergic disease, low FEV₁, increased peak flow variation and airway histamine responsiveness (1). Eosinophilia is an indicator of inflammation that is most closely related to the clinical severity of childhood asthma (2). Increased interleukin (IL)-4 concentrations have been reported both in allergic infants and allergic children (3–6). Several authors have also described low interferon- γ production in children with allergic disease in addition to elevated IL-4 (7–9). Less is known whether the eosinophilia and cytokine imbalance found in the allergic children appear prior to the clinical manifestations of disease, or merely are the result of disease. Elevated eosinophil counts have been described as a marker of allergy in newborns

(10), but this was not confirmed in other study (11). A significantly increased production of IL-4 at 6 months of age was recently observed in children developing allergies compared with the non-allergic children (12). Also reduced interferon- γ production has been suggested as a risk factor of allergic disorders (13, 14).

In a prospective study, enrolling 67 families with ($n = 46$) and without ($n = 21$) a history of allergic disease, we found that elevated eosinophil counts in the peripheral blood of healthy infants at 3 months were associated with a subsequent diagnosis of allergic disease by the age of 18 months (15). Low levels of IL-4 were detected in 10 of 63 cord blood samples (16). The levels then increased, both in the healthy and allergic infants reaching a peak at either 6 or

9 months and then decreased up to 18 months of age. The infants who became allergic had significantly higher IL-4 than those who did not. As allergic respiratory symptoms-like rhinoconjunctivitis and asthma often develop later in life, the children were again evaluated when they were 6 years old, thus testing the hypothesis that eosinophilia at 3 months of age is associated with allergic disease development up to 6 years of age, and detectable levels of IL-4 in serum in apparently healthy infants are associated with the development of allergic disease.

Material and methods

The study group comprised 67 children who were subject to a physical examination and sampling of cord blood at birth and venous blood at 3, 6, 9 and 18 months, as described previously (15–16). The family history of allergic disease was evaluated through interviews and skin prick testing (SPT, $n = 62$) of both parents. Thirty-eight of the children had at least two allergic members of the immediate family, i.e. both parents, or one parent and at least one sibling, while 15 children had one allergic family member and nine children had a negative family history.

A follow-up was carried out when the children were between 5 and 7 years old (range: 5.5–7.3 years). This included a questionnaire, physical examination, skin prick and lung function tests, venous blood sampling and nasal smear. The analysis of the nasal smear showed that children having detectable nasal metachromatic cells at every examination were more often allergic than children with no detectable cells at any time during the 6-year follow-up period (17). The parents of all the 67 children completed the questionnaires, while 63 children were subject to a physical examination, 61 to SPT and 57 to venipuncture.

Skin prick tests were performed in duplicate using the following allergens: egg and milk (Dome/Hollister-Stier/Bayer, extracts 1:20 w/v), fish (Dome/Hollister-Stier/Bayer, extracts 1:10 w/v), cat, dog, timothy, house dust mite (*Dermatophagoides farinae*), mould (*Cladosporium*) and birch (SQTM extracts from ALK, Hørsholm, Denmark). Histamine hydrochloride, 10 mg/ml, served as a positive control. The size of the wheal was marked with a felt-tip pen after 15 min and transferred onto a micropore tape for measuring. The SPT was regarded as positive when the mean diameter (half of the sum of the largest diameter and the diameter perpendicular to it) was at least 3 mm.

The classification of the children as allergic, probably allergic and non-allergic at the age of 6, was based on the SPT results in combination

with the case history and physical examination. The children were also classified in regards to clinical symptoms.

Atopic eczema/dermatitis syndrome (AEDS) was classified as flexural itching dermatitis lasting for more than 1 month (18). Urticaria was defined as allergic if it appeared at least twice within 1 hour after exposure to a particular antigen. The diagnosis of asthma was based on a history of recurrent bronchial obstruction, at least once verified by a physician, in combination with a 15% reduction in forced expiratory volume at 1 second (FEV₁) after a simple running exercise test at the 6 year follow-up and dry cough at night not because of cold or chest infection (19). Children who reported airway obstructive symptoms in conjunction with a respiratory infection or had a history of recurrent wheezing, but who failed to demonstrate a significant fall of FEV₁ were classified as having probable asthma. Allergic rhinoconjunctivitis (ARC) was defined as rhinitis and/or conjunctivitis appearing at least twice after exposure to a particular allergen and not related to infection.

In children fulfilling some, but not all the diagnostic criteria for a disease, probable AEDS, asthma, ARC and urticaria respectively was indicated.

Peripheral blood eosinophils were counted in a Fuchs-Rosenthal chamber after staining with 0.1 ml Magdala Red 10% in 0.1 ml sodium carbonate 10% (Merck, Darmstadt, Germany), 6 ml acetone (Merck) and 45 ml distilled water. Blood samples were available at 3 ($n = 62$), 6 ($n = 60$), 9 ($n = 63$) and 18 months ($n = 60$) and 6 years ($n = 57$). The missing samples were because of technical failure or refusal to venipuncture. Eosinophilia was defined as more than 4×10^8 eosinophils/l (20). Serum IL-4 levels were measured with an ELISA kit (Intertest-4; Genzyme Corporation, Boston, MA, USA). The detection level was 100 ng/l.

The Regional Ethics Committee for Human Research at the Linköping University Hospital approved the study and written consent was obtained from the parents.

Statistics

The Mann–Whitney *U*-test for comparisons between allergic and non-allergic individuals was used because eosinophil numbers could not be assumed to be normally distributed. The number of allergic subjects in the two groups was compared using chi-squared analysis with Yates' correction. A probability level of $< 5\%$ was considered to be significant.

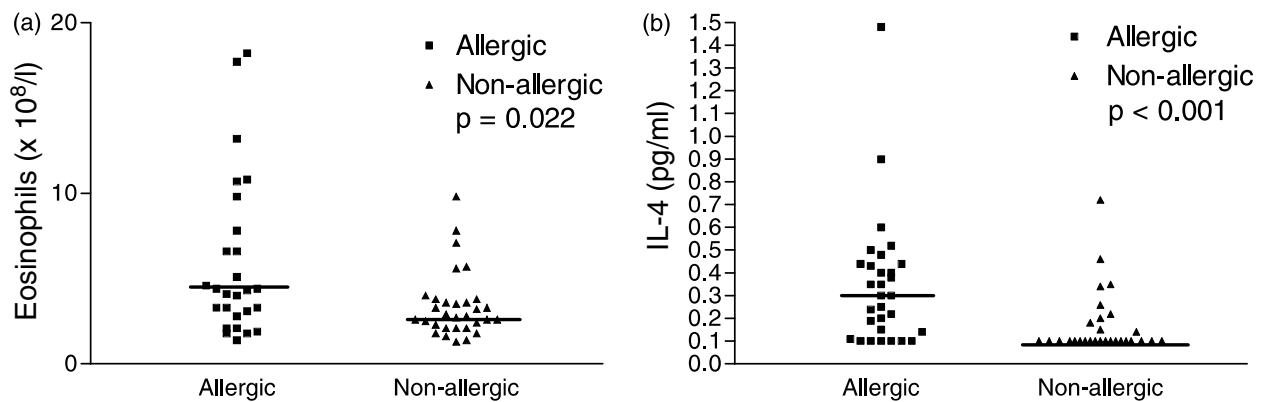


Fig. 1. Eosinophil counts at 3 months of age (a) and interleukin (IL)-4 levels at 18 months of age (b) in relation to allergic disease at 6 years. Squares represents allergic and triangles represents non-allergic individuals.

Results

The prevalence of allergic disease was 43% (29 of 67) at 6 years of age. Thirteen of them had more than one allergic diagnosis. AEDS ($n = 19$) was the most common diagnosis, followed by ARC ($n = 16$), asthma ($n = 11$) and urticaria ($n = 5$). Five children were regarded as probably allergic and 33 as non-allergic. Eighteen children had a positive SPT at 6 years.

Forty-two children had the same clinical classification at 18 months and 6 years of age. Six children with allergic manifestation during the first 18 months had recovered by the age of 6, while seven previously healthy children had become allergic. The remaining 12 children were classified as probably allergic, either at 18 months or 6 years of age, or were not possible to evaluate.

Results from eosinophil counts at 3 months of age were available in 27 of the 29 children who were classified as allergic at 6 years. The mean counts at 3 months for this group were $5.9 \times 10^8/l$ (range: 1.4–18.2) (Fig. 1a). The corresponding number was 3.4×10^8 eosinophils/l (range: 1.3–9.8) in the 31 children who were classified as non-allergic at 6 years of age ($p = 0.022$ Mann–Whitney). In contrast, eosinophil counts at 6 years were similar in allergic and non-allergic children, i.e. 4.1 (range 0.5–9.3) $\times 10^8/l$ vs. 3.5 (range 0.3–14) $\times 10^8/l$.

Of 23 infants with eosinophilia at 3 months of age, 15 had allergic disease, two were probably allergic and six non-allergic at 6 years when compared with 12 allergic, two probably allergic and 25 non-allergic of the 39 infants with normal eosinophil counts ($\chi^2 = 6.7$, $p < 0.01$).

In 19 of the 23 children with eosinophilia at 3 months, an eosinophil count was also obtained at 6 years. Nine of them (47%) continued to have

eosinophilia, while the counts were normal in the remaining 10 children. Six of the nine children with continuous eosinophilia were allergic at 6 years and three were non-allergic.

In 10 of 63 infants, IL-4 was detected in cord blood. Only two of them were allergic at 6 years. In contrast, 23 of 34 children with detectable IL-4 at 18 months were allergic at 6 years, when compared with six of the 24 children with undetectable IL-4 ($\chi^2 = 8.6$, $p < 0.01$). Furthermore, the IL-4 levels at 18 months of age were higher in children who were allergic at 6 years when compared with those who were not (0.35 $\mu g/l$, 95% CI: 0.10–1.48 vs. 0.17 $\mu g/l$, 95% CI: 0.10–0.72, $p < 0.001$) (Fig. 1b). At 6 years of age, nine of 57 children had detectable levels of IL-4. Five of them were classified with an allergic disease and four as not.

Discussion

Children with an allergic disease at the age of 6 more often had blood eosinophilia and detectable IL-4 in serum during infancy, when compared with healthy children.

Infancy seems to represent a critical time window for the initiation of immune responses to allergens leading to tolerance or clinically manifest allergy. IL-4 is of interest in this respect as it enhances isotype switching to immunoglobulin E and adhesion of eosinophils to endothelium (21). Van der Velden and coworkers (12) reported increased IL-4 levels at 6 months who subsequently became allergic. These children were followed until 12 months of age and remarkably, IL-4 levels in the allergic children decreased between 6 and 12 months of age. Our study show a similar decrease, but over a longer time period. Therefore, we hypothesize that IL-4

may be essential in the skewing of the immune systems towards the TH₂ cytokine profile. According to our data, IL-4 does seem to play a less important role as effector cytokine in the established allergic disease, as only nine children of the total group had detectable levels and five of them were classified as allergic. On the contrary, Ohshima and co-workers have reported significantly elevated serum levels of IL-4 in allergic children in a cross-sectional study, particularly at the age of 13–24 months (3). It is however, difficult to directly compare our findings, as their study was not prospective.

The role of eosinophil counts as a predictor of allergic disease has been subject to considerable attention. Thus, Öymar and coworkers (22) showed eosinophil counts to be one of the best variables for predicting wheezing symptoms. Lönnkvist et al. (23) have shown that eosinophil counts can be used to estimate the short-term risk of deterioration in cases of mild and moderate allergic asthma. However, eosinophil counts were not related to asthma severity according to recent work of Siroux and coworkers (24) which is also in accordance with our findings that eosinophilia might be more useful in predicting allergic disease rather than reflecting current disease.

The difference between these studies and ours is that the children in our study had not developed symptoms of allergic disease when the eosinophils counts were performed at 3 months of age. In order to assess the predictive value of an analysis, the study should be prospective. Even if this was the case with our study, it was too small to allow an accurate estimate of sensitivity and specificity.

In conclusion, children who develop allergic disease during the first 6 years of life have eosinophilia and increased levels of IL-4 during infancy and early childhood in comparison with non-allergic children.

Acknowledgments

Special thanks are due to Lena Lindell, Kerstin Hagersten and Lisbeth Hjälle, Linköping for excellent assistance and Lars Söderström, Pharmacia Diagnostics, Uppsala for statistical advice. This study was supported by the Swedish Research Council (no. 7510), the National Foundation for the National Heart and Lung Association and The National Swedish Association for the Prevention of Asthma and Allergy.

References

- HEYMAN PW, RAKES GP, HOGAN AD, INGRAM JM, HOOVER GE, PLATTS-MILLS TAE. Assessment of eosinophils, viruses and IgE antibody in wheezing infants and children. *Int Arch Allergy Immunol* 1995; 107: 380–2.
- HUGHES JM, RIMMER SJ, SALOME CM, et al. Eosinophilia, interleukin-5 and tumour necrosis factor- α asthmatic children. *Allergy* 2001; 56: 412–8.
- OHSHIMA Y, KATAMURA K, MIURA M, MIKAWA H, MAYUMI M. Serum levels of interleukin 4 and soluble CD23 in children with allergic disorders. *Eur J Pediatr* 1995; 154: 723–8.
- KIM K-M, MAYUMI M, MIKAWA H. IgE, IL-4 and soluble Fc ϵ RII in the serum of atopic and non-atopic children. *Pediatr Allergy Immunol* 1992; 3: 11–5.
- BETTIOL J, BARTSCH P, LOUIS R, et al. Cytokine production from peripheral whole blood in atopic and nonatopic asthmatics: relationship with blood and sputum eosinophilia and serum IgE levels. *Allergy* 2000; 55: 1134–41.
- TANG M, KEMP A, VARIGOS G. IL-4 and interferon-gamma production in children with atopic disease. *Clin Exp Immunol* 1993; 92: 120–4.
- JUJO K, RENZ H, ABE J. Decreased interferon gamma and increased interleukin-4 production in atopic dermatitis promotes IgE synthesis. *J Allergy Clin Immunol* 1992; 90: 323–31.
- SCHMITT M, NIGGEMANN B, KLEINAU I, NASERT S, KAPP A, WAHN U. Lymphocyte subsets, sIL-2-R and sICAM-1 in blood during allergen challenge tests in asthmatic children. *Pediatr Allergy Immunol* 1993; 4: 208–13.
- HOLTER W, EMMINGER W, MAJDIC O, KNAPP W. Enhanced production of IL-4 but not of IFN γ and IL-10 by peripheral blood mononuclear cells from atopic children in response to CD2 plus CD28 stimulation. *Pediatr Allergy Immunol* 1996; 7: 75–82.
- CALBI M, SCARPELLINO B, GIACHETTI L. Usefulness of neonatal eosinophil counts as a marker of atopy? *Pediatr Allergy Immunol* 1993; 4: 86–8.
- HAUS M, HALL JM, DE HEESE HV, WEINBERG EG, BERMAN D. Cord blood and maternal total eosinophil counts in relation to infant allergy. *Pediatr Allergy Immunol* 1992; 3: 23–7.
- VAN DER VELDEN VHJ, LAAN MP, BAERT MRM, DE WAAL MALEFYT R, NEIJENS HJ, SAVELKOU HFJ. Selective development of a strong Th2 cytokine profile in high-risk children who develop atopy: risk factors and regulatory role of IFN-gamma, IL-4 and IL-10. *Clin Exp Allergy* 2001; 31: 997–1006.
- HALONEN M, MARTIANEZ FD. A deficient capacity to produce interferon-gamma: is it a risk for asthma and allergies? *Clin Exp Allergy* 1997; 27: 1234–6.
- KONDO N, KOBAYASHI Y, SHINODA S, et al. Reduced interferon gamma production by antigen-stimulated cord blood mononuclear cells is a risk factor of allergic disorders – 6-year follow-up study. *Clin Exp Allergy* 1998; 28: 1340–4.
- BORRES MP, ODELRAM H, IRANDER K, KJELLMAN N-IM, BJÖRKSTÉN B. Peripheral blood eosinophilia in infants at 3 months of age is associated with subsequent development of atopy in early childhood. *J Allergy Clin Immunol* 1995; 3: 694–8.
- BORRES MP, EINARSSON R, BJÖRKSTÉN B. Serum levels of interleukin-4, soluble CD23 and IFN gamma in relation to the development of allergic disease during the first 18 months of life. *Clin Exp Allergy* 1995; 25: 543–8.
- BORRES MP, IRANDER K, BJÖRKSTÉN B. Nasal metachromatic cells in infancy in relation to the appearance of atopic disease during the first 6 years of life. *Allergy* 1997; 52: 770–4.

18. HANIFIN JM, RAIJKA G. Diagnostic features of atopic dermatitis. *Acta Dermatol Venereol* 1980; 92 (Suppl.): 44–7.
19. ASHER M, KEIL U, ANDERSON HR, et al. International Study of Asthma and Allergies in Childhood (ISAAC): rationale and methods. *Eur Respir J* 1995; 8: 483–9.
20. ORFANAKIS NG, OSTLUND RE, BISHOP CR, ATHENS JW. Normal blood leukocyte concentration values. *Am J Clin Path* 1970; 53: 6647–51.
21. FITCH PS, BROWN V, SCHOCK BC, ENNIS M, SHIELDS MD. Interleukin-4 and interleukin-4 soluble receptor alpha levels bronchoalveolar lavage from children with asthma. *Ann Allergy Asthma Immunol* 2003; 90: 429–33.
22. ÖYMAR K, HAVEN J, HALVORSEN T, BJERKNES R. Eosinophil counts and urinary eosinophil protein X in children hospitalised for wheezing during the first year of life: prediction of recurrent wheezing. *Acta Pediatr* 2001; 90: 843–9.
23. LÖNNKVIST K, HELLMAN C, LUNDAHL J, HALLDÉN G, HEDLIN G. Eosinophil markers in blood, serum, and urine for monitoring the clinical course in childhood asthma: impact of budesonide treatment and withdrawal. *J Allergy Clin Immunol* 2001; 107: 812–7.
24. SIROUX V, ORYSZCZYN MP, PATY E, KAUMANN F, PISON C, VERVLOET D. Relationships of allergic sensitisation, total immunoglobulin and blood eosinophils to asthma severity in children of the EGEA Study. *Clin Exp Allergy* 2003; 33: 746–51.