Allergy 2005: 60: 391–395

ALLERGY
DOI: 10.1111/j.1398-9995.2005.00733.x

Short communication

$CD4^+IL\text{-}13^+$ cells in peripheral blood well correlates with the severity of atopic dermatitis in children

Background: In atopic dermatitis (AD) a Th1/Th2 imbalance has been reported, and interleukin (IL)-13 seems to play a pivotal role in the inflammatory network. We tried to assess the correlation between the immunological marker $CD4^+IL\text{-}13^+$ and the clinical phase of extrinsic AD in children.
Methods: Twenty children with AD were studied. Assessed parameters were: clinical severity (SCORAD index), total serum immunoglobulin E (IgE), blood eosinophil count, and percentage of $CD4^+IFN\gamma^+$, $CD4^+IL\text{-}4^+$, $CD4^+IL\text{-}13^+$ T cells. Determinations were carried out in the acute phase and after clinical remission were achieved. Ten nonatopic-matched children served as controls.
Results: At baseline, AD was mild in 25%, moderate in 50% and severe in 25% of children. In the acute phase a significant relationship between the eosinophil count and the SCORAD index was found ($P = 0.0001$). Blood $CD4^+IL\text{-}4^+$ were significantly higher in the AD group (median 17.0, range: 13.7–21.4) than in controls (12.6, 6.4–17.2, $P < 0.0001$). $CD4^+IL\text{-}13^+$ cells in the AD group well correlated ($P = 0.0007$) with SCORAD index. At remission, a significant correlation between SCORAD index and eosinophil count was found ($P < 0.03$) and the percentage of $CD4^+IL\text{-}13^+$ cells globally decreased ($P < 0.0001$), while no difference was found among SCORAD classes.
Conclusion: This study confirms the Th2 profile predominance in the peripheral blood of children with AD, and evidences close relationship between the number of $CD4^+IL\text{-}13^+$ T cells and the disease's severity.

S. La Grutta[1], P. Richiusa[2], G. Pizzolanti[2], A. Mattina[2], G. B. Pajno[3], R. Citarrella[2], G. Passalacqua[4], C. Giordano[2]

[1]Allergy Unit, Children Hospital – ARNAS, Palermo; [2]Laboratory of ImmunoEndocrinology, Endocrinology Section, Faculty of Medicine, Università di Palermo, Palermo; [3]Department of Pediatrics, Università di Messina, Messina; [4]Allergy and Respiratory Diseases, Department of Internal Medicine, Università di Genova, Genova, Italy

Key words: allergy; atopic dermatitis; CD4 T cells; interleukin-13; Th1/Th2 cells.

Carla Giordano
Laboratory of ImmunoEndocrinology
Endocrinology Section
Faculty of Medicine
Università di Palermo
Palermo
Italy

Accepted for publication 6 August 2004

Atopic dermatitis (AD) is a chronic inflammatory skin disease affecting about 15% of children, with consequent costs and morbidity. The AD has its onset during the first 6 months of life in 45%, in the first year of life in 60% and before 5 years in at least 85% of affected children (1, 2). The criteria proposed by Hanifin more than 20 years ago, still maintain their validity for diagnosing the disease (3). In addition, the severity score atopic dermatitis (SCORAD) index provides a standardized and reproducible method to quantify the disease's severity (4, 5). Nowadays, the exact role of allergy/atopy in the pathogenesis of AD is still controversial, but it is true that the immunological mechanisms underlying the disease (6) somewhere resemble those of other allergic disorders. It is also well-known that many children with AD are sensitized to inhalant/food allergens, so that AD is called 'extrinsic'. Many of the abnormalities found in patients with AD [e.g. increased serum immunoglobulin E (IgE) and peripheral blood eosinophilia] are considered the result of a predominant production of Th2 cytokines, namely interleukin (IL)-4, IL-5 and IL-13 [with concomitant reduction of interferon (IFN)-γ production] (7–9). More recently IL-13 has emerged as a pivotal mediator of Th2-dominant immune responses (10) and also as a predictive marker of AD development in children (11), thus supporting that IL-13 plays an important role also in AD pathogenesis (12). Of note, IL-13 cooperates to many biological activities of IL-4, another typical Th2 cytokine (13, 14). In addition, *in vivo* and *in vitro* data have shown that the pattern of cytokine expression correlates with disease severity (15, 16). In the acute skin lesions of AD the number of IL-13 mRNA positive cells is usually higher than in chronic AD and the role of this cytokine as marker of disease's activity has been consistently demonstrated (17).

This study aimed at evaluating the relationship between clinical and immunological variables, during

Abbreviations: AD, atopic dermatitis; SCORAD, severity score atopic dermatitis; PBMC, peripheral blood mononuclear cells; mAb, monoclonal antibody; PE, phycoerythrin conjugate; FITC, fluorescein conjugate.

the acute and remission phases of AD. We studied therefore the production of IL-13 by activated purified T cells in order to relate this marker of Th2 predominance to SCORAD index.

Material and methods

Study design

Twenty children were recruited from an outpatient clinic and their clinical baseline characteristics are shown in Table 1. All children fulfilled the diagnostic criteria for acute extrinsic AD. At visit 1, patients had no asthma or rhinitis. The AD children were free of antihistamines, systemic steroids or topical steroids during the previous 2 weeks. At visit 2, all children were in remission phase and cleared of eczema. Ten age-matched children with no family history for atopy, served as control group (Table 1).

The clinical severity of AD (measured by the SCORAD index) and several immunological parameters (total serum IgE, blood eosinophil count, Th1 and Th2 cells) were assessed (visit 1). When a clinical remission was obtained, with 1–2 week bursts of topical corticosteroid (mometasone furoate) once-daily (18), the same parameters were reassessed (visit 2). A washout period of at least 2 weeks from the last application of the topical steroid was elapsed at visit 2. All parents signed a written informed consent.

Clinical scoring system

The severity of AD was measured through the SCORAD index (4). The AD was considered as mild if the SCORAD index was <25, moderate if index was between 25 and 50, and severe form if SCORAD index was >50. All patients were clinically assessed at both visits (acute phase – visit 1 and remission phase – visit 2), by the same supervisor (SLG) trained in SCORAD index evaluation and unaware of the immunological parameters.

Allergy tests

All patients (including controls) underwent skin prick tests for the geographically relevant aeroallergens (*Dermatophagoides pteronyssinus*, parietaria pollen, grasses, olive tree, cat/dog dander and *Alternaria alternata)* and the most common food allergens (cow milk, egg, peanut, cod fish, wheat and soybean). The skin response was measured according to EAACI criteria (19). Blood samples were collected at visit 1 (acute phase) and after the clinical remission of skin lesions (visit 2), for the determination of serum total IgE (UniCAp Total IgE; Pharmacia, Uppsala, Sweden), serum-specific IgE (UniCap Specific IgE; Pharmacia Upjohn, Uppsala, Sweden), total white blood cells and eosinophil count. For specific IgE, a level of 0.35 kU/l or greater was considered positive. In the control group, total and specific IgE assays and eosinophil count were performed.

Cells and cytokines

Purified T cells, isolated from peripheral blood by Ficoll density gradient, were stimulated for 4 h with phorbol-12-myristate-13-acetate (PMA, 25 ng/ml; Sigma, Milan, Italy) and ionomycin (1 μg/ml; Sigma) in the presence of Brefeldin A (10 μg/ml; Sigma). After activation, 1×10^6 cells were incubated with peridinin chlorophyll protein (PerCP)-conjugated CD4 monoclonal antibody (mAb) (30 min). After surface staining and washing, the cells were permeabilized (1X flow cytometry cell sorter (FACS) Permeabilizing Solution; Becton Dickinson, San Diego, CA, USA) and stained with fluorescein conjugate (FITC)- or phycoerythrin conjugate (PE)-anti-intracellular cytokine antibody and incubated for 30 min at 4°C in the dark. Antihuman FITC-IFN-γ, FITC-IL-2, PE-IL-4, PE-IL-13 mAbs (Becton-Dickinson Pharmingen, San Diego, CA, USA) were used to evaluate Th1 and Th2 phenotypes. Isotype matched normal, PerCP, PE and FITC antibodies were used as negative controls. Cells were analysed by three-colour flow cytometric analysis using FACScalibur (Becton Dickinson).

Statistical analysis

Data are reported as median and range. All the statistical evaluations were performed by tests for nonparametric data. The level of significance was set as P-values <0.05. The STATVIEW statistical software (SAS Institute Inc., Cary, NC, USA) was used for all statistical computations.

Results

The baseline clinical and laboratory characteristics in the AD group on admission in the study (acute phase) and in the control subjects, matched for age and sex, are shown in Table 1. The overall median SCORAD index was 38.0 (range: 15.3–88.1). The AD was mild in five (25%) patients, moderate in 10 (50%); and severe in five (25%). All patients had positive skin test for house dust mite (HDM), and three also displayed skin sensitivity for hen egg. The skin positive tests were confirmed by the radioallergosorbent test (RAST) assay. Total serum IgE levels were increased (>2 SD for age) in 16 of the 20 patients (80%).

A significant relationship ($P = 0.0001$, Spearman rank correlation) between the eosinophil count and the

Table 1. Clinical and laboratory characteristics of AD patients and control subjects

Patients	Mild SCORAD <25	Moderate SCORAD 25–50	Severe SCORAD >50	Total	Control subjects
N	5	10	5	20	10
Mean age (years)	5.4	4.1	6.6		5.5
Age range (years)	4–9	2–7	2–9		2–10
Sex (M/F)	2/3	5/5	3/2	10/10	5/5
Total IgE (median and range)	250 (105–334)	185 (90–680)	340 (160–600)		38.5 (23.5–79.0)
Blood eosinophils (median and range)	280 (220–420)	550 (350–950)	950 (830–980)		35.5 (14–72)
RAST to mites (kU/l) (median and range)	3.45 (0.8–16.4)	4.5 (1.1–15.7)	5.6 (1.2–15.2)		<0.35

AD, atopic dermatitis; IgE, immunoglobulin E; RAST, radioallergosorbent test; SCORAD, severity score atopic dermatitis.

SCORAD index value was found in the acute phase as shown in Fig. 1A. Furthermore, eosinophil count significantly decreased during the remission period ($P < 0.0001$, Wilcoxon signed rank test, Fig. 1B). No significant difference in IgE level was found between the acute and the remission phase, and no correlation was found between total IgE and eosinophils, total IgE and SCORAD index (data not shown).

Concerning the distribution of Th1 and Th2 phenotypes in the acute phase, we found no significant difference in the percentage of CD4$^+$IFNγ^+ cells (Th1 cells), between AD patients and controls (19.9%, 10.1–27.5 *vs* 20.1, 12.0–29.8, P = ns, Mann–Whitney U-test, Fig. 2A).

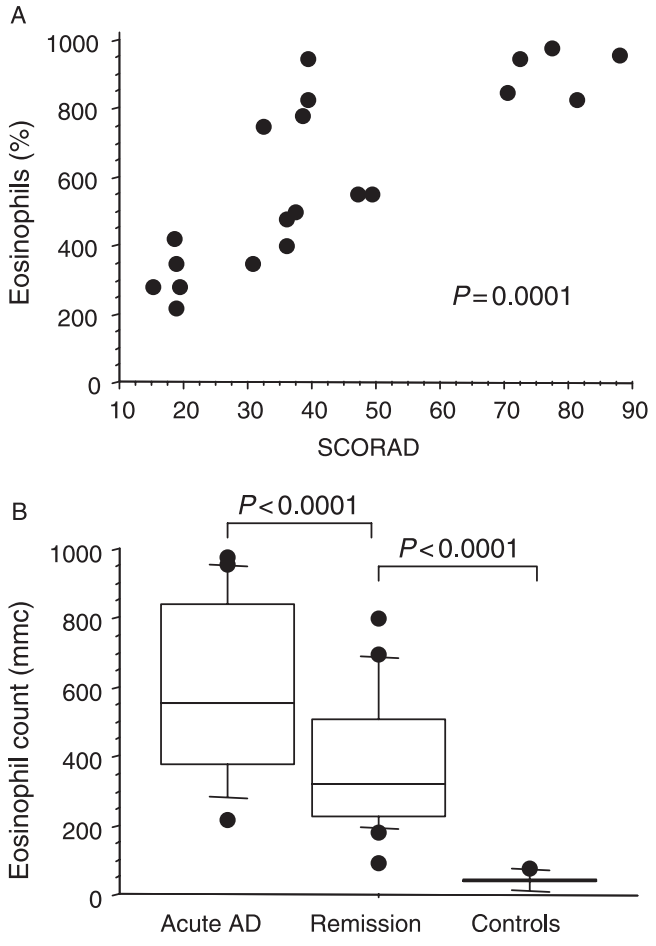

Figure 1. (A) Correlation between atopic dermatitis (AD) severity score atopic dermatitis (SCORAD) index and blood eosinophil count in the acute phase ($P = 0.0001$, Spearman rank correlation). (B) Absolute eosinophil counts in acute and remission AD, and in control subjects. Bars indicate (from the bottom to the top) 10th, 25th, 50th (median), 75th and 90th percentiles. Values below 10th and above 90th percentiles are plotted as circles. Differences between AD acute phase and remission phase (Wilcoxon signed rank test) and between AD remission phase and controls (Mann–Whitney U-test) were significant.

However, CD4$^+$IL-4$^+$ cells (Th2 cells) were significantly higher in the AD group than in controls (17.0, 13.7–21.4% *vs* 12.8, 6.4–17.2%, $P < 0.0001$, Fig. 2B).

In the acute phase of AD, we found that the percentage of CD4$^+$IL-13$^+$ T cells was significantly different among the three SCORAD severity classes reaching the higher levels in severe AD class ($P < 0.0001$, Kruskall–Wallis test, Fig. 3A). In fact, the CD4$^+$IL-13$^+$ T cells were significantly correlated to the SCORAD index ($P = 0.0007$, Fig. 3B). The percentage of CD4$^+$IL-13$^+$ T cells decreased during clinical remission, and no significant difference was found among SCORAD severity classes at remission ($P = 0.095$, Kruskall–Wallis test, Fig. 3C). In fact, we found a strong correlation between ΔIL-13 (difference between the acute phase and the remission phase) and CD4$^+$IL-13$^+$ percentage cells in the acute phase ($P < 0.0001$, Spearman rank correlation), as shown in Fig. 3D.

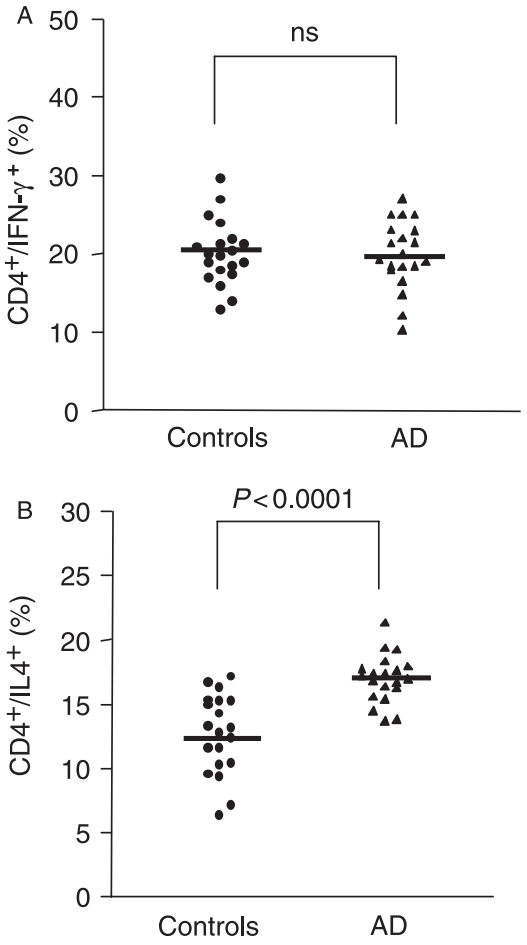

Figure 2. (A) Percentages of CD4$^+$IFNγ^+ cells and (B) CD4$^+$IL-4$^+$ cells in the acute phase of atopic dermatitis (AD) and in control subjects. The percentage of CD4$^+$IL-4$^+$ cells in the acute phase of AD was significantly higher than in controls (Mann–Whitney U-test).

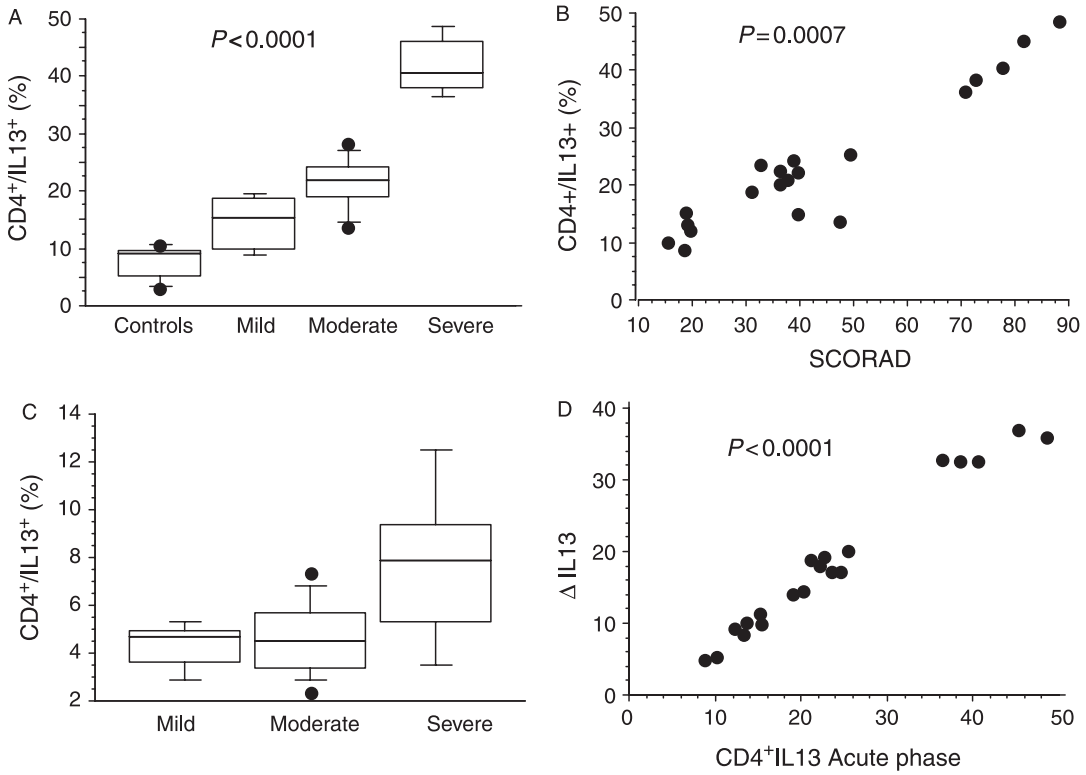

Figure 3. (A) $CD4^+IL\text{-}13^+$ cells in acute atopic dermatitis (AD) distinguished in mild, moderate and severe according to the score atopic dermatitis (SCORAD) index and in control group. Differences among severity SCORAD groups and control group were significant ($P < 0.0001$, Kruskall–Wallis test). Box plots as in Fig. 1B; (B) linear correlation between $CD4^+IL\text{-}13^+$ cells and AD SCORAD index (Spearman rank correlation); (C) $CD4^+IL\text{-}13^+$ cells in the three severity groups in the remission period (differences not significant); (D) correlation between the delta IL-13 (ΔIL-13) (percentage of $CD4^+IL\text{-}13^+$ in the acute phase–remission phase) and the percentage of $CD4^+IL\text{-}13^+$ in the acute phase. Significant *P*-values are shown upon the bars. Bars indicate (from the bottom to the top) 10th, 25th, 50th (median), 75th and 90th percentiles. Values below 10th and above 90th percentiles are plotted as circles.

Discussion

The AD is a very common disease of the infancy, and its association with atopy is well recognized since many years (6, 7). In fact, in patients with AD, the sensitization to environmental allergens is frequent, and allergen avoidance strategies may improve the clinical outcome (20). Nevertheless, despite intensive research a unifying pathogenetic concept of AD has not been yet established.

The present study shows that in the acute phase of AD, a Th2 phenotype predominates as demonstrated by the relative increase of $CD4^+IL\text{-}4^+$ and $CD4^+IL\text{-}13^+$ cells. Interestingly, $CD4^+IL\text{-}13^+$ cells exhibited a very strict correlation with the SCORAD index, and the remission of clinical symptoms was accompanied by a reduction of $CD4^+IL\text{-}13^+$ cells. The levels of $CD4^+IL\text{-}13^+$ cells during the acute and the remission phases mirror the clinical time course of AD. We found a strict correlation between the number of $CD4^+IL\text{-}13^+$ cells in the AD group and the severity of the disease as measured by the SCORAD index ($P = 0.0007$). Of note we suggest that $CD4^+IL\text{-}13^+$ cell percentages could characterize the acute phase of AD. Previous attempts to identify the most reliable marker of disease activity were made. Several eosinophil-associated parameters (e.g. eosinophilic cationic protein, urinary eosinophil protein X, peripheral eosinophilia and IL-5 production) have been considered good candidates (21, 22). More recently also selectins and intracellular adhesion molecule (ICAM)-1 (23, 24), which mediate the cellular traffic, have been proposed as hallmark of severity. In this sense, $CD4^+IL\text{-}13^+$ cell count could be considered a good biological marker, since it behaves dynamically according to the clinical AD course. Indeed, the possible correlation of IL-13 with severity of AD was suggested since 1997 in a study comparing children with allergic or nonallergic asthma and AD (25). It is to be stressed that although the highest $CD4^+IL\text{-}13^+$ values are related to a severe AD SCORAD index at onset of the disease, this does not appear to predict the subsequent remission. In addition, the decrease in $CD4^+IL\text{-}13^+$ percentage is not dependent on the baseline value.

Our results strongly suggest that in AD a merely clinical scoring method, as SCORAD is, has an immunological counterpart: in fact, the number $CD4^+IL\text{-}13^+$ cells is related to the clinical scoring method. Moreover,

the clinical recovery is confirmed by the return of $CD4^+IL\text{-}13^+$ percentage towards levels not different from controls. Future approaches in clinically oriented research will be needed to complete our understanding of this skin disease.

Acknowledgments

The authors wish to thank Dr F. Cibella, Institute of Biomedicine and Molecular Immunology – CNR, Palermo, Italy for the statistical analysis.

References

1. Schultz-Larsen F, Diepgen T, Svensson A. The occurrence of atopic dermatitis in north Europe: an international questionnaire study. J Am Acad Dermatol 1996;**34**:760–764.
2. Girolomoni G, Abeni D, Masini C, Sera F, Ayala F, Belloni-Fortina A et al. The epidemiology of atopic dermatitis in Italian schoolchildren. Allergy 2003;**58**:420–425.
3. Hanifin JM. Atopic dermatitis. J Am Acad Dermatol 1982;**70**:452–457.
4. Kunz B, Oranje AP, Labreze L, Stalder JF, Ring J, Taieb A. Clinical validation and guidelines for the SCORAD index: consensus report of the European Task Force on atopic dermatitis. Dermatology 1997;**195**:10–19.
5. Gelmetti E, Colonna C. The value of SCORAD and beyond. Towards a standardized evaluation of severity? Allergy 2004;**59** (Suppl. 78):61–65.
6. Leung DYM. Atopic dermatitis: new insights and opportunities for therapeutic intervention. J Allergy Clin Immunol 2000;**105**:860–876.
7. Leung DYM, Soter NA. Cellular and immunologic mechanisms in atopic dematitis. J Am Acad Dermatol 2001;**44**:S1–S12.
8. Campbell DE, Fryga AS, Bol S, Kemp AS. Intracellular interferon-gamma (IFN-gamma) production in normal children and in children with atopic dermatitis. Clin Exp Immunol 1999;**115**:377–382.
9. Kimura M, Tsuruta S, Yoshida T. Unique profile of IL-4 and IFN-γ production by peripheral blood mononuclear cells in infants with atopic dermatitis. J Allergy Clin Immunol 1998;**102**:238–244.
10. Wynn TA. IL-13 effector functions. Annu Rev Immunol 2003;**21**:425–456.
11. Lange J, Ngoumou G, Berkenheide S, Moseler M, Mattes J, Kuehr J et al. High interleukin-13 production by phytohaemagglutinin- and Der p 1-stimulated cord blood mononuclear cells is associated with the subsequent development of atopic dermatitis at the age of 3 years. Clin Exp Allergy 2003;**33**:1537–1543.
12. Ohshima Y, Yasutomi M, Omata N, Yamada A, Fujisawa K, Kasuga K et al. Dysregulation of IL-13 production by cord blood CD4+ T cells is associated with the subsequent development of atopic disease in infants. Pediatr Res 2002;**51**:195–200.
13. de Vries JE. The role of IL-13 and its receptor in allergy and inflammatory responses. J Allergy Clin Immunol 1998;**102**:165–169.
14. Kelly-Welch AE, Hanson EM, Boothby MR, Keegan AD. Interleukin-4 and interleukin-13 signaling connections maps. Science 2003;**300**:1527–1528.
15. Takamatsu Y, Hasegawa M, Sato S, Takehara K. IL-13 production by peripheral blood mononuclear cells from patients with atopic dermatitis. Dermatology 1998;**196**:377–381.
16. Hamid Q, Naseer T, Minshall EM, Song YL, Boguniewicz M, Leung DY. In vivo expression of IL-12 and IL-13 in atopic dermatitis. J Allergy Clin Immunol 1996;**98**:225–231.
17. Katagiri K, Itami S, Hatano Y, Takayasu S. Increase levels of IL-13 mRNA, but not IL-4 mRNA, are found in vivo in peripheral blood mononuclear cells (PBMC) of patients with atopic dermatitis (AD). Clin Exp Immunol 1997;**108**:289–294.
18. Eichenfield LF. Consensus guidelines in diagnosis and treatment of atopic dermatitis. Allergy 2004; **59**(Suppl. 78):53–60.
19. The European Academy of Allergology and Clinical Immunology. Position paper: Allergen standardization and skin tests. Allergy 1993;**48**:48–82.
20. Capristo C, Romei I, Boner A. Environmental prevention in atopic eczema dermatitis syndrome (AEDS) and asthma: avoidance of indoor allergens. Allergy 2004;**59** (Suppl. 78):53–60.
21. Pucci N, Lombardi E, Novembre E, Farina S, Bernardini R, Rossi E et al. Urinary eosinophil protein X and serum cationic protein in infants and young children with atopic dermatitis: correlation with disease activity. J Allergy Clin Immunol 2000;**15**:353–357.
22. Kimura M, Tsuruta S, Yoshida T. Correlation of house dust mite-specific lymphocyte proliferation with IL- production, eosinophilia, and the severity of symptoms in infants with atopic dermatitis. J Allergy Clin Immunol 1998;**101**:84–89.
23. Halmerbauer G, Frischer T, Koller DY. Monitoring of disease activity by measurement of inflammatory markers in atopic dermatitis in childhood. Allergy 1997;**52**:765–769.
24. Wolkerstorfer A, Laan MP, Savelkoul HF, Neijens HJ, Mulder PG, Oudesluys-Murphy AM et al. Soluble E-selectin, other markers of inflammation and disease severity in children with atopic dermatitis. Br J Dermatol 1998;**138**:431–435.
25. Koning H, Neijens HJ, Baert MR, Oranje AP, Savelkoul HF. T cell subsets and cytokines in allergic and non-allergic children: I. Analysis of IL-4, IFN-gamma and IL-13 mRNA expression and protein production. Cytokine 1997;**9**:416–426.