

Increased expression of CC chemokine ligand 18 in extrinsic atopic dermatitis patients

Chang Ook Park¹, Hee Jung Lee¹, Ju Hee Lee^{1,2}, Wen Hao Wu^{2,3}, Nam Soo Chang², Min-Geol Lee^{1,2,3} and Kwang Hoon Lee^{1,2,3}

¹Department of Dermatology, Yonsei University College of Medicine, Seoul, Korea;

²Cutaneous Biology Research Institute, Yonsei University College of Medicine, Seoul, Korea;

³Brain Korea 21 Project for Medical Science, Yonsei University College of Medicine, Seoul, Korea

Correspondence: Kwang Hoon Lee, MD, PhD, Department of Dermatology and Cutaneous Biology Research Institute, Yonsei University College of Medicine, 134 Shinchon-Dong, Seodaemoon-Gu, Seoul 120-752, Korea, Tel.: 82 2 2228 2280, Fax: 82 2 393 9157, e-mail: kwanglee@yumc.yonsei.ac.kr

Accepted for publication 14 September 2007

Background: Although most patients with atopic dermatitis (AD) show high total and allergen-specific serum immunoglobulin E (IgE) levels, a small subgroup of AD patients have normal total IgE levels and negative serum allergen-specific IgE. This subgroup has been termed 'intrinsic' AD (IAD) as a counterpart to 'extrinsic' AD (EAD). However, the difference of chemokines between IAD and EAD has not yet been evaluated.

Objective: We investigated the expression of CC chemokine ligand (CCL) 17, CCL22, and CCL18 in patients with IAD and EAD, which are known to be highly expressed in AD patients.

Methods: We assessed the protein levels of these chemokines in peripheral blood mononuclear cells (PBMCs), sera and lesional skins. We also evaluated CCL18 mRNA levels in monocytes, Langerhans cell (LC)-like dendritic cells (DCs) and inflammatory dendritic epidermal cell (IDEC)-like DCs from both types of AD patients.

Results: There were no significant differences in CCL17 and CCL22 expression in PBMCs, sera and lesional skins of patients with IAD and EAD. CCL18 expression did not differ in PBMCs, sera and LC-like DCs from the two subgroups, but strong CCL18 expression was observed in lesional skins and IDEC-like DCs in patients with EAD. Lastly, serum CCL18 levels significantly decreased after immunotherapy.

Conclusion: This study suggests that the chemokine microenvironment, especially the level of CCL18, is different between EAD and IAD patients. High FcεRI surface-expressing DCs, such as IDEC, were the major source of CCL18, and produced a prominent CCL18 microenvironment in EAD patients compared with IAD patients.

Key words: atopic dermatitis – CCL18 – inflammatory dendritic epidermal cell – Langerhans cell

Please cite this paper as: Increased expression of CC chemokine ligand 18 in extrinsic atopic dermatitis patients. *Experimental Dermatology* 2007.

Introduction

Atopic dermatitis (AD) is a highly pruritic chronic relapsing skin disease characterized typically by distributed skin lesions with multifactorial pathophysiological aspects (1,2). An allergy or immunoglobulin E (IgE)-mediated hypersensitivity has long been thought to be important in the pathogenesis of AD because most patients have high concentrations of total and allergen-specific serum IgE, and positive skin prick tests reactions of the immediate type to common inhalant and food allergens (3,4). However, some AD patients show the typical skin lesions and distribution pattern of AD with no detectable sensitization and low serum IgE levels (5). The term 'intrinsic' AD (IAD) was proposed for this non-allergic type as a counterpart to

'extrinsic' AD (EAD) (3). The frequency of IAD ranges from 16% to 45% and a female predominance is found in IAD patients (4).

Several immunologic parameters, including Th2 cytokines and IgE receptors, have been investigated in peripheral blood mononuclear cells (PBMCs), lesional skin biopsies, epidermal cell suspensions and sera of IAD and EAD patients (6–9). Interleukin (IL)-4 and IL-5 were elevated in patients with EAD, but patients with IAD showed high levels of IL-5 with low IL-4 levels (6). These elevated IL-5 levels in both groups might explain blood eosinophilia (10), but more prominent dermal infiltration of eosinophils and eosinophil granular proteins was observed in EAD patients in comparison with IAD patients (11,12). Skin-derived T cells from patients with EAD displayed an increased IL-13

production, whereas lower levels of IL-13 were observed in T cells from patients with IAD (7). Furthermore, CD1a-positive epidermal dendritic cells and monocytes in EAD patients showed increased FcεRI surface expression compared with IAD patients (8,9). Many chemokines, including CC chemokine ligand (CCL) 17/thymus and activation-regulated chemokine (TARC), CCL22/macrophage-derived chemokine (MDC), and CCL18, are known to be highly expressed in AD patients, suggesting an important role of chemokines in the pathogenesis of AD (13–16). However, the differences of these chemokines between IAD and EAD have not yet been evaluated. In this study, we investigated the expression of CCL17, CCL22 and CCL18 in patients with IAD and EAD to provide better insight into the different pathogenesis of IAD and EAD.

Methods

Participants

Blood samples were obtained with informed consent from AD patients according to the criteria of Hanifin and Rajka (17). IAD was diagnosed by the following criteria: (i) a clinical phenotype of AD, (ii) low or moderate total serum IgE levels (<150 kU/l) in combination with negative *in vitro* IgE screening for aeroallergens and food allergens, as well as negative prick test results for standard aeroallergens and food allergens, and (iii) absence of other atopic diseases such as allergic asthma and rhinoconjunctivitis. Ten patients with IAD, 25 patients with EAD and nine nonatopic healthy controls were evaluated. Clinical severity was documented independently and blindly by clinicians using the eczema area and severity index (EASI) (18), Skin biopsy specimens were taken from the patients with IAD ($n = 9$), EAD ($n = 17$) and healthy individuals ($n = 6$). The patients did not receive any systemic or topical treatment with immunosuppressive drugs for at least 4 weeks before collection of blood samples and skin biopsies. All participants provided written informed consent, and the institutional review board approved this study.

Flow cytometry

Peripheral blood mononuclear cells from human blood were stained for 30 min on ice with mouse anti-CCL17, anti-CCL22 and anti-CCL18 monoclonal antibody (R&D Systems, Minneapolis, MN, USA) in phosphate-buffered saline containing 0.4% bovine serum albumin. After washing, cells were treated with fluorescein isothiocyanate-conjugated secondary antibody, followed by fixing and permeabilizing the cells with a formaldehyde/saponin solution (Sigma, St Louis, MO, USA). Analysis was performed on a FACSCalibur flow cytometer (Becton Dickinson, Mountain View, CA, USA). Results were expressed as the relative fluorescence intensity (RFI) calculated from

the mean fluorescence intensity (MFI) as follows:

$$RFI = (MFI_{\text{parameter evaluated}} - MFI_{\text{control}}) / MFI_{\text{control}}$$

Enzyme-linked immunosorbent assay

Serum CCL17, CCL22 and CCL18 levels were determined using commercially available enzyme-linked immunosorbent assay (ELISA) kits (R&D Systems) according to the manufacturer's instructions.

Immunohistochemistry

Acetone-fixed 6-μm cryostat sections were stained with mouse anti-CCL17, anti-CCL22, anti-CCL18 monoclonal antibody, or isotype control antibody (R&D Systems). The samples were costained with anti-CD206 (BD Biosciences, San Jose, CA, USA) or anti-CD207 (Beckman Coulter, Fullerton, CA, USA) monoclonal antibody. Development of the staining was performed with a Histostain-DS kit (Zymed Laboratories, San Francisco, CA, USA) according to the manufacturer's recommendations. The intensities of the stainings were evaluated by two independent investigators from low (0) to high (6).

Cell culture

The culture media contained RPMI 1640 (Gibco Laboratories, Grand Island, NY, USA), 2 μM L-glutamine (Gibco Laboratories), 100 IU/ml penicillin (Gibco Laboratories), 100 μg/ml streptomycin (Gibco Laboratories), and 10% fetal bovine serum (Hyclone, Logan, UT, USA). Monocytes from study participants were isolated from PBMCs via the cell attachment method (19). PBMCs were attached to six-well plates for 40 min. The supernatant and the floating cells were discarded, and the attached cells were used for culture. Monocytes were plated in six-well plates at a final concentration of 3×10^6 cells in 3 ml of culture medium. For the generation of Langerhans cell (LC)-like dendritic cells (DCs), cells were cultured in medium supplemented with 1000 U/ml GM-CSF (R&D Systems), 500 U/ml IL-4 (R&D Systems) and 10 ng/ml transforming growth factor-β1 (R&D Systems) at days 0, 2, 4, and 6, plus 10 ng/ml tumor necrosis factor-α at day 6 (20,21). Inflammatory dendritic epidermal cell (IDEC)-like DCs were generated by culturing monocytes for 6 days in medium supplemented with 500 U/ml GM-CSF, 1000 U/ml IL-4 at days 0, 2, 4, 6, and the reducing agent 2-mercaptoethanol (50 μM; Merk, Munchen, Germany) at days 4 and 6 (22,23). At days 2, 4 and 6, one-third of the medium was removed, and an equivalent volume of fresh medium supplemented with the above-mentioned cytokines.

Reverse transcriptase-polymerase chain reaction

Total RNA was extracted after the seventh day of culture from LC-like DCs or IDEC-like DCs using the RNeasy mini kit (Qiagen, Hilden, Germany) following the manu-

facturer's protocol. Reverse transcription reactions were performed using 1 µg of total RNA. The following primers were used: CCL18: sense, 5'-ATC ATG AAG GGC CTT GCA GCT GC-3'; antisense, 5'-TCA GGC ATT CAG CTT CAG GTC GC-3', which yielded a 273-bp product and β -actin: sense, 5'-GGC GGA CTA TGA CTT AGT TG-3'; antisense 5'-AAA CAA CAA TGT GCA ATC AA-3', resulting in a 238-bp product. Amplification was performed on a GeneAmp polymerase chain reaction (PCR) system 2700 (Applied Biosystems, Foster city, CA, USA). PCR was conducted under the following conditions: for CCL18, denaturation at 94°C for 30 s, annealing at 67°C for 30 s, and extension at 72°C for 30 s for 40 cycles, followed by a final extension at 72°C for 7 min; for β -actin, 94°C for 30 s, 53°C for 30 s, and 72°C for 30 s for 25 cycles, followed by a final extension at 72°C for 7 min. Specific PCR fragments were separated on a 1% agarose gel and visualized using ethidium bromide staining. The amounts of PCR products were determined by densitometry using TINA 2.10e software (Raytest, Straubhardt, Germany) and evaluated semi-quantitatively by grading the ratio between the specific products and the β -actin band.

Statistical analysis

Statistical differences were analysed with the Mann-Whitney *U*-test for unpaired samples and with the Wilcoxon signed-rank test for paired samples. $P < 0.05$ was considered statistically significant.

Results

Clinical and laboratory features of IAD and EAD patients

The clinical and laboratory findings of this study are presented in Table 1. There was no significant difference in clinical severity between the two subgroups according to EASI score. The total serum IgE level was markedly elevated in EAD patients. There was also a female predominance in IAD patients.

CCL17, CCL22 and CCL18 expression in PBMCs, sera and lesional skins of patients with IAD and EAD

There were no significant differences in CCL17, CCL18 and CCL22 expression in PBMCs of patients with IAD and EAD (Fig. 1). In particular, CCL18 expression in PBMCs of both groups was barely detected. The sera of both AD patient groups had increased CCL17, CCL18 and CCL22 expression compared with healthy controls, but no significant differences between IAD and EAD were observed (data not shown). CCL17 and CCL22 expression in lesional skins using immunohistochemistry revealed no significant differences between the two subgroups (Table 2), but lesional

Table 1. Characteristics of the study participants

	EAD	IAD
No. of patients (M:F)	25 (14:11)	10 (2:8)
Age (years)	17.5 $\pm$ 7.5	20.9 $\pm$ 10.8
Onset of AD (%)		
Infant	56	30
Early childhood	12	40
Late childhood	24	0
Adolescent	4	10
Adult	4	20
Total IgE	2558.3 $\pm$ 1813.0 ¹	78.4 $\pm$ 84.6
EASI score	18.3 $\pm$ 14.5	15.9 $\pm$ 16.9

Data of age, total IgE and EASI score are presented as mean $\pm$ SD. AD, atopic dermatitis; EAD, extrinsic AD; IAD, intrinsic AD.

¹Statistically significant in comparison with IAD.

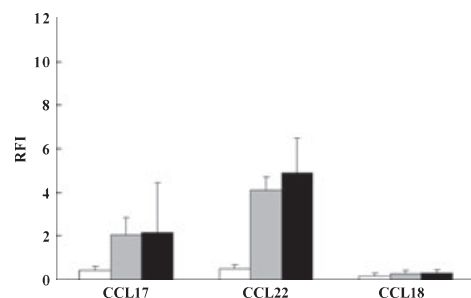


Figure 1. Flow cytometry analyses of CCL17, CCL22 and CCL18 in PBMCs of EAD ($n = 10$, black column), IAD ($n = 5$, grey column) patients and healthy controls ($n = 6$, white column). There were no significant differences in CCL17, CCL22 and CCL18 between EAD and IAD groups. CCL22 was highly expressed among these chemokines and CCL18 expression was barely detected in both AD groups.

skins of patients with EAD expressed more CCL18 than those of IAD patients (Fig. 2a,b). CCL18 was expressed in CD206-expressing DCs and CD207-expressing LCs of AD patients (Fig. 2c,d).

CCL18 expression in LC-like DCs and IDEC-like DCs of IAD and EAD patients

Monocytes from the two AD subgroups did not express CCL18, but LC-like DCs in both groups expressed a low level of CCL18, which did not differ between IAD and EAD patients. Strong CCL18 expression was induced in IDEC-like DCs in EAD patients, but a lower level of CCL18 was detected in IAD patients (Fig. 3).

CCL17, CCL22 and CCL18 expression in the sera of EAD patients undergoing immunotherapy

The initial sera of 10 EAD patients with specific IgE to *Dermatophagoides* were compared to the follow-up sera with an improved EASI score after hyposensitization with

Table 2. Immunohistochemistry analysis of CCL17, CCL22 and CCL18 in lesional skins of EAD and IAD patients

	CCL17	CCL22	CCL18
EAD	1.37 ± 1.15	3.03 ± 0.93	2.71 ± 0.71 ¹
IAD	1.42 ± 0.58	3.21 ± 0.56	1.71 ± 0.71

The data are presented as mean ± SD from 17 EAD patients and 9 IAD patients. The staining intensities were evaluated by two independent investigators from low (0) to high (6) grade.

¹Statistically significant in comparison with IAD.

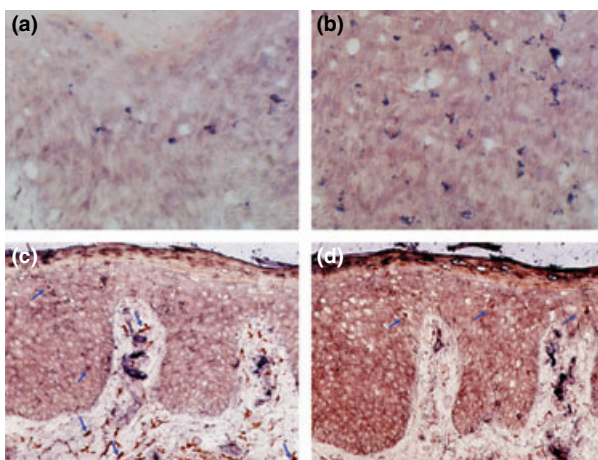


Figure 2. CCL18 expression in lesional skin of 17 EAD and 9 IAD patients. (a, b) Lesional skin of an EAD patient (b) expressed more CCL18 than that of an IAD patient (a); (c) CCL18-positive staining (black) in CD206-expressing DCs (light brown) from lesional skin of an AD patient; (d) CCL18-positive staining (black) in CD207-expressing LCs (light brown) from an EAD patient. Representative results are shown.

Alavac (Bencard, Brenford, UK) for more than 1 year (Fig. 4). Eight patients showed a CCL17 and CCL22 decrease at follow-up visits after Alavac therapy compared with initial presentations, but two patients showed a slight increase of these chemokines. In the case of CCL18, all patients showed a decrease after immunotherapy.

Discussion

Previous studies have demonstrated several immunologic differences between EAD and IAD, such as expression level of Th2 cytokines and IgE receptors (6–9). Although various chemokines were suggested to mediate leucocyte recruitment during atopic skin inflammation (24,25), the differences of these chemokines between IAD and EAD have not yet been evaluated. CCL17, secreted by keratinocytes and dermal endothelial cells, may mediate the recruitment of Th2 cells expressing the CC chemokine receptor (CCR) 4 (26,27). An increased CCL17 serum

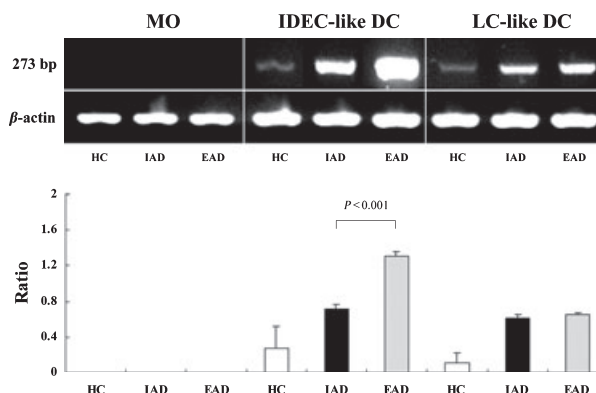
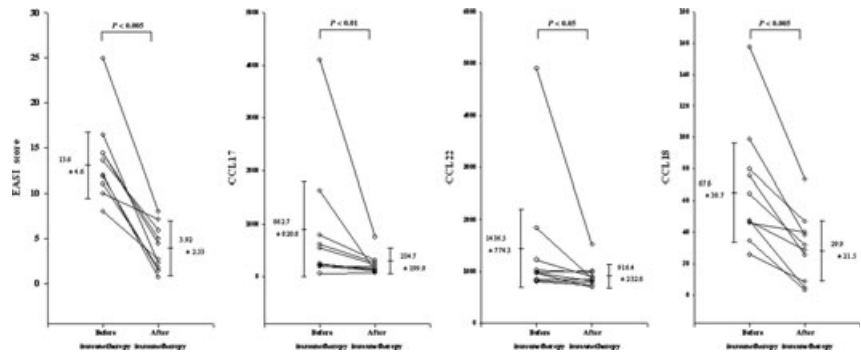


Figure 3. RT-PCR analysis of CCL18 in monocytes (MO), IDEC-like DCs and LC-like DCs of healthy control (HC) ($n = 5$), IAD ($n = 5$) and EAD ($n = 5$) patients. *Upper panel:* representative photograph shows bands corresponding to RT-PCR amplification of CCL18 mRNA; *lower panel:* graph shows mean ratio of CCL18 expression in three groups. Monocytes from the two AD subgroups did not express CCL18, but LC-like DCs in both groups expressed a low level of CCL18, which did not differ between IAD and EAD patients. Significant CCL18 expression was detected in IDEC-like DCs in EAD patients compared with IAD patients.

level was observed in AD patients in comparison with healthy controls and was correlated with disease activity (13). CCL22 also has a chemotactic effect on Th2 cells moving into the skin and serum CCL22 levels correlated with the clinical severity of AD (14,28). CCL18 was known to be specifically associated with AD, which was the most highly expressed chemokine in epidermal and dermal DCs, the major source of CCL18 in AD patients (15). A DNA microarray also identified CCL18 as one of the genes showing the strongest association with AD (29,30). Recent study showed CCL18 induces homing of memory T cells to human skin *in vivo* (16).

In this study, CCL17 and CCL22 expression showed no significant differences in PBMCs, sera and lesional skins of patients with EAD and IAD. CCL18 serum levels of both groups were also not significantly different, and there was very low CCL18 detection in PBMCs from both AD patients. In particular, lesional skins of EAD patients showed increased CCL18 expression compared with IAD patients and also showed more prominent CCL18 expression in IDEC-like DCs from EAD relative to IAD patients. In the previous report, CCL18 was significantly induced in AD patients with a history of house dust mite allergy after atopy patch tests with *Dermatophagoides* species, indicating a role for this chemokine in an allergen-specific immune response (15). Our results suggest that high FcεRI surface-expressing DCs, such as IDEC in EAD patients, is the major source of CCL18, which produce a prominent CCL18 microenvironment in the skin of EAD patients compared with that of IAD patients. Although the CCL18 receptor is still unknown, it was observed that CCL18 binds to cutaneous lymphocyte-associated antigen-positive mem-

Figure 4. EASI score and ELISA analysis of CCL17, CCL22 and CCL18 in the sera of 10 EAD patients with specific IgE to *Dermatophagoides*, before and 1-year after hyposensitization with Alavac (Bencard, Brenford, UK). EASI score improved in all patients. Levels of CCL17, CCL22 and CCL18 decreased after immunotherapy, showing the same direction as EASI score. Although CCL17 and CCL22 showed a slight increase in two patients, CCL18 decreased in all patients after immunotherapy.



ory T cells in the peripheral blood of AD patients *in vitro* and induces the migration of memory T cells into human skin *in vivo* (16). It should be determined whether CCL18 efficiently mediates the recruitment of Th2 cells that secrete cytokines such as IL-4 and IL-13 in parallel with the high serum IgE levels of EAD patients.

We also evaluated the changes of CCL18 expression in EAD patients who showed improvement of EASI scores after specific immunotherapy. The significant serum CCL18 decrease after immunotherapy also supports the important role of CCL18 in EAD. Although comparable studies on the effectiveness of specific immunotherapy (SIT) against House Dust Mite (HDM) sensitizations in patients with AD are rare, SIT has been an attractive therapeutic option for long time treatment in AD (31). The most recent and relevant study was a placebo-controlled multicentre study performed in a total of 89 adult patients with AD, in which a significant reduction of the Scoring of Atopic Dermatitis (SCORAD) after 4 months of weekly injections of HDM allergens has been observed (32). Beyond that, recent review suggests that proper selection of patients and introduction of highly purified allergen and improvement in the route of administration facilitates the introduction of SIT as an additional safe therapeutic option (31).

It is still unknown whether EAD and IAD represent the extreme ends of clinical manifestations of a continuous AD spectrum, or if there are different immunologic mechanisms in IAD and EAD. The former hypothesis is supported by recent studies that children with IAD became EAD with a positive skin prick test to allergens within a decade (33). Furthermore, positive atopy patch test reactions to aeroallergens were induced in IAD patients without allergen-specific serum IgE, and allergen-specific IgE to microbial components was determined in some IAD patients (34,35). Some adult IAD patients, however, are present persistently without any specific allergen, and there are many different immunologic mechanisms between EAD and IAD, indicating a different underlying pathophysiology between the two subgroups.

LCs and IDECs in the lesional skin of AD patients have an important function in the pathophysiology of AD. LCs

prime naïve T cells into Th2 cells during the initial phase of AD, inducing the release of chemotactic signals and the recruitment of IDECs. IDECs lead to the release of Th1 cytokines in the chronic phase of AD, thereby amplifying the allergic immune response (36). High CCL18 expression in IDEC-like DCs of EAD patients suggests that CCL18 may play a pivotal role in the maintenance of allergen-mediated skin inflammation, especially in patients with EAD. Therefore, development of drug targeting CCL18 might be a new therapeutic approach in AD, such as CCR3 antagonists. Observing an EAD patient's serum CCL18 level after immunotherapy also could be helpful in monitoring disease severity. However, the number of patients and healthy controls included in this study is not enough to make epidemiological conclusions, because it is known that there are large inter-individual variations within the patient groups. A large-scale study is needed to confirm this finding.

In conclusion, this study demonstrated different chemokine micromilieu between EAD and IAD. Furthermore, IDEC might maintain allergic response through CCL18, attracting T cells in EAD patients.

Funding sources

This work was supported by the Brain Korea 21 Project for Medical Science, Yonsei University.

Conflicts of interest

None identified.

Acknowledgements

We thank the patients and volunteers for their participation and cooperation.

References

- 1 Leung D Y, Bieber T. Atopic dermatitis. *Lancet* 2003; **361**: 151–160.

- 2 Novak N, Bieber T, Leung D Y. Immune mechanisms leading to atopic dermatitis. *J Allergy Clin Immunol* 2003; **112** (Suppl.): S128–S139.
- 3 Schmid-Grendelmeier P, Simon D, Simon H U, Akdis C A, Wuthrich B. Epidemiology, clinical features, and immunology of the 'intrinsic' (non-IgE-mediated) type of atopic dermatitis (constitutional dermatitis). *Allergy* 2001; **56**: 841–849.
- 4 Novak N, Bieber T. Allergic and nonallergic forms of atopic diseases. *J Allergy Clin Immunol* 2003; **112**: 252–262.
- 5 Wuthrich B. Atopic dermatitis flare provoked by inhalant allergens. *Dermatologica* 1989; **178**: 51–53.
- 6 Kagi M K, Wuthrich B, Montano E, Barandun J, Blaser K, Walker C. Differential cytokine profiles in peripheral blood lymphocyte supernatants and skin biopsies from patients with different forms of atopic dermatitis, psoriasis and normal individuals. *Int Arch Allergy Immunol* 1994; **103**: 332–340.
- 7 Akdis C A, Akdis M, Simon D *et al.* T cells and T cell-derived cytokines as pathogenic factors in the nonallergic form of atopic dermatitis. *J Invest Dermatol* 1999; **113**: 628–634.
- 8 Oppel T, Schuller E, Gunther S *et al.* Phenotyping of epidermal dendritic cells allows the differentiation between extrinsic and intrinsic forms of atopic dermatitis. *Br J Dermatol* 2000; **143**: 1193–1198.
- 9 Novak N, Kruse S, Kraft S *et al.* Dichotomic nature of atopic dermatitis reflected by combined analysis of monocyte immunophenotyping and single nucleotide polymorphisms of the interleukin-4/interleukin-13 receptor gene: the dichotomy of extrinsic and intrinsic atopic dermatitis. *J Invest Dermatol* 2002; **119**: 870–875.
- 10 Wedi B, Raap U, Lewrick H, Kapp A. Delayed eosinophil programmed cell death in vitro: a common feature of inhalant allergy and extrinsic and intrinsic atopic dermatitis. *J Allergy Clin Immunol* 1997; **100**: 536–543.
- 11 Jeong C W, Ahn K S, Rho N K *et al.* Differential in vivo cytokine mRNA expression in lesional skin of intrinsic vs. extrinsic atopic dermatitis patients using semiquantitative RT-PCR. *Clin Exp Allergy* 2003; **33**: 1717–1724.
- 12 Rho N K, Kim W S, Lee D Y, Lee J H, Lee E S, Yang J M. Immunophenotyping of inflammatory cells in lesional skin of the extrinsic and intrinsic types of atopic dermatitis. *Br J Dermatol* 2004; **151**: 119–125.
- 13 Kakinuma T, Nakamura K, Wakugawa M *et al.* Thymus and activation-regulated chemokine in atopic dermatitis: serum thymus and activation-regulated chemokine level is closely related with disease activity. *J Allergy Clin Immunol* 2001; **107**: 535–541.
- 14 Kakinuma T, Nakamura K, Wakugawa M *et al.* Serum macrophage-derived chemokine (MDC) levels are closely related with the disease activity of atopic dermatitis. *Clin Exp Immunol* 2002; **127**: 270–273.
- 15 Pivarcsi A, Gombert M, Dieu-Nosjean M C *et al.* CC chemokine ligand 18, an atopic dermatitis-associated and dendritic cell-derived chemokine, is regulated by staphylococcal products and allergen exposure. *J Immunol* 2004; **173**: 5810–5817.
- 16 Gunther C, Bello-Fernandez C, Kopp T *et al.* CCL18 is expressed in atopic dermatitis and mediates skin homing of human memory T cells. *J Immunol* 2005; **174**: 1723–1728.
- 17 Hanifin J M, Rajka G. Diagnostic features of atopic dermatitis. *Acta Derm Venereol (Stockh)* 1980; **92** (Suppl.): S44–S47.
- 18 Hanifin J M, Thurston M, Omoto M *et al.* The eczema area and severity index (EASI): assessment of reliability in atopic dermatitis. *Exp Dermatol* 2001; **10**: 11–18.
- 19 Choi G S, Kang J M, Lee M G. Analysis of methods for the generation of dendritic cells from human peripheral blood monocytes. *Yonsei Med J* 2000; **41**: 642–650.
- 20 Reich K, Heine A, Hugo S *et al.* Engagement of the FcεRI stimulates the production of IL-16 in Langerhans cell-like dendritic cells. *J Immunol* 2001; **167**: 6321–6329.
- 21 Geissmann F, Dieu-Nosjean M C, Dezutter C *et al.* Accumulation of immature Langerhans cells in human lymph nodes draining chronically inflamed skin. *J Exp Med* 2002; **196**: 417–430.
- 22 Sallusto F, Lanzavecchia A. Efficient presentation of soluble antigen by cultured human dendritic cells is maintained by granulocyte/macrophage colony-stimulating factor plus interleukin 4 and down-regulated by tumor necrosis factor α. *J Exp Med* 1994; **179**: 1109–1118.
- 23 Novak N, Kraft S, Haberstick J, Geiger E, Allam P, Bieber T. A reducing microenvironment leads to the generation of FcεRI^{high} inflammatory dendritic epidermal cells (IDEC). *J Invest Dermatol* 2002; **119**: 842–849.
- 24 Pivarcsi A, Homey B. Chemokine networks in atopic dermatitis: traffic signals of disease. *Curr Allergy Asthma Rep* 2005; **5**: 284–290.
- 25 Homey B, Steinhoff M, Ruzicka T, Leung D Y. Cytokines and chemokines orchestrate atopic skin inflammation. *J Allergy Clin Immunol* 2006; **118**: 178–189.
- 26 Vestergaard C, Bang K, Gesser B, Yoneyama H, Matsushima K, Larsen CG. A Th2 chemokine, TARC, produced by keratinocytes may recruit CLA+CCR4+ lymphocytes into lesional atopic dermatitis skin. *J Invest Dermatol* 2000; **115**: 640–646.
- 27 Imai T, Nagira M, Takagi S *et al.* Selective recruitment of CCR4-bearing Th2 cells toward antigen-presenting cells by the CC chemokines thymus and activation-regulated chemokine and macrophage-derived chemokine. *Int Immunol* 1999; **11**: 81–88.
- 28 Katou F, Ohtani H, Nakayama T *et al.* Macrophage-derived chemokine (MDC/CCL22) and CCR4 are involved in the formation of T lymphocyte-dendritic cell clusters in human inflamed skin and secondary lymphoid tissue. *Am J Pathol* 2001; **158**: 1263–1270.
- 29 Nomura I, Gao B, Boguniewicz M, Darst M A, Travers J B, Leung D Y. Distinct patterns of gene expression in the skin lesions of atopic dermatitis and psoriasis: a gene microarray analysis. *J Allergy Clin Immunol* 2003; **112**: 1195–1202.
- 30 Plager D A, Leontovich A A, Henke S A, Davis M D, McEvoy M T, Scialis G F. Early cutaneous gene transcription changes in adult atopic dermatitis and potential clinical implications. *Exp Dermatol* 2006; **16**: 28–36.
- 31 Bussmann C, Boeckenhoff A, Henke H, Werfel T, Novak N. Does allergen-specific immunotherapy represent a therapeutic option for patients with atopic dermatitis? *J Allergy Clin Immunol* 2006; **118**: 1292–1298.
- 32 Werfel T, Breuer K, Rueff F *et al.* Usefulness of specific immunotherapy in patients with atopic dermatitis and allergic sensitization to house dust mites: a multi-centre, randomized, dose-response study. *Allergy* 2006; **61**: 202–205.
- 33 Novembre E, Cianferoni A, Lombardi E, Bernardini R, Pucci N, Vierucci A. Natural history of 'intrinsic' atopic dermatitis. *Allergy* 2001; **56**: 452–453.
- 34 Kerschenlohr K, Decard S, Darsow U, Ollert M, Wollenberg A. Clinical and immunologic reactivity to aeroallergens in 'intrinsic' atopic dermatitis patients. *J Allergy Clin Immunol* 2003; **111**: 195–197.
- 35 Novak N, Allam J P, Bieber T. Allergic hyperreactivity to microbial components: a trigger factor of 'intrinsic' atopic dermatitis? *J Allergy Clin Immunol* 2003; **112**: 215–216.
- 36 Novak N, Valenta R, Bohle B *et al.* FcεRI engagement of Langerhans cell-like dendritic cells and inflammatory dendritic epidermal cell-like dendritic cells induces chemotactic signals and different T-cell phenotypes in vitro. *J Allergy Clin Immunol* 2004; **113**: 949–957.