

Serum mucosa-associated epithelial chemokine (MEC/CCL28) in atopic dermatitis: a specific marker for severity

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Abstract

Background Mucosa-associated epithelial chemokine (MEC; CCL28) is considered to be pivotal in mediating the migration of CC chemokine receptor 3- and 10- (CCR3- and CCR10)-expressing, skin-homing, memory, cutaneous lymphocyte-associated antigen-positive (CLA⁺) T cells. CCL28 is selectively and continuously expressed by epidermal keratinocytes, but highly upregulated in inflammatory skin diseases, such as atopic dermatitis (AD).

Aim This controlled longitudinal study was designed to evaluate the expression of CCL28 in serum in childhood AD and bronchial asthma (BA), and its possible relationship to disease severity and activity.

Methods Serum CCL28 levels were measured in 36 children with AD, 23 with BA, 14 with both conditions, and 21 healthy age- and sex-matched controls. Sixteen patients in the AD group were followed up and resampled for serum CCL28 after clinical remission. Serum CCL28 levels were correlated with some AD disease activity and severity variables.

Results Serum CCL28 levels in AD, whether during flare [median, 1530 pg/mL; mean $\pm$ standard deviation (SD) = 1590.4 $\pm$ 724.3 pg/mL] or quiescence (median, 1477 pg/mL; mean $\pm$ SD = 1575.2 $\pm$ 522.1 pg/mL), were significantly higher than those in healthy children (median, 301 pg/mL; mean $\pm$ SD = 189.6 $\pm$ 92.8 pg/mL); however, the levels during flare and quiescence were statistically comparable. The serum levels in BA (median, 340 pg/mL; mean $\pm$ SD = 201.6 $\pm$ 109.5 pg/mL) were significantly lower than those in the AD group, and comparable with those in healthy controls. Serum CCL28 levels in severe AD were significantly higher than those in mild and moderate cases, and correlated positively with the calculated severity scores [Leicester Sign Score (LSS) and Scoring Atopic Dermatitis (SCORAD)]. CCL28 levels during the exacerbation of AD were positively correlated with the corresponding values during remission, the peripheral absolute eosinophil counts, and serum lactate dehydrogenase levels. Serum CCL28 levels were not correlated with the serum total immunoglobulin E values in AD.

Conclusions Our data reinforce the concept that CCL28 might contribute to the pathogenesis of AD, probably through the selective migration and infiltration of effector/memory T-helper-2 cells in the skin. CCL28 may also represent an objective prognostic marker for disease severity. Further studies may pave the way for CCL28 antagonism among adjuvant therapeutic strategies.

Introduction

Atopic dermatitis (AD) is a common chronic inflammatory skin disorder of unknown etiology expressed early in life, with a peak incidence in early childhood. Disease development is primarily determined by as yet unknown genetic factors, leading to the accumulation of peripheral activated T lymphocytes in the skin. It has been hypothesized that AD is associated with an unbalanced establishment of the peripheral T-lymphocyte system. Cutaneous lymphocyte-associated antigen (CLA)⁺ is a cell surface glycoprotein which has been postulated to play an important role in T-cell migration and

homing to the skin. It has been proposed that interaction between T cells and epidermal keratinocytes plays a central role in the pathogenesis of AD.²

Mucosa-associated epithelial chemokine (MEC or CCL28) is a CC chemokine that has been identified and characterized recently, and is a functional ligand for CC chemokine receptor 3 and 10 (CCR3 and CCR10). The CCL28 gene is located on chromosome 5, and the corresponding cDNA encodes a 127-amino-acid precursor protein with a putative 22-amino-acid signal sequence. The signal sequence is cleaved to generate the 105-amino-acid mature protein. Among human chemokines, CCL28 is most similar to cutaneous T-cell-attracting

chemokine (CTACK or CCL27), sharing 40% amino acid identity.³

CCL28 has been reported to chemoattract transfectants expressing the seven-transmembrane G-protein-coupled CCR3 and CCR10. Desensitization experiments have confirmed CCR10 specificity. CCR10, previously known as orphan G-protein-coupled receptor (GPR-2), has been detected on the surface of T lymphocytes, dermal microvascular endothelial cells, and dermal fibroblasts, but mRNA expression studies suggest that it may be present on additional cell types. CCL28 induces calcium mobilization in human CCR10-expressing transfectants by CCL27, indicating that these chemokines share the new receptor, CCR10.⁴

CCL28 is expressed in a wide variety of human cell types and tissues. It appears to be predominantly produced by epithelial cells. Its mRNA expression is most abundant in salivary and mammary glands, human saliva, and breast milk.⁵ Significant mRNA expression has also been observed in other tissues associated with mucosal epithelial surfaces, including the stomach, colon, rectum, nose, and trachea. Interleukin (IL)-10/– knockout mice display increased CCL28 expression in the stomach and Peyer's patches, indicating that IL-10 may be CCL28 suppressive.⁶ Studies showing CCL28 production in nasal and bronchial epithelial cell lines have suggested that it may also play a role in allergic respiratory diseases, such as bronchial asthma (BA).

To our knowledge, no clinical studies have been performed on serum CCL28 levels in allergic diseases in the pediatric age group to date. Moreover, no data are available on CCL28 levels in other biologic fluids of allergic children. Therefore, this study was performed to explore the expression of CCL28 in AD and BA, and hence to determine its role in the pathogenesis of such diseases. We aimed to identify the clinical relevance of the measurement of serum CCL28 in terms of disease activity and/or severity.

Methods

This follow-up controlled study was conducted over a period of 9 months from 1 January to 30 September 2005. All patients were enrolled at the Pediatric Allergy and Immunology Unit and Outpatient Clinic of Ain Shams University Children's Hospital, Cairo, Egypt. Informed consent was obtained from the parents or caregivers of each child before enrollment. The children were divided into the following groups.

1 Atopic dermatitis (AD). This group comprised 36 children with AD diagnosed by standardized diagnostic criteria.⁷ Their ages ranged between 8 and 120 months [mean $\pm$ standard deviation (SD) = 47 $\pm$ 22 months]. They comprised 20 boys (55.5%) and 16 girls (44.5%). The parents completed a questionnaire concerning the precipitating factor(s). A possible cause could be traced in 18 children exposed to food allergens [egg (six), milk (five), banana (four), and fish (three)]. In 18 children, the cause was

unclear. For convenience, the severity of AD was evaluated according to two scores. First, the Leicester Sign Score (LSS; range, 0–108), in which severity is scored by six clinical features (erythema, purulence, excoriation/crusting, dryness/scaling, cracking/fissuring, and lichenification) graded at six defined body sites on a scale of 0 (none) to 3 (severe).^{8,9} Second, the Scoring Atopic Dermatitis (SCORAD) index system, which was used to evaluate the severity of eczema. This index includes an evaluation of the extent, intensity, and subjective symptoms to a maximum score of 103 points.¹⁰ Children with AD were classified into three groups (mild, moderate, and severe) according to a proposal for severity grading of AD using only objective criteria (mild AD, SCORAD score < 15; moderate AD, SCORAD score = 15–40; severe AD, SCORAD score > 40).¹¹ Sixteen children (44.5%) presented with severe AD, 11 (30.5%) with moderately severe AD, and nine (25%) with mild illness. In the 16 patients with severe AD, serum CCL28 levels were measured before and after treatment with topical corticosteroids in combination with oral antihistamines. The response to treatment was scored using the Physician's Global Assessment (PGA) score. PGA is an overall assessment (scale, 0–5) of a patient's eczema, taking into consideration the quality and extent of the lesions relative to the baseline assessment [0, clear (100% improvement); 1, almost clear (90–99% improvement); 2, marked improvement (50–89% improvement); 3, modest improvement (< 50% improvement); 4, no change; 5, worse]. Twenty-two patients had AD only. The remaining 14 children had concomitant asthma (personal history and/or clinical evidence of respiratory allergy).

2 Bronchial asthma (BA). This group comprised 23 children enrolled while presenting with an acute asthmatic exacerbation. Their ages ranged between 14 and 90 months (mean $\pm$ SD = 49 $\pm$ 16 months), and they comprised 12 boys (52%) and 11 girls (48%). From the history, the asthma exacerbations were triggered by exposure to allergens (food, animal allergens, or both) in 12 children and upper respiratory tract infections in five. In six children, the triggering agent was unclear. Ten children (43.5%) presented with acute severe asthma, and the remaining 13 (56.5%) presented with acute exacerbations of mild to moderate severity based on clinical data and peak expiratory flowmetry (PEFR).^{12,13}

3 Control group. This group comprised 21 healthy children without past, current, or family history of allergic disorders. There were 12 males (57%) and nine females (43%). Their ages ranged between 12 and 120 months (mean $\pm$ SD = 77 $\pm$ 26 months). They underwent clinical examination to exclude any concomitant illness, particularly allergic disorders and parasitic infestations. Children with peripheral blood eosinophilia or elevated serum immunoglobulin E (IgE) for age were excluded.¹⁴

Study measurements

Blood sampling

Four milliliters of venous blood were collected under complete aseptic conditions from each patient or control subject, and were

divided into two equal parts. One sample was mixed with ethylenediaminetetraacetic acid (EDTA) as an anticoagulant for blood counting, and the other was left to clot for 30 min for serum CCL28 assay. After coagulation, the latter sample was centrifuged for 15 min at 1000 *g*. The separated sera were divided into three aliquots and stored at -20°C for the subsequent assay of serum CCL28, lactate dehydrogenase (LDH), and total IgE. Repeated thawing and freezing was avoided. Hemolyzed samples were excluded.

Serum CCL28/MEC assay

A human CCL28 immunoassay kit was used (Quantikine R&D Systems, Inc., Minneapolis, MN, USA). This assay employs the quantitative sandwich enzyme immunoassay technique using *Escherichia coli*-expressed recombinant human CCL28 and antibodies raised against the recombinant factor. Results obtained using natural human CCL28 showed linear curves that were parallel to the standard curves obtained using the Quantikine kit standards.

A monoclonal antibody specific for CCL28 was precoated onto a microplate. Standards and samples were pipetted into the wells and any CCL28 present was bound by the immobilized antibody. After washing away any unbound substances, a horseradish peroxidase-linked monoclonal antibody specific for CCL28 was added to the wells. Following a wash to remove any unbound antibody–enzyme reagent, a substrate solution of hydrogen peroxide–tetramethylbenzidine was added to the wells, and color developed in proportion to the amount of CCL28 bound in the initial step. The color development was stopped by 2 N sulfuric acid and the intensity of the color was measured at 450 and 540 nm. Readings at 540 nm were subtracted. For the calculation of the results, a standard curve was constructed on a log/log scale by plotting the mean absorbance for each standard on the *y*-axis against the concentration on the *x*-axis, and drawing a best-fit curve through the points on the graph. The minimum detectable dose (MDD) ranged from 0.58 to 6.05 pg/mL. The mean MDD was 2.58 pg/mL.

Serum total IgE assay

This was performed in all subjects by quantitative enzyme immunoassay (Medix Biotech, San Carlos, CA, USA). The results were expressed in IU/mL. Owing to the variability in serum total IgE levels with age in childhood, we calculated the percentage values from the reference ranges by dividing the subject's actual level by the highest normal for age multiplied by 100.¹⁴ IgE levels used in the correlations were both the measured and calculated percentage values. The serum total IgE level that exceeded the highest normal for age was considered to be elevated.

Complete blood count

A Coulter counter was used (Coulter MicroDiff 18, Fullerton, CA, USA). The differential leukocyte counts were estimated manually from the blood film and expressed as absolute count values.

Infants and children whose absolute eosinophil counts exceeded the normal reference values for age were considered to have peripheral blood eosinophilia. Blood sampling of all subjects was performed at the same time (10.00 h) daily to avoid diurnal variations in eosinophil counts.¹⁴

Serum total LDH assay

Serum total LDH (U/L) was measured only in the AD group during flare and in the healthy subjects. LDH assay was performed on an automatic chemistry analyzer (Synchron CX5 System, Beckman, Inc., Fullerton, CA, USA).

Statistical analysis

All statistical analysis of the data was carried out using SPSS (Statistical Package for the Social Sciences) version 9.02 for Windows (SPSS Inc., Chicago, IL, USA). Data were expressed as the mean, SD, median, and interquartile ranges (IQRs) (25th and 75th percentiles). Student's *t*-test was used to compare parametric data. For nonparametric comparison of serum CCL28 among different groups, the Mann–Whitney *U*-test was used. The relationship between the various numerical parameters was studied using Pearson's correlation coefficient (*r*) with graphic representation using a linear regression line; $r < 0.5$ was considered to be weak, $r = 0.5$ – 0.75 to be moderate, and $r > 0.75$ to be strong. $P < 0.05$ for all tests was considered to be significant.

Results

Serum CCL28 levels of the studied groups (Figs 1 and 2)

Serum CCL28 levels of AD patients during flare ranged between 687 and 2256 pg/mL (median, 1530 pg/mL; mean $\pm$ SD = 1590.4 ± 724.3 pg/mL). These values were statistically comparable with the corresponding values of the same patients during quiescence, which ranged from 970 to 1980 pg/mL (median, 1477 pg/mL; mean $\pm$ SD = 1575.2 ± 522.1 pg/mL) ($Z = 1.1$; $P > 0.05$). Healthy children had much lower serum CCL28 levels (range, 10–460 pg/mL; median, 301 pg/mL; mean $\pm$ SD = 189.6 ± 92.8 pg/mL) when compared with AD patients, whether during flare ($Z = 4.1$; $P < 0.0001$) or quiescence ($Z = 3.7$; $P < 0.0001$) (Fig. 1).

The serum CCL28 levels of asthmatic patients during exacerbations ranged between 153 and 620 pg/mL (median, 340 pg/mL; mean $\pm$ SD = 201.6 ± 109.5 pg/mL). These values were statistically comparable with the values for the controls ($Z = 0.4$; $P > 0.05$), but significantly lower than the values for children with AD, whether during flare ($Z = 3.1$; $P < 0.0001$) or quiescence ($Z = 2.9$; $P < 0.0001$) (Fig. 2).

Within the AD group, 14 subjects had concomitant asthma. Their serum CCL28 levels ranged from 832 to 2059 pg/mL (median, 1425 pg/mL; mean $\pm$ SD = 1601 ± 428.3 pg/mL). These values were statistically comparable with the values for the AD-only group, whether during flare ($Z = 0.7$; $P > 0.05$) or quiescence ($Z = 0.9$; $P > 0.05$), but were

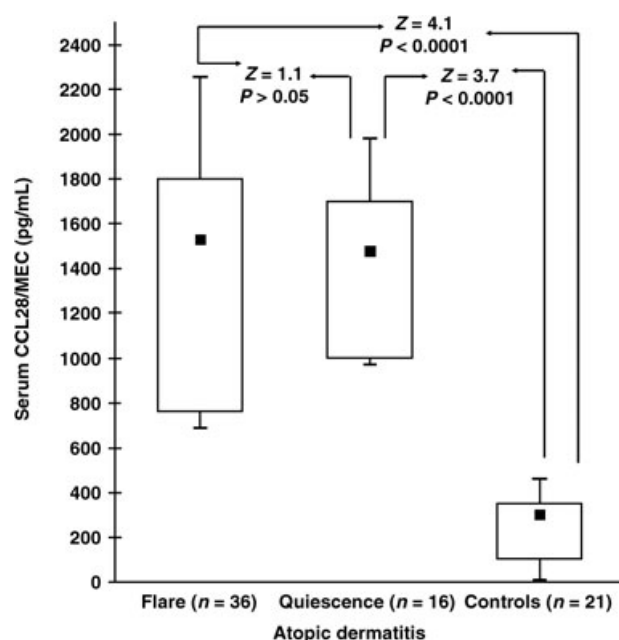


Figure 1 Box-plot summary of serum mucosa-associated epithelial chemokine (MEC/CCL28) levels (pg/mL) in atopic dermatitis (AD) and control subjects. The Mann–Whitney *U*-test was used in the comparisons. Filled squares represent the median and the boxes encompass the interquartile ranges (25th and 75th percentiles). The ranges are marked as maximum and minimum

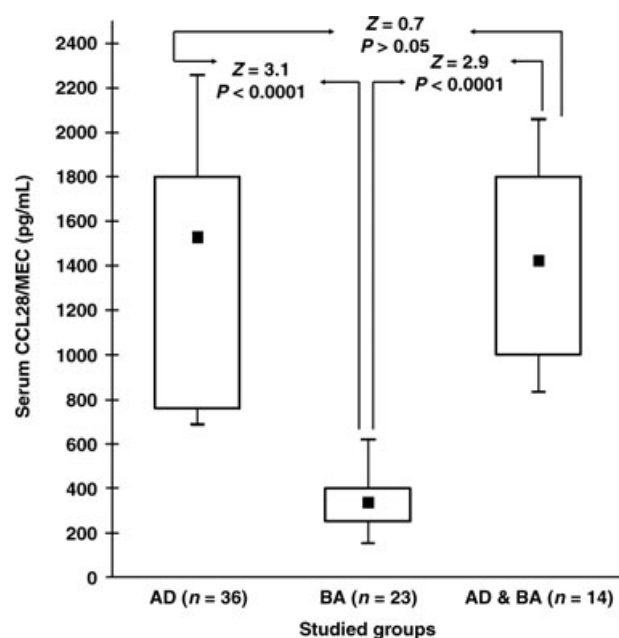


Figure 2 Box-plot summary of serum mucosa-associated epithelial chemokine (MEC/CCL28) levels (pg/mL) in the studied groups. The Mann–Whitney *U*-test was used in the comparisons

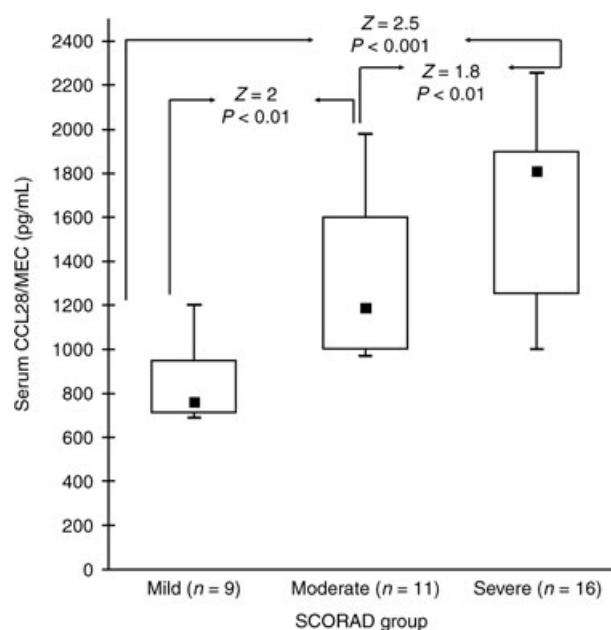


Figure 3 Box-plot summary of the variation in serum mucosa-associated epithelial chemokine (MEC/CCL28) levels (pg/mL) as a function of the Scoring Atopic Dermatitis (SCORAD) index of severity. The Mann–Whitney *U*-test was used in the comparisons

significantly higher than the values for the subjects with BA only ($Z = 2.9$; $P < 0.0001$) (Fig. 2) and the controls ($Z = 3.9$; $P < 0.0001$).

Serum CCL28 levels in relation to other clinical and laboratory variables (Figs 3–6)

In our study, the mean SCORAD scores ranged between 10 and 65 (mean $\pm$ SD = 24.8 ± 10.6). The median and mean $\pm$ SD of the serum CCL28 levels in the severe group (1809 pg/mL; 1754.8 ± 1110 pg/mL) were significantly higher than the corresponding values in the mild group (759 pg/mL; 785.1 ± 153.3 pg/mL; $Z = 2.5$; $P < 0.001$) or the moderate group (1190 pg/mL; 1165.9 ± 586.5 pg/mL; $Z = 1.8$; $P < 0.01$). Similarly, moderate cases had significantly higher values than mild cases (Fig. 3). Moreover, serum CCL28 levels correlated positively with the LSS and SCORAD scores ($r = 0.89$ and $r = 0.70$, respectively; $P < 0.001$ for both) (Fig. 4), indicating CCL28 overexpression with increased severity.

A positive correlation was found between the CCL28 serum levels during AD exacerbation and the corresponding values during remission, indicating that the higher the level became during acute attacks, the higher it remained after remission ($r = 0.91$; $P < 0.0001$).

The mean $\pm$ SD absolute eosinophil counts were statistically comparable among subjects with AD, BA, and AD associated with BA (650 ± 235 , 590 ± 197 , and 660 ± 301 cells/mm³, respectively); however, each value was significantly

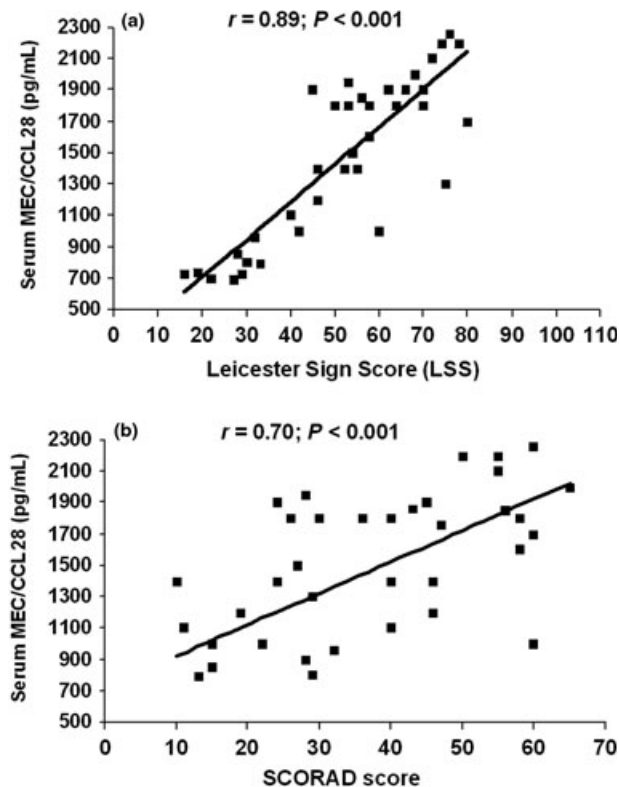


Figure 4 Serum mucosa-associated epithelial chemokine (MEC/CCL28) levels (pg/mL) as a function of the atopic dermatitis severity scores: (a) the Leicester Sign Score (LSS); (b) the Scoring Atopic Dermatitis (SCORAD) index

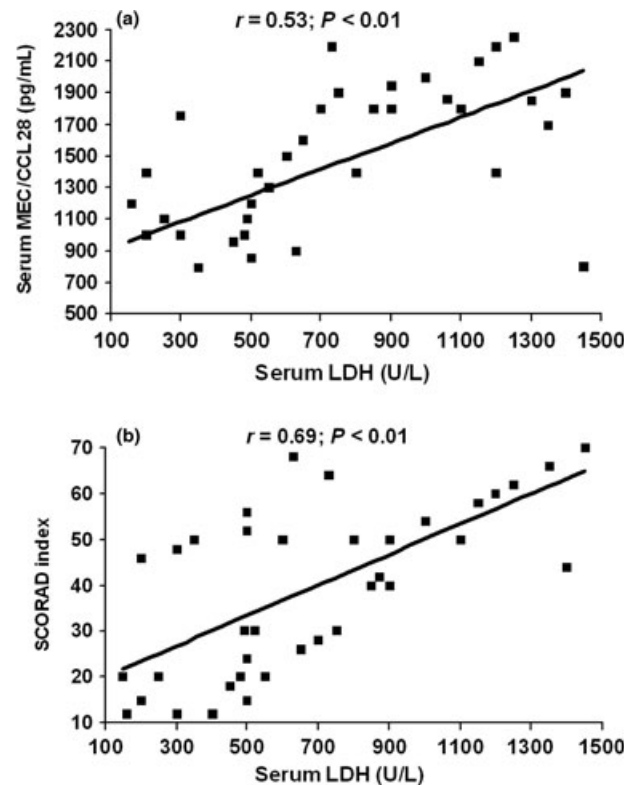


Figure 6 Positive correlations between serum total lactate dehydrogenase (LDH) (U/L) and serum CCL28 (pg/mL) levels (a) and the Scoring Atopic Dermatitis (SCORAD) index (b) in atopic dermatitis during flare

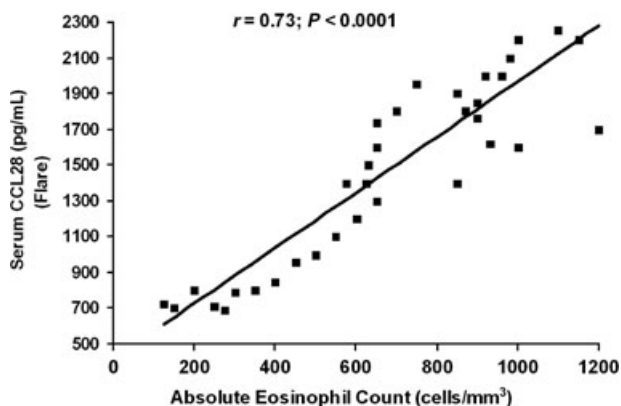


Figure 5 Significant positive correlation between serum mucosa-associated epithelial chemokine (MEC/CCL28) levels (pg/mL) and the absolute eosinophil count (cells/mm³) in the atopic dermatitis group during flare

higher when compared with the control group (220 ± 90 cells/mm³; $P < 0.01$ for all groups). Serum CCL28 levels correlated positively with the absolute eosinophil count in all groups during flare [$r = 0.73$, $P < 0.0001$ in AD (Fig. 5);

$r = 0.42$, $P < 0.01$ in BA; and $r = 0.65$, $P < 0.01$ in AD associated with BA]. A similar correlation was found in the AD group during quiescence ($r = 0.87$; $P < 0.01$). Another positive correlation was found between the SCORAD score and the absolute eosinophil count in the AD group ($r = 0.46$, $P < 0.05$).

The studied patient groups (AD, BA, and AD associated with BA) were statistically comparable in terms of serum IgE levels (500.6 ± 385.6 , 655 ± 492.5 , and 627.9 ± 336.6 IU/mL, respectively), and each mean value was significantly higher than that of the healthy controls (140.6 ± 19.7 IU/mL; $P < 0.001$). Serum CCL28 values and SCORAD scores could not be related by correlation coefficient to IgE concentrations or IgE percentage values from normal in the studied groups.

Serum total LDH levels of AD patients during flare ranged between 130 and 1450 U/L (mean $\pm$ SD = 1036.6 ± 486.2 U/L). These values were significantly higher than the control values (range, 40–110 U/L; mean $\pm$ SD = 70 ± 25 U/L; $t = 1.2$; $P < 0.05$). LDH values correlated positively with each of the serum CCL28 levels and the SCORAD scores (Fig. 6).

Serum CCL28 levels could not be correlated with age, weight centiles, or height centiles of subjects in each patient group. CCL28 expression was not influenced by age, gender,

or family history. In the asthma group, CCL28 levels did not vary according to the PEFR values of the patients.

Discussion

MEC, or CCL28, has been implicated in the homing of lymphocytes to inflammatory sites. It has been assumed to play an important role in T-cell migration to skin in adult AD, psoriasis vulgaris, and bullous pemphigoid;¹⁵ however, the expression of CCL28 in both normal children and children with atopic diseases has not been studied extensively. To evaluate a possible role for CCL28 estimation in pediatric allergy, we measured serum CCL28 levels in phenotypically well-defined groups of allergic children (AD, BA, and AD associated with BA) in comparison with a group of healthy, nonatopic, age- and sex-matched control subjects.

We observed that serum CCL28 levels were specifically elevated in patients with AD, irrespective of allergic respiratory comorbidity. The study revealed that the levels in AD, whether during flare or exacerbation, were significantly higher than those of control (Fig. 1) or asthmatic (Fig. 2) groups. Our results, which conform with those of Kagami *et al.*,¹⁵ probably reflect the upregulation of CCL28 in AD, presenting a potentially useful marker for the presence of an atopic reaction. CCL28 regulation by allergen exposure and microbial products suggests an important role for CCL28 in the initiation and amplification of the atopic skin response. The failure to demonstrate a significant reduction in serum CCL28 levels after remission of flare (Fig. 1) could be a result of the ongoing process of subclinical allergic cutaneous inflammation. Moreover, it could be indicative of the need for more intensive nonconventional anti-inflammatory therapy. Therefore, we suggest that serum CCL28 levels could be used as a marker of the ongoing inflammatory activity in the skin.

Among human chemokines, CTACK (CCL27) is most similar to MEC (or CCL28), sharing 40% amino acid identity.³ Like CCL28, CCL27 is also known to recruit CLA⁺ memory T-helper-2 (Th2) lymphocytes.¹⁶ Dose-dependent chemotaxis of identical lymphocyte populations in response to CCL28 is nearly indistinguishable from that exhibited by CCL27, except that CCL28 has a higher maximal activity.^{17,18} CCL27 has been found to be expressed at high levels in the epidermis of lesional skin biopsy specimens from patients with AD compared with nonlesional skin.^{19,20} Owing to the tissue-specific expression profile and receptor specificity of both chemokines (CCL27 and CCL28), a definite role of CCL28 in AD is strongly suggested.

CCL28 is expressed in a variety of human tissues, and appears to be predominantly produced by epithelial cells.⁵ It is strongly expressed constitutively in lesional epidermal keratinocytes of patients with AD and psoriasis vulgaris.¹⁵ *In vitro*, CCL28 displays chemotactic activity for resting CD4⁺ CD8⁺ T cells.⁴ These cells can be detected in significant

proportions in the peripheral blood of children with AD.¹ This may suggest a faulty maturation of the T-lymphocyte system as a basic pathophysiologic change in AD, leading to skin inflammation with CD4⁺ CD8⁺ T lymphocytes resembling immature T cells.²¹ This is likely to lead to skewing of many immune reactions in these patients.

CCL28 is also displayed by cutaneous venules, and is strongly expressed on endothelial cells. It is thought to trigger vascular arrest of circulating skin-homing memory T cells, which uniformly express CCR10, particularly CLA⁺CCR10⁺ memory T cells. Chronic cutaneous inflammation induces CD4⁺ T cells expressing E-selectin-binding activity in draining lymph nodes. CLA is a ligand for E-selectin, an adhesion molecule, which is critical in the recruitment of T cells. These E-selectin ligand⁺ T cells migrate efficiently to CCL28 and CCL27.^{22,23}

This could reinforce the hypothesis that CCL28 and CCL27 may work in a sequential way, with CCL28 acting mainly in the first steps of T-cell recruitment by inducing integrin-dependent adhesion and transendothelial migration of T cells, and CCL27 playing a major role in the migration of T cells into the upper layers of the skin. Finally, T cells infiltrating the epidermis may result in the induction of apoptosis in keratinocytes, resulting in spongiosis, the histologic hallmark of eczema.

To test the specificity of the CCL28 response in AD, we sought to investigate its expression in a group of asthmatic subjects during acute exacerbations. The serum CCL28 levels of asthmatic patients were statistically comparable with those of the controls, but were significantly lower than the values of children with AD, whether during flare or quiescence. The normal values of serum CCL28 in our asthmatic patients indicate that the results of studies using bronchial cell lines and mouse models^{24,25} cannot simply be extrapolated to human studies *in vivo*. It is also possible that the local production of CCL28 in mucosal epithelial cells may be insufficient to lead to a systemic increase.

In children presenting with concomitant cutaneous allergic and respiratory diseases (BA and AD), the overexpression of CCL28 can be ascribed to the presence of AD, providing further evidence for the implication of CCL28 in the atopic cutaneous response. Our data may thus reflect the upregulation of CCL28 in AD specifically; however, our findings are still limited by the sample size.

Serum CCL28 levels in subjects categorized with severe AD were significantly higher than those in the moderate and mild groups, with a positive correlation to LSS and SCORAD scores (Figs 3 and 4). This substantiates the usefulness of this parameter as a skin-specific objective marker of disease severity in AD.

A large variety of laboratory measurements^{26,27} have been linked to disease activity and severity in AD; however, most lack specificity.

The high levels of IgE in AD have not been explained satisfactorily, but can be ascribed to a deficiency of IgE isotype-specific "suppressor" T-cell function. It has not been established whether AD is exclusively an IgE-mediated allergic disorder, and it is difficult to demonstrate consistently a role for allergens in the pathogenesis. Although patients with AD frequently possess IgE antibody specific for inhalants or food allergens, it is not generally possible to induce skin lesions or AD by intradermal injection of the suspected allergen.^{28,29} Moreover, typical lesions of AD may occur in individuals with X-linked agammaglobulinemia, who have virtually no IgE.⁷

The serum CCL28 levels and SCORAD scores in the current study could not be correlated with the serum total IgE concentrations or percentage values from normal during AD flare. Our results are not concordant with some reports.^{27,28} The discrepancy can be ascribed to the small number of cases with elevated IgE (six children only). We also recruited subjects at different stages of disease activity, and serum total IgE levels are known to fluctuate throughout the stages of disease.

In contrast, the serum CCL28 concentrations and SCORAD scores correlated positively with the absolute eosinophil counts during both AD flare and quiescence. Previous studies have identified eosinophil chemoattractant properties of CCL28 using human cells.^{4,24} It is unclear whether CCL28 may have a role in regulating the constitutive numbers of eosinophils in peripheral blood by inducing eosinophil chemotaxis in a CCR3- and CCR10-dependent manner. Animal studies have confirmed that CCL28 is a chemoattractant for murine eosinophils. There appears to be a temporal relationship of CCL28 production with eotaxin and other CCR3 ligands in animal models.²⁴ Perhaps they play an important role in the localization of eosinophils around inflammatory sites. Interestingly, anti-CCL28-treated animals also showed reduced eosinophil accumulation.³⁰ Therefore, it could be concluded that CCL28, a CCR3 ligand, is important for various stages of eosinophil migration and/or activation within tissues. In the future, it might be possible to target tissue eosinophilia in severe allergic diseases by the manipulation of CCL28-CCR3 interactions.

The serum total LDH levels of our AD patients during flare were significantly higher than those of controls. Moreover, significant positive correlations were found between serum LDH levels during flare and serum CCL28 concentrations and SCORAD scores (Fig. 6). Elevated levels of LDH can be attributed to its release from damaged lesional epidermal cells into peripheral blood, reflecting the extent of skin involvement and the degree of epidermal injury. LDH has been reported previously as a useful, but nonspecific, marker for the severity of skin eruption, being elevated in many other diseases.^{31,32}

In conclusion, it seems that serum CCL28 expression is upregulated in children with AD, irrespective of allergic

respiratory comorbidity. It may be an important chemokine in lesion formation in AD, and may have a distinct role in the development of atopy/allergy skin phenotypes. It could be a useful specific inflammatory marker for assessing AD severity in children. Further research using immunohistochemical staining of CCL28 in lesional AD skin and other cutaneous hypersensitivities is warranted, as well as studies addressing the relationship between its expression and the conventional lines of treatment used. A potential role of CCL28 in respiratory allergy deserves further evaluation on a wider scale. Moreover, the effect of CCL28 antagonists and other immunotherapeutics, such as monoclonal antibodies, on CCL28 kinetics is worthy of evaluation. CCL28 antagonism may provide a conceptual basis for the development of new therapeutic strategies in severe AD.

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