

Expression of CCL1 and CCL18 in atopic dermatitis and psoriasis

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doi:10.1111/j.1365-2230.2011.04295.x

Summary

Background. Recent studies have shown that chemoattractive proteins play an important role in the organization of the innate and adaptive immune responses. There are some reports that chemokine (C-C motif) ligand (CCL)1 and CCL18, members of a family of chemoattractive proteins, have increased expression in atopic dermatitis (AD).

Aim. To evaluate the quantity and pattern of CCL1 and CCL18 expression in lesions and blood of patients with AD, and compare them with those of patients with psoriasis.

Methods. Biopsy specimens were taken from atopic skin and normal-appearing skin of patients with AD and from the psoriatic skin only of patients with psoriasis. Quantitative real-time PCR analysis and immunohistochemistry of CCL1 and CCL18 expression were performed, and the quantities of expressed CCL1 and CCL18 in acute AD were compared with those of normal-appearing atopic skin and psoriatic skin. The serum level of CCL1 and CCL18 was assessed by ELISA.

Results. Expression of CCL1 mRNA and protein was significantly higher in the acute lesional skin of patients with AD than in their nonlesional skin or in the lesional skin of patients with psoriasis. Both CCL18 mRNA and protein were abundant in acute AD lesions and in psoriatic lesions, but were lower in the nonlesional skin of patients with AD. The serum levels of CCL1 and CCL18 were not different in patients with AD and patients with psoriasis.

Conclusions. CCL1 is a chemokine that is associated with AD. Both CCL1 and CCL18 may play important roles in the initiation and progression of atopic skin inflammation.

Introduction

Atopic dermatitis (AD) is a chronic inflammatory disease characterized by recurrent pruritis. Various inflammatory cells, including both T cells and eosinophils expressing cutaneous lymphocyte-associated antigen (CLA), CD4+ or CD8+, play an important role in initiating and progressing inflammatory reactions in AD tissue.¹ It has been shown that various cytokines and chemokines are involved in inflammatory-cell infiltration into the lesion, and that their receptors are

expressed on inflammatory cells or keratinocytes.^{2–5} Colantonio *et al.*⁶ showed that expression of CCL1 and CCL18 is higher in atopic skin than in normal or pruritic skin. It has been documented that the secretion of the chemokine RANTES (regulated upon activation, normal T-cell expressed and secreted) is increased by antigens or staphylococcal enterotoxin B in peripheral monocytes in patients with AD.⁷ Park *et al.*⁸ reported that CCL18 is strongly expressed on inflammatory dendritic epidermal cell (IDEC)-like dendritic cells present in patients with extrinsic AD. Gros *et al.*⁴ suggested recently that CCL1, CCL17 and CCL18 were associated with eczema development, and that increased CCL1, CCL13, CCL17 and CCL18 expression was specific for patients with chronic AD compared with patients with psoriasis. However, there is still disagreement regarding the relationship between the severity of AD and the serum levels of CCL1 and CCL18. In addition, there have

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Conflict of interest: none declared.

Accepted for publication 8 October 2011

been few genetic studies on the differences in expression of CCL1 and CCL18 between atopic and nonatopic lesions.

We conducted a study to investigate the expression of CCL1 and CCL18 in the lesional and nonlesional skin of patients with AD and in the lesional skin of patients with psoriasis, in order to compare the serum levels of these chemokines between AD and psoriasis, and to determine if there was a correlation between these serum levels and the severity of AD.

Methods

The study was approved by the institutional review board of Kangnam Sacred Heart Hospital, and carried out in accordance with the Declaration of Helsinki. Informed consent was obtained from all patients.

Patient demographics and assessment of clinical severity

We recruited 79 patients with AD (36 men, 43 women; mean $\pm$ SD age 18.33 ± 11.71 years, range 3–36) and 52 patients with psoriasis (22 men, 30 women; mean $\pm$ SD age 24.09 ± 10.84 years, range 17–54) who visited the Department of Dermatology at Hallym University between January 2008 and July 2009. AD was diagnosed according to the criteria of Hanifin and Rajka.⁹ The clinical severity of AD was assessed by the Eczema Area and Severity Index (EASI); the mean EASI was 12.1 ± 6.4 (range 5.6–22.6). Based on a positive ImmunoCAP test (Phadia, Uppsala, Sweden), the patients were separated into those with intrinsic AD ($n = 26$) and those with extrinsic AD ($n = 53$).

Skin biopsy samples (4 mm) were taken from 8 patients with AD (5 men, 3 women; range 19–30; 3 with intrinsic AD, 5 with extrinsic AD; mean EASI 13.7 ± 4.8), both from the acute inflammatory lesional skin (group A) and the nonlesional skin (group B), and were also taken from the plaque lesions of 11 patients with psoriasis (6 men, 5 women; range 20–35), who were designated as group C. An acute inflammatory lesion was defined as an eczematous area with moderate to severe intensity of erythema, induration and excoriation, and with no to mild lichenification based on the EASI. There were no significant differences in age and gender distributions between biopsied patients with AD and those with psoriasis.

Blood samples were taken from all participants for assessment of complete blood count, total serum IgE, specific IgE against common inhalant and food allergens, and levels of CCL1 and CCL18.

Immunohistochemistry

For immunohistochemistry of chemokine and chemokine receptor expression, skin sections were fixed with acetone and preprocessed with H_2O_2 , followed by an avidin–biotin blocking step (Avidin/Biotin Blocking Kit; Vector Laboratories Inc., Burlingame, CA, USA). Sections were stained with antibodies against human CCL1 (goat IgG; Santa Cruz Biotechnology Inc., Santa Cruz, CA, USA) and CCL18 (D6, mouse IgG; German Cancer Research Center, Heidelberg, Germany). Development of the staining was performed with an avidin-biotinylated enzyme complex (Vectastain ABC Kit; Vector Laboratories) and 3-amino-9-ethyl-carbazole solution (AEC Kit; Vector Laboratories). Sections were counterstained with haematoxylin.

Quantitative real-time reverse transcriptase PCR analysis

Quantitative real-time PCR analyses were performed as previously described.¹⁰ Skin biopsies were homogenized in liquid nitrogen and RNA was extracted (TRIzol reagent; Invitrogen Life Technologies, Carlsbad, CA, USA) according to the manufacturer's instructions. A quantity (2 μ g) of RNA was treated with enzyme (DNase I; Boehringer Mannheim, Mannheim, Germany) and reverse transcribed with oligo dT₁₈ (Invitrogen Life Technologies) and random hexamer primers (Promega Corp., Madison, WI, USA) using standard protocols. cDNA (25 ng) was amplified in the presence of 12.5 μ L of TaqMan Universal Mastermix (Applied Biosystems, Carlsbad, CA, USA), 0.625 μ L of gene-specific TaqMan probe, 0.5 μ L of gene-specific forward and reverse primers, and 0.5 μ L of water. cDNA (25 ng) was amplified in the presence of target-specific primer combinations and SYBR Green Mastermix (Applied Biosystems). Gene-specific PCR products were measured (ABI PRISM 7700 or 5700 Sequence Detection System; Applied Biosystems, Foster City, CA, USA) continuously during the PCR amplification of 40 cycles (denaturing at 95 °C for 15 s; annealing at 60 °C for 1 min with commercial primers (CCL1: Hs00171072_m1; CCL18: Hs00268113_m1; and 18s as endogenous control) (Applied Biosystems).

ELISA

For the detection of the CCL1 protein in cell culture, specific ELISA kits (human CCL1 DuoSet and human CCL18/PARC DuoSet; R&D Systems Inc., Minneapolis, MN, USA) were used according to the manufacturer's instructions. Dilution series of recombinant human

CCL1 and CCL18 protein (R&D Systems) were used for standard curves.

Statistical analysis

For statistical analysis, the Student *t*-test or Mann–Whitney *U*-test was used for comparison between two groups; the Tukey test was used for comparison between three or more groups, and the Spearman rank correlation test was used for correlation. $P < 0.05$ was considered significant.

Results

Expression of CCL1 and CCL18 in the three groups

Using immunocytochemistry, the number of cells with expression of these two chemokines were counted twice within four high-power fields. The number of cells with CCL1 expression was 91.8 ± 14.6 in group A,

14.6 ± 5.1 in group B and 8.1 ± 8.6 in group C; it was significantly larger in group A ($P < 0.05$). Immunohistochemical staining was strongly positive for group A, but negative for groups B and C. In the biopsy specimens, CCL1 was expressed in the basal layer of the epidermis and around the vessels of the dermis, especially in infiltrated lymphocytes and mast cells. CCL18 expression was 7.8 times higher in group A than in group B, and 1.2 times higher in group A than in group C ($P < 0.05$) (Figs 1,2). Slightly higher CCL1 and 18 expression was seen in the lesional skin of patients with extrinsic AD compared with patients with intrinsic AD (data not shown).

Expression of CCL1 and CCL18 mRNA in the three groups

The number of biopsy specimens meeting the criteria for mRNA expression was six in group A, four in group B and five in group C. The amount of CCL1 mRNA (expressed as fg per 25 ng cDNA) was 10.1 ± 4.2 in

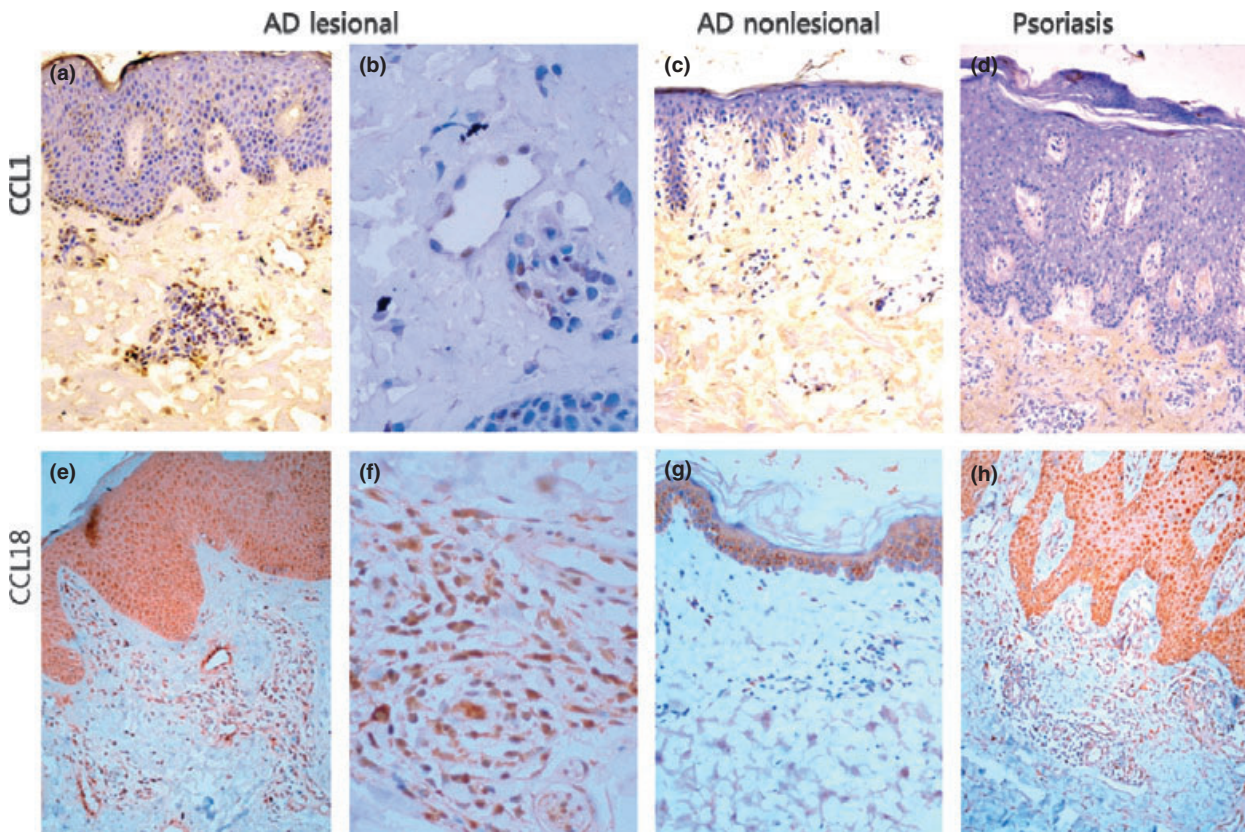


Figure 1 Increased expression of CCL1 and CCL18 in the lesional skin but not in the nonlesional skin of patients with atopic dermatitis (AD). (a–h) Using immunohistochemistry, marked expression of CCL1 was found in (a,b) the lesional skin of patients with AD [(b) is a magnification of (a)], while it was absent in (c) the nonlesional skin of the same patients or (d) lesional skin of patients with psoriasis. There was marked expression of CCL18 in the lesional skin of (e,f; magnified) patients with AD and (g) those with psoriasis but not in (h) the nonlesional skin of those with AD. * $P < 0.05$. Original magnification (a,c,d,e,g,h) $\times 100$; (b,f) $\times 400$.

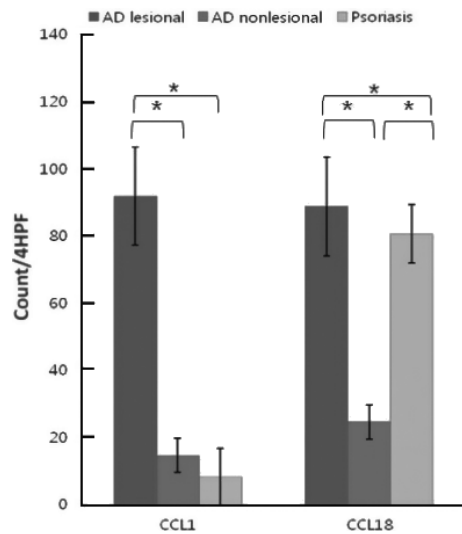


Figure 2 Immunohistochemistry was used to identify expression of CCL1 and CCL18 in the lesional and nonlesional skin of patients with atopic dermatitis (AD) and patients with psoriasis. The number of cells with expression of these chemokines was counted twice within four high-power fields. * $P < 0.05$.

group A, 0.5 ± 0.5 in group B and 0.6 ± 0.6 in group C; it was significantly larger in group A ($P < 0.05$) (Fig. 3). The amount of CCL18 mRNA (expressed as fg per 25 ng cDNA) was 445.3 ± 489.1 in group A, 58.8 ± 55.4 in group B and 383.5 ± 395.8 in group C; it was significantly larger in groups A and C than in group B ($P < 0.05$) (Fig. 3). There were no significant

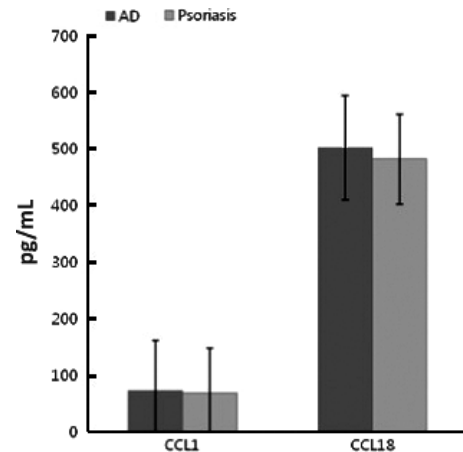


Figure 4 Serum levels of CCL1 and CCL18 in patients with atopic dermatitis (AD) and those with psoriasis; no significant difference found.

differences in CCL1 and CCL18 mRNA expression in lesional skin of patients with either intrinsic or extrinsic AD.

Serum levels of CCL1 and CCL18 in patients with atopic dermatitis and patients with psoriasis

The serum CCL1 and CCL18 concentrations measured by ELISA were 72.3 ± 90.2 and 502.8 ± 92.4 $\mu\text{g/mL}$, respectively, in patients with AD, and 64.6 ± 79.6 and 481.2 ± 79.6 $\mu\text{g/mL}$ in patients with psoriasis, but the

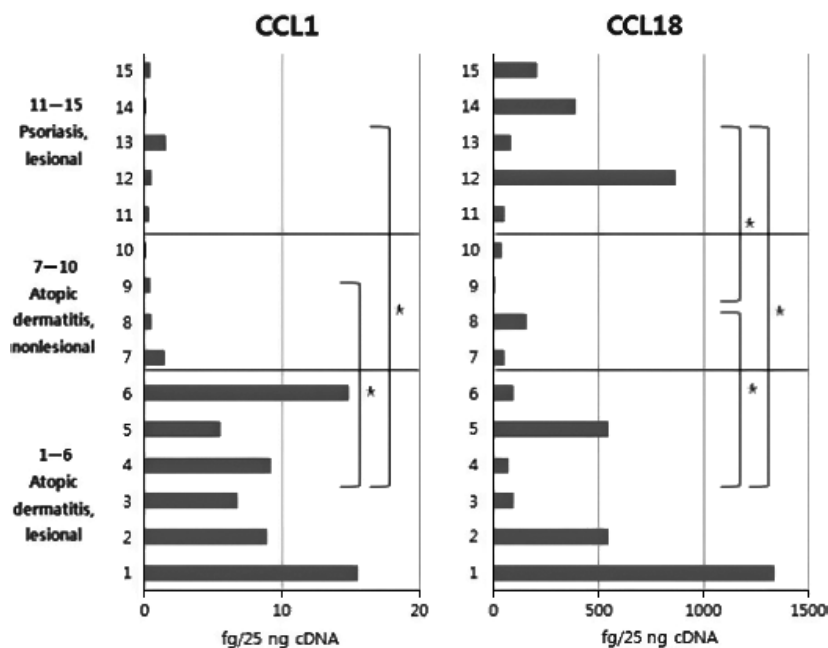


Figure 3 Quantitative real-time PCR analysis of CCL1 and CCL18 expression in the lesional ($n = 6$) and nonlesional skin ($n = 4$) of patients with atopic dermatitis compared with the lesional skin of patients with psoriasis ($n = 5$). Values are expressed in fg of the target gene per 25 ng of total cDNA. * $P < 0.05$, Student t -test.

difference was not significant in either case ($P < 0.05$) (Fig. 4). There were no significant differences in CCL1 and CCL18 in the sera of patients with either intrinsic or extrinsic AD.

Relationship between the serum levels of CCL1 and CCL18 and the Eczema Area and Severity Index

The EASI did not significantly correlate with the serum levels of CCL1 and CCL18.

Discussion

Chemokines belong to a family of cytokines, and > 50 types of chemokines have been identified to date.¹¹ Chemokines play a role in recruiting appropriate cells during inflammatory responses,¹ and are categorized into four groups, according to the cysteine located at the N-terminal, as C, CC, CXC and CX3C chemokines.

CC chemokines mainly recruit lymphocytes and monocytes; CCL5 (RANTES), CCL3 [macrophage inflammatory protein (MIP)-1 α], CCL4 (MIP-1 β), CCL2 (monocyte chemoattractant protein-1) and CCL1 (I-309) belong to the CC chemokine class. The CXC chemokine class includes CXCL8 [interleukin (IL)-8], which mainly recruits neutrophils, and CXCL9 (monokine induced by interferon gamma; Mig) and CXCL10 (interferon gamma-induced protein-10), which recruit monocytes and lymphocytes.¹¹ CXCL1 (lymphotactin) belongs to the C chemokine class, and CX3CL1 (fractalkin) belongs to the CX3C chemokine class.

Chemokines are involved in inflammatory processes, neovascularization, haematopoiesis, allergic reactions, wound healing and viral infections.¹¹ Maeda *et al.*¹² have documented that the expression of chemokine receptors on the surfaces of immunocytes exerts a significant effect on immunity and the prevention of infections.

Chemokines play an important role in inflammatory-cell infiltration into the skin in patients with AD.^{2,4} T cells are attracted into the skin by a variety of chemokines. In particular, T-helper (Th)2 cells containing CCR4 are attracted to the skin by macrophage-induced chemokine (MDC) and by thymus and activation-regulated chemokine (TARC).¹³ A recently identified cytokine, IL-31, is known to be involved in the induction of pruritus in patients with extrinsic AD by the secretion of IL-31 by Th2 cells.¹⁴ RANTES, monocyte chemoattractant protein-4, and eotaxin, which belong to the CC chemokine class, are often increased in patients with AD, and are closely involved in the chemotaxis of eosinophils and Th2 cells containing CCR3 receptors.¹⁵

Ying¹ reported that CCL1 expression is higher in patients with AD than in those with psoriasis, and is also higher in lesional skin than in nonlesional skin of patients with AD. Gluck and Rogala¹⁶ showed that CCL1 binds to CCR8 receptors on T cells and dendritic cells. They also reported that CCL1 is expressed mainly on dendritic cells, mast cells and endothelial cells of the dermis, and that serum CCL1 levels are significantly higher in patients with AD than in normal subjects. In our study, CCL1 expression was higher in the lesional skin of patients with AD than in the nonlesional skin of the same patients, but not in the lesional skin of those with psoriasis. Plausible reasons for this discrepancy are that there may have been experimental errors in both previous studies and the current study, or that the severity of AD was relatively lower in our patients than in those of the previous studies, resulting in a lower rate of development of systemic reactions. In other words, although the severity of AD was not described in previous studies, it is possible that the severity may have been higher in patients in previous studies than in our patients, because chemokines are involved in local skin reactions. Further studies, with a larger sample size, assessing the relationship between the serum CCL1 levels and the severity of AD, are needed to confirm this assumption.

CCL18 is known to be expressed most strongly in patients with AD. Recent studies have shown that CCL18 is secreted from dendritic cells in the dermis, and in Langerhans cells and IDECs in the epidermis of patients with AD.^{7,8} These studies also stated that serum CCL18 levels are not increased in the nonlesional skin of patients with AD or in the lesional skin of patients with psoriasis, and that CCL18 binds to CLA⁺ T cells in peripheral blood and is increased by certain antigens or by staphylococcal enterotoxin B. In our study, CCL18 expression was higher in the lesional skin of patients with AD than in the nonlesional skin of the same patients, which is similar to the results of previous studies. Expression of CCL18 mRNA and receptors was lower in the lesional skin of patients with psoriasis but higher in the nonlesional skin of patients with AD. These results suggest that CCL18 is expressed in various types of dermatitis rather than in AD alone. Further studies with a larger sample size are warranted to confirm this assumption. Previous studies have shown that serum CCL18 levels are higher in patients with AD than in those with psoriasis and in normal subjects.^{4,7} In our study, the serum CCL18 levels were not significantly higher in patients with AD than in those with psoriasis. This may be for the same reasons as given for CCL1.

Taken together, expression of both CCL1 and CCL18 was higher in the lesional skin of patients with AD than in the nonlesional skin of the same patients. However, expression of CCL18 was higher in the lesional skin of patients with psoriasis, whereas the expression of CCL1 was lower. Thus, we believe CCL1 to be a more specifically acting chemotactic factor for AD than CCL18.

The results of this study are subject to some limitations. Firstly, the sample size was small because the skin biopsy was difficult to perform. Secondly, only two chemokines were investigated. Further studies with a larger sample size and more chemokines are needed to provide useful data for establishing the criteria for severity and types of AD.

Conclusion

The results of this study suggest that CCL1 may be a more specific chemokine than CCL18 for the acute inflammation of AD because CCL1 and CCL18 are involved in the acute inflammatory stage of AD and also because CCL18, has higher expression in the lesional skin of patients with psoriasis.

What's already known about this topic?

- There is still disagreement regarding the relationship between the severity of AD and the serum levels of CCL1 and CCL18.
- In addition, there have been few genetic studies on the difference in the expression of CCL1 and CCL18 between atopic and nonatopic lesions.

What does this study add?

- The results suggest that CCL1 may be a more specific chemokine than CCL18 for the acute inflammation of AD.

Acknowledgements

This research was supported by Basic Science Research Program through the National Research Foundation of Korea(NR) funded by the Ministry of Education, Science and Technology (No. 2011-0013003, 2011-0004436) and by a grant from Amore Pacific Co. Ltd., 2011.

References

- 1 Ying S. C-C chemokine expression in atopic and nonatopic asthma. *Chem Immunol* 2000; **78**: 178–88.
- 2 Fujisawa T, Nagao M, Hiraguchi Y *et al.* Serum measurement of thymus and activation-regulated chemokine/CCL17 in children with atopic dermatitis: elevated normal levels in infancy and age-specific analysis in atopic dermatitis. *Pediatr Allergy Immunol* 2009; **20**: 633–41.
- 3 Ezzat MH, Shaheen KY. Serum mucosa-associated epithelial chemokine in atopic dermatitis: a specific marker for severity. *Indian J Dermatol* 2009; **54**: 229–36.
- 4 Gros E, Bussmann C, Bieber T *et al.* Expression of chemokines and chemokine receptors in lesional and nonlesional upper skin of patients with atopic dermatitis. *J Allergy Clin Immunol* 2009; **124**: 753–60.e1.
- 5 Lai GM, Lin SH. [Expression of chemokine receptor CXCR3 mRNA in patients with atopic dermatitis] (in Chinese). *Nan Fang Yi Ke Da Xue Xue Bao* 2007; **27**: 1084–5.
- 6 Colantonio L, Iellem A, Sinigaglia F *et al.* Skin-homing CLA+ T cells and regulatory CD25+ T cells represent major subsets of human peripheral blood memory T cells migrating in response to CCL1/I-309. *Eur J Immunol* 2002; **32**: 3506–14.
- 7 Pivarcsi A, Gombert M, Dieu-Nosjean MC *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**: 5810–17.
- 8 Park CO, Lee HJ, Lee JH *et al.* Increased expression of CC chemokine ligand 18 in extrinsic atopic dermatitis patients. *Exp Dermatol* 2008; **17**: 24–9.
- 9 Hanifin JM. Diagnostic criteria for atopic dermatitis: consider the context. *Arch Dermatol* 1999; **135**: 1551.
- 10 Muller A, Homey B, Soto H *et al.* Involvement of chemokine receptors in breast cancer metastasis. *Nature* 2001; **410**: 50–6.
- 11 Niederlova J, Koubek K. [Chemokines and chemokine receptors] (in Czech). *Sb Lek* 1999; **100**: 169–89.
- 12 Maeda S, Okayama T, Omori K *et al.* Expression of CC chemokine receptor 4 (CCR4) mRNA in canine atopic skin lesion. *Vet Immunol Immunopathol* 2002; **90**: 145–54.
- 13 Panina-Bordignon P, Papi A, Mariani M *et al.* The C-C chemokine receptors CCR4 and CCR8 identify airway T cells of allergen-challenged atopic asthmatics. *J Clin Invest* 2001; **107**: 1357–64.
- 14 Sonkoly E, Muller A, Lauerma AI *et al.* IL-31: a new link between T cells and pruritus in atopic skin inflammation. *J Allergy Clin Immunol* 2006; **117**: 411–17.
- 15 Ying S, Meng Q, Zeibecoglou K *et al.* Eosinophil chemotactic chemokines (eotaxin, eotaxin-2, RANTES, monocyte chemoattractant protein-3 (MCP-3), and MCP-4), and C-C chemokine receptor 3 expression in bronchial biopsies from atopic and nonatopic (intrinsic) asthmatics. *J Immunol* 1999; **163**: 6321–9.
- 16 Gluck J, Rogala B. Chemokine RANTES in atopic dermatitis. *Arch Immunol Ther Exp (Warsz)* 1999; **47**: 367–72.