

ORIGINAL ARTICLE

Exploring CCL18, eczema severity and atopy

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Abstract

Background: Childhood atopic dermatitis (AD) is a distressing disease associated with pruritus, sleep disturbance, and impaired quality of life. The pathophysiology of AD is complex, and the chemokine CCL18/pulmonary and activation-regulated chemokine (PARC) may be involved.

Objective: To evaluate whether CCL18 was associated with disease severity, quality of life, nocturnal scratching, serum eosinophil, and IgE levels.

Patients and methods: Patients with AD aged 20 yr or younger were recruited. Disease severity was assessed with the SCORing Atopic Dermatitis (SCORAD) index, quality of life with the Children's Dermatology Life Quality Index (CDLQI), and nocturnal scratching with a wrist motion monitor. Concentrations of plasma CCL18/PARC, serum total IgE, and eosinophil counts were measured in these patients.

Results: One hundred and eight patients with AD (mean [s.d.] age of 10.5 [4.4] yr) were recruited. The mean (s.d.) plasma concentration of CCL18/PARC was 162.2 (129.0) pg/ml, respectively. CCL18/PARC was significantly correlated with objective SCORAD ($r = 0.44$, $p < 0.001$), extent ($r = 0.45$, $p < 0.001$), intensity ($r = 0.43$, $p < 0.001$), the symptoms of pruritus ($r = 0.20$, $p = 0.04$), and sleep loss ($r = 0.19$, $p = 0.049$) but not with CDLQI or nocturnal scratching activities. CCL18/PARC levels were also correlated with eosinophil counts ($r = 0.37$, $p < 0.001$) and IgE_{log} ($r = 0.27$, $p = 0.005$). Positive correlation with SCORAD was present even in patients without bronchial hyper-reactivity.

Conclusions: Serum levels of CCL18 correlate with the clinical severity score, serum eosinophil, and IgE levels. CCL18 is associated with AD and atopy.

Atopic dermatitis (AD) is a distressing disease associated with pruritus, sleep disturbance, and impaired quality of life. Its pathophysiology is complex and involves the interplay of Th2 and Th1 lymphocytes and chemokines (1). Chemokine (C-C motif) ligand 18 (CCL18) is a small cytokine belonging to the CC chemokine family that was previously called pulmonary and activation-regulated chemokine (PARC) (2,3). CCL18 is approximately 60% identical in amino acid sequence to CCL13. It is expressed at high levels in lung and at lower levels in certain lymphoid tissues, such as the lymph nodes, and is chemotactic for activated T cells and non-activated lymphocytes (3). The gene for human CCL18 contains three exons and is located on chromosome 17 (4). CCL18 is present in skin biopsies of patients with AD but not in normal or psoriatic skin (5). The chemokine binds to CLA⁺ T cells in peripheral blood of patients with AD and healthy individuals and induces migration of AD-derived memory T cells *in vitro* and in human skin-transplanted SCID mice. In a previous study of 79 proteins using antibody array, CCL18

or PARC was found to be a significant marker of atopy in children with AD (6). Thus, the biomarker is not confined to the pulmonary system as the nomenclature of PARC might imply but is involved in atopic and cutaneous pathophysiology. We speculated that levels of CCL18 may correlate with various clinical parameters of disease severity and common laboratory markers of atopy in patients with AD regardless of the status of airway hyper-reactivity. Thus, the purpose of this study was to evaluate whether CCL18 levels were associated with disease severity, quality of life, nocturnal scratching, and laboratory markers of disease activity in AD.

Patients and methods

Patients with AD younger than 20 yr of age were recruited from the pediatric dermatology clinic of a university teaching hospital. AD was diagnosed according to criteria proposed by Hanifin and Rajka (7), and its severity and symptomatology including pruritus and sleep loss were evaluated using

SCORing Atopic Dermatitis (SCORAD) index (8,9). Quality of life of these patients with AD was measured with a validated Chinese version of the Children Dermatology Life Quality Index (CDLQI) (10). Data on bronchial challenge testing with methacholine inhalation were available in older children who managed to perform this test. Subjects were excluded if information regarding pruritus or sleep loss could not be recalled by patients and caregivers. Apart from the subjective symptoms of pruritus and sleep loss as reported by the patients in the SCORAD, we also documented the objective signs of nocturnal itch and scratching. Enrolled patients were instructed to wear the DigiTrac monitor (IM Systems, Baltimore, MD) on their dominant wrist before sleeping. In our pilot testing on patients with AD (11), the DigiTrac was found to be able to provide essential data on a wide spectrum of frequencies of wrist movements and quantity of movements in terms of acceleration or *g* values. The monitor was programmed to record limb motion between 10 PM and 8 AM the following morning. The data were then downloaded to a computer using DigiTrac 3.9 (IM Systems, Baltimore, MD, USA). Wrist activities were expressed in unit of average value of acceleration (*g*/min). We also found that wrist activities between 1 and 3 Hz for the first 3 h of sleep were good indicators of nocturnal itch as well as markers of AD severity in children (12). The average activities over the first 3 h of sleep ($\bar{A}$) were used for the analyses of correlations.

Peripheral venous blood was collected from patients with AD, centrifuged at 4°C and 2041 *g* for 10 minutes, and then stored immediately at -70°C until analysis. Plasma concentrations of CCL18 (13–15) were measured by enzyme-linked immunosorbent assays according to the manufacturer's recommendations and expressed in picograms per milliliter (R&D Systems, Abingdon, UK). The level of sensitivity was 7.82 pg/ml. Serum concentration of total IgE was log-transformed before analysis. Eosinophil counts were measured by Coulter STKS counter (Beckman-Coulter, Miami, FL, USA). Informed written consent was obtained from parents, and Clinical Research Ethics Committee of our University approved this study.

Data were expressed as proportion or mean and standard deviation (s.d.) unless otherwise stated. Chi-square was used to compare proportions, and Student *t*-test or ANOVA was used to analyze numerical variables. The correlations between different SCORAD components, inflammatory markers, and DigiTrac data were analyzed by Pearson correlation coefficients. Distribution of plasma CCL18 concentrations in patients with different degrees of AD severity was displayed in a box plot. All comparisons were made two-tailed, and *p*-values < 0.05 were considered to be statistically significant.

Results

One hundred and eight patients with AD, with mean (s.d.) age being 10.5 (4.4) yr, were recruited. Their mean (s.d.) SCORAD and CDLQI were 48.7 (20.8) and CDLQI 8.7 (5.4), respectively. The mean (s.d.) plasma CCL18 concentration was 162.2 (129.0) pg/ml. Plasma CCL18 showed significant correlation with objective SCORAD ($r = 0.440$,

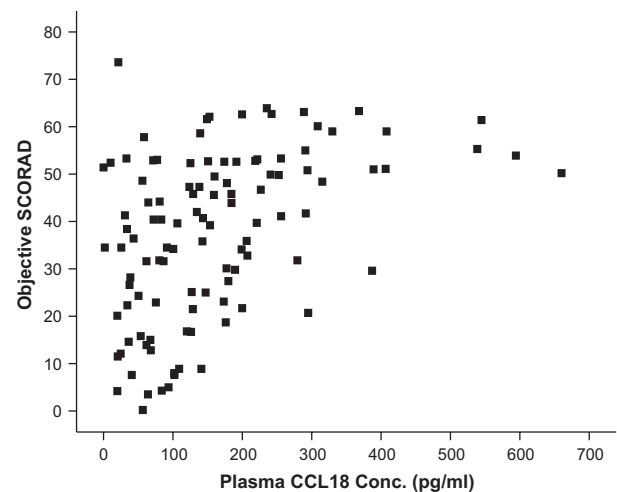


Figure 1 Scatter plot of objective SCORing Atopic Dermatitis against plasma CCL18 concentrations in our patients ($r = 0.424$, $p < 0.001$).

$p < 0.001$; Fig. 1), the symptoms of pruritus ($r = 0.201$, $p = 0.040$), and sleep loss ($r = 0.193$, $p = 0.049$) but not with CDLQI or average nocturnal wrist scratching activity (Table 1). Plasma CCL18 levels were also correlated with eosinophil percentage ($r = 0.373$, $p < 0.001$) and IgE ($r = 0.267$, $p = 0.005$). When patients with AD were classified by their objective SCORAD, plasma CCL18 concentrations showed a significant and positive trend with increasing disease severity. The respective mean (s.d.) levels in pg/ml among patients with mild ($n = 15$), moderate ($n = 38$), and

Table 1 Pearson correlation coefficients between clinical parameters and laboratory markers in patients with atopic dermatitis ($n = 108$)

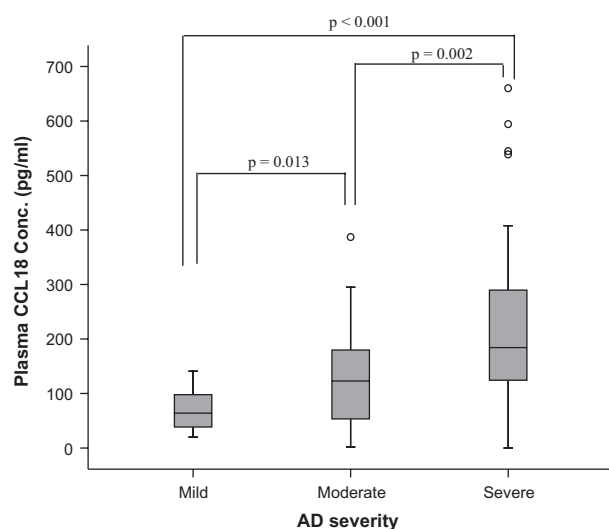
Clinical parameters	Plasma CCL18	<i>p</i>
Age	-0.040	0.680
Total SCORing Atopic Dermatitis (SCORAD) index	0.424	<0.001
Objective SCORAD	0.440	<0.001
SCORAD components		
Extent	0.449	<0.001
Intensity	0.432	<0.001
Pruritus	0.201	0.040
Sleep loss	0.193	0.049
CDLQI	0.153	0.127
NESS	0.582	<0.001
IgE _{log}	0.267	0.005
Eosinophil percentage	0.373	<0.001
Average wrist activity $\bar{A}$	0.058	0.645

IgE_{log}, log-transformed serum total IgE level
 Pearson correlation coefficients between SCORAD and CDLQI = 0.534 ($p < 0.001$); average wrist activity and CDLQI = 0.212 ($p = 0.085$).

Table 2 Plasma concentrations of CCL18 in atopic dermatitis patients with and without bronchial hyper-responsiveness (BHR)

	BHR (n = 22)	No BHR (n = 37)	p
SCORAD	54.8 ± 21.9	50.6 ± 19.5	0.466
CDLQI	8.2 ± 5.1	10.4 ± 7.1	0.229
IgE _{log}	3.66 ± 0.49	3.26 ± 0.81	0.042
Eosinophil percentage	12.6 ± 6.6	9.6 ± 5.3	0.081
CCL18/pulmonary and activation-regulated chemokine	205.5 ± 151.7	136.9 ± 111.4	0.073

Correlation between SCORing Atopic Dermatitis (SCORAD) and CCL18 is 0.302 in BCT-negative subjects ($p = 0.070$) and 0.371 in BCT-positive subjects ($p = 0.089$).

**Figure 2** Distribution of plasma CCL18 concentrations in patients with different degree of atopic dermatitis severity ($p < 0.001$ for trend; data are in parametric distribution).

severe ($n = 55$) AD were 68.3 (36.5), 127.5 (85.7), and 211.9 (147.6) ($p < 0.001$ for trend; Fig. 2).

Results of methacholine bronchial challenges were available in 59 of our patients (Table 2). The correlation between CCL18 and objective SCORAD was not significant in patients with ($n = 22$) or without ($n = 37$) bronchial hyper-responsiveness.

Discussion

This study demonstrates that CCL18 appears to be an important CC chemokine that correlates with the extent, intensity, pruritus, and sleep disturbance of patients with AD. Extent and intensity represent the objective signs as observed by the

investigator, whereas pruritus and sleep disturbance are the symptoms experienced by the patients. It is apparent that better correlations exist between CCL18 and the objective signs than the subjective symptoms. In addition, serum level also correlates with two markers of allergy, namely eosinophil counts and IgE levels. The role of CCL18 in skin disease is rarely studied. CCL18 is a chemokine, with unknown receptor counterpart, frequently associated with inflammatory Th2-type responses (16). The complex pathophysiology of AD involves the interplay of Th2 and Th1 lymphocytes and chemokines (1). The production of CCL18 by antigen-presenting cells is enhanced by Th2 cytokines like IL-4 and IL-13 and suppressed by IFN- γ (17–19). Gunther et al. (5) demonstrated that CCL18 contributes to the leukocytic infiltration associated with AD and that patients with AD have elevated levels of CCL18 in serum and lesional skin, which mediates skin homing of human memory T cells. The present study demonstrates that CCL18 is definitely involved in its pathophysiology and correlates with various clinical and laboratory parameters. Nevertheless, the marker might not be involved in the itch pathway as it does not correlate with nocturnal wrist movements (12,20). Further studies are required to evaluate whether this marker correlates with various itch-associated (12,20) and Th2 pathway markers that we previously studied (14).

CCL18 has also been investigated in other non-dermatological diseases. In immune diseases, serum CCL18 levels are positively correlated with disease activity score in rheumatoid arthritis (21). Circulating levels of the chemokine CCL18 but not CXCL16 are elevated and correlate with disease activity in rheumatoid arthritis (22). Increased expression of CCL18, CCL19, and CCL17 by dendritic cells from patients with rheumatoid arthritis and regulation by Fc gamma receptors are demonstrated (23). It has also been implicated in hypersensitivity pneumonitis (24) and contact hypersensitivity (25). In pulmonary diseases, the chemokine is an indicator of pulmonary fibrotic activity in idiopathic interstitial pneumonias and systemic sclerosis (26). CCL18 level in patients with systemic sclerosis is a sensitive indicator of active pulmonary fibrosis (27). CCL18/PARC is elevated in sputum specimens from patients with asthma and may play important roles in the development of airway eosinophilic inflammation in asthma (28), although the old nomenclature of pulmonary and activation-regulated chemokine may imply the exclusive role of CCL18 in the pulmonary system. In this study, we included a bronchial hyper-responsiveness-negative group and documented positive correlation with AD severity even in patients without bronchial hyper-reactivity. Although the old nomenclature of pulmonary and activation-regulated chemokine may imply the exclusive role of CCL18 in the pulmonary system, we included a BHR-negative group in this study and documented positive correlation with AD severity even in patients without bronchial hyper-reactivity. Thus, CCL18 is associated with the atopic skin disease and its role in atopy is definitely beyond the pulmonary system.

References

1. Leung AK, Hon KL, Robson WL. Atopic dermatitis. *Adv Pediatr* 2007; **54**: 241–73.
2. Adema GJ, Hartgers F, Verstraten R, et al. A dendritic-cell-derived C-C chemokine that preferentially attracts naive T cells. *Nature* 1997; **387**: 713–7.
3. Hieshima K, Imai T, Baba M, et al. A novel human CC chemokine PARC that is most homologous to macrophage-inflammatory protein-1 alpha/LD78 alpha and chemotactic for T lymphocytes, but not for monocytes. *J Immunol* 1997; **159**: 1140–9.
4. Tasaki Y, Fukuda S, Iio M, et al. Chemokine PARC gene (SCYA18) generated by fusion of two MIP-1alpha/LD78alpha-like genes. *Genomics* 1999; **55**: 353–7.
5. 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**: 1723–8.
6. Hon KL, Ching GK, Wong KY, Leung TF, Leung AK. A pilot study to explore the usefulness of antibody array in childhood atopic dermatitis. *J Natl Med Assoc* 2008; **100**: 500–4.
7. Hanifin JM, Rajka G. Diagnostic features of atopic dermatitis. *Acta Derm Venereol (Stockh)* 1980; **2**: 44–7.
8. Severity scoring of atopic dermatitis: the SCORAD index. Consensus Report of the European Task Force on Atopic Dermatitis. *Dermatology* 1993; **186**: 23–31.
9. 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**: 10–9.
10. Hon KL, Kim WY, Lam A, et al. CDLQI, SCORAD and NESS: are they Correlated? *Qual Life Res* 2006; **15**: 1551–8.
11. Hon KL, Lam MC, Kam WY, et al. Non-invasive home-based assessment of itch in children with atopic dermatitis. *Br J Dermatol* 2005; **153** (Suppl. 1): 89.
12. Hon KL, Lam MC, Leung TF, et al. Nocturnal wrist movements are correlated with objective clinical scores and plasma chemokine levels in children with atopic dermatitis. *Br J Dermatol* 2006; **154**: 629–35.
13. Leung TF, Ma KC, Hon KL, Lam CW, et al. Serum concentration of macrophage-derived chemokine may be a useful inflammatory marker for assessing severity of atopic dermatitis in infants and young children. *Pediatr Allergy Immunol* 2003; **14**: 296–301.
14. Hon KL, Leung TF, Ma KC, Li A, Wong YF. Serum levels of cutaneous T-cell attracting chemokine (CTACK) as a laboratory marker of the severity of atopic dermatitis in children. *Clin Exp Dermatol* 2004; **29**: 293–6.
15. Hon KL, Leung T, Ma KC, Li A, Wong Y, et al. Urinary leukotriene E4 correlates with severity of atopic dermatitis in children. *Clin Exp Dermatol* 2004; **29**: 277–81.
16. Gunther C, Kopp T, Carballido JM, Pfeiffer C. CCL18 is expressed in patients with bullous pemphigoid and parallels disease course. *Br J Dermatol* 2009; **160**: 747–55.
17. Kodolija V, Muller C, Politz O, Hakij N, Orfanos CE, Goerdt S. Alternative macrophage activation-associated CC-chemokine-1, a novel structural homologue of macrophage inflammatory protein-1 alpha with a Th2-associated expression pattern. *J Immunol* 1998; **160**: 1411–8.
18. Vulcano M, Struyf S, Scapini P, et al. Unique regulation of CCL18 production by maturing dendritic cells. *J Immunol* 2003; **170**: 3843–9.
19. de Nadai P. Involvement of CCL18 in Inflammatory Allergic Diseases. *J Allergy Clin Immunol* 2004; **113**: S262.
20. Hon KL, Lam MC, Wong KY, Leung TF, Ng PC. Pathophysiology of nocturnal scratching in childhood atopic dermatitis: the role of brain-derived neurotrophic factor and substance P. *Br J Dermatol* 2007; **157**: 922–5.
21. Serum CCL18 levels are positively correlated with disease activity score in RA. *Nat Clin Pract Rheumatol* 2008; **4**: 10–1.
22. van Lieshout AWT. Circulating levels of the chemokine CCL18 but not CXCL16 are elevated and correlate with disease activity in rheumatoid arthritis. *Ann Rheumatic Dis* 2007; **66**: 1334–8.
23. Radstake TRDJ. Increased expression of CCL18, CCL19, and CCL17 by dendritic cells from patients with rheumatoid arthritis, and regulation by Fc gamma receptors. *Ann Rheum Dis* 2005; **64**: 359–67.
24. Pardo A, Smith KM, Abrams J, et al. CCL18/DC-CK-1/PARC up-regulation in hypersensitivity pneumonitis. *J Leukoc Biol* 2001; **70**: 610–6.
25. Goebeler M, Trautmann A, Voss A, Brocker EV, Toksoy A, Gillitzer R. Differential and sequential expression of multiple chemokines during elicitation of allergic contact hypersensitivity. *Am J Pathol* 2001; **158**: 431–40.
26. Prasse AM, Toews GB, Schafer MM. CCL18 as an Indicator of Pulmonary Fibrotic Activity in Idiopathic Interstitial Pneumonias and Systemic Sclerosis. *Arthritis Rheum* 2007; **56**: 1685–93.
27. Kodera MM. Serum Pulmonary and Activation-Regulated Chemokine/CCL18 Levels in Patients With Systemic Sclerosis: a Sensitive Indicator of Active Pulmonary Fibrosis. *Arthritis Rheum* 2005; **52**: 2889–96.
28. Kim HBM. Protein Microarray Analysis in Patients With Asthma: elevation of the Chemokine PARC/CCL18 in Sputum. *Chest* 2009; **135**: 295–302.