

## Original article

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

**Background:** Mucosa-associated epithelial chemokine (MEC; CCL28) is considered pivotal in mediating migration of CCR3 and CCR10-expressing skin-homing memory CLA<sup>+</sup> T cells. CCL28 is selectively and continuously expressed by epidermal keratinocytes, but highly upregulated in inflammatory skin diseases such as atopic dermatitis (AD).

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

**Methods:** Serum CCL28 levels were measured in 36 children with AD, 23 with BA, and 14 who had both conditions as well as in 21 healthy age and sex-matched subjects serving as controls. Sixteen patients in the AD group were followed up and re-sampled 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; mean  $\pm$  SD = 1590.4  $\pm$  724.3 pg/ml) or quiescence (median = 1477, mean  $\pm$  SD = 1575.2  $\pm$  522.1 pg/ml) were significantly higher than the healthy children values (median = 301; 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; mean  $\pm$  SD = 201.6  $\pm$  109.5 pg/ml) were significantly lower than the AD group and comparable to the healthy control values. Serum CCL28 levels in severe AD were significantly higher as compared to mild and moderate cases and correlated positively to the calculated severity scores (LSS and SCORAD). CCL28 levels during exacerbation of AD could be positively correlated to the corresponding values during remission, the peripheral absolute eosinophil counts and serum lactate dehydrogenase levels. Serum CCL28 did not vary with the serum total IgE values in AD.

**Conclusion:** Our data reinforce the concept that CCL28 might share in the pathogenesis of AD probably through selective migration and infiltration of effector/memory Th2 cells into the skin. It may also represent an objective prognostic marker for disease severity. Further studies may pave way for CCL28 antagonism among the adjuvant therapeutic strategies.

**Key words:** Mucosa-associated epithelial chemokine, CCL28, allergic diseases, atopic dermatitis, bronchial asthma

**Mohamed H. Ezzat,  
Karim Y. Shaheen\*.**

From the Departments  
of Pediatrics and  
Clinical Pathology\*,  
Faculty of Medicine,  
Ain Shams University,  
Cairo, Egypt.

### Correspondence:

Mohamed Hesham  
Ezzat  
25 El-Sebak Street  
Cairo 11451, Egypt  
E-mail: ezzatmhm  
@hotmail.com

## INTRODUCTION

Atopic dermatitis (AD) is a common chronic inflammatory skin disorder of unknown etiology expressed early in life with 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 was hypothesized that AD is associated with an unbalanced establishment of the peripheral T-lymphocyte system. Cutaneous lymphocyte-associated antigen (CLA)<sup>1</sup> 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<sup>2</sup>.

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

similar to cutaneous T cell-attracting chemokine (CTACK or CCL27), sharing 40% AA identity.<sup>3</sup>

CCL28 has been reported to chemoattract transfectants expressing the seven-transmembrane G-protein coupled CCR3 & CCR10. Desensitization experiments had 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.<sup>4</sup>

CCL28 is expressed in a variety of human cell types and tissues but 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.<sup>5</sup> Significant mRNA expression has also been observed in other tissues associated with mucosal epithelial surfaces, including stomach, colon, rectum, nose and trachea. IL-10 *-/-* knock-out mice display increased CCL28 expression in the stomach and Peyer's patches, indicating that IL-10 may be CCL28 suppressive.<sup>6</sup> Studies showing CCL28 production in nasal and bronchial epithelial cell lines have suggested that it might also play a role in allergic respiratory diseases such as bronchial asthma (BA).

To our knowledge, there are no clinical studies on serum CCL28 levels in allergic diseases in the pediatric age groups to date. Also, no data on CCL28 levels in other biological fluids of allergic children could be cited in literature. Therefore, this study was done to explore the expression of CCL28 in AD and BA and hence its pathogenic role in such diseases and to identify the clinical relevance of measuring 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 the first of January to the end of September 2005. All patients were enrolled from the Pediatric Allergy and Immunology Unit and outpatient clinic of Ain Shams University Children's Hospital, Cairo. An informed consent was obtained from the parents or caregivers of each child before enrollment. Children were divided into the following groups:

**Atopic Dermatitis (AD):** This group comprised 36 children with AD diagnosed by standardized diagnostic criteria<sup>7</sup>. Their ages ranged between 8

and 120 months (mean  $\pm$  SD = 47  $\pm$  22 months). They comprised 20 boys (55.5%) and 16 (44.5%) girls. The parents were subjected to a questionnaire concerning the precipitating factor(s). A possible cause could be traced in 18 children being exposed to food allergens [egg (6), milk (5), banana (4), and fish (3)]. In 18 children, the cause was not clear. For convenience, the severity of AD was evaluated according to two scores. First, the Leicester Sign Score (LSS; range, 0 to 108), in which severity is scored by 6 clinical features (erythema, purulence, excoriation/crusting, dryness/scaling, cracking/fissuring, and lichenification) graded at 6 defined body sites on a scale of 0 (none) to 3 (severe).<sup>8, 9</sup> Second, the Scoring Atopic Dermatitis (SCORAD) index system that was used to evaluate the severity of eczema. This index includes evaluation of extent, intensity, and subjective symptoms to a maximum score of 103 points.<sup>10</sup> AD children were classified into 3 groups (mild, moderate, and severe) according to a proposal for severity grading of AD by using only objective criteria (mild AD, SCORAD score < 15; moderate AD, score = 15-40; and severe AD, score > 40).<sup>11</sup> Sixteen children (44.5%) presented with severe AD, 11 (30.5%) with moderate severity, and 9 (25%) were categorized as having 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 anti-histamines. The response to treatment was scored by using the Physician's Global Assessment (PGA) score. The PGA is an overall assessment from 0 to 5 of a patient's eczema, taking into consideration the quality and extent of lesions relative to the baseline assessment (0 = clear [100%], 1 = almost clear [90% to 99% improvement], 2 = marked improvement [50% to 89%], 3 = modest improvement [ $<$ 50%], 4 = no change, and 5 = worse). Twenty-two patients had AD only. The remaining 14 children had concomitant asthma (personal history and/or clinical evidences of respiratory allergy).

**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 (48%) girls. From history, the asthma exacerbations were triggered by exposure to allergens (food, animal allergens or both) in 12 children and upper respiratory tract infections in 5 children. In 6 children, the triggering agent was not clear. 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).<sup>12, 13</sup>

**Control group:** This group comprised 21 healthy children without past, current or family history of allergic disorders. They were 12 (57%) males and 9 (43%) females. Their ages ranged between 12 and 120 months with a mean  $\pm$  SD of  $77 \pm 26$  months. They underwent clinical examination to exclude any concomitant illness particularly allergic disorders and parasitic infestations. Children with peripheral blood eosinophilia or elevated serum IgE for age were excluded.<sup>14</sup>

#### **Study measurements:**

**Blood sampling:** Four ml of venous blood were collected under complete aseptic conditions from each patient or control subject and were divided into 2 equal parts. One sample was mixed with EDTA as an anticoagulant for blood counting, and the other was left to clot for 30 minutes for serum CCL28 assay. After coagulation, the latter sample was centrifuged for 15 minutes at 1000 x g. The separated sera were divided into 3 aliquots and stored at  $-20^{\circ}\text{C}$  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:** The kit used was a human CCL28 immunoassay (*Quantikine R&D Systems, Inc. McKinley Place N.E., Minneapolis, Minnesota, MN 55413 U.S.A.*). This assay employs the quantitative sandwich enzyme immunoassay technique using E 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 has been pre-coated 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 2N sulfuric acid and the intensity of the color was measured at 450 nm and 540 nm. Readings at 540 nm were subtracted. For calculation of 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 - 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*). 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.<sup>14</sup> IgE levels used in the correlations were both the measured and the calculated percentage values. The serum total IgE level that exceeded the highest normal for age was considered elevated.

**Complete blood count:** Peripheral blood cells were counted by a Coulter counter (*Coulter MicroDiff 18, Fullerton CA, USA*). The differential leukocytic counts were estimated manually from the blood film and expressed in absolute count values. Infants and children whose absolute eosinophil counts (AEC) 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 am) daily to avoid diurnal variations in eosinophil counts.<sup>14</sup>

**Serum total lactate dehydrogenase (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, California U.S.A.*).

#### **Statistical Analysis:**

All statistical analysis of the data was carried out using SPSS (Statistical Package for the Social Science) version 9.02 for Windows system. Data were expressed as mean, standard deviation (SD), median, and interquartile ranges IQR (25<sup>th</sup> and 75<sup>th</sup> percentiles). Student's "t" test was used for comparing parametric data. For non-parametric comparison of serum CCL28 among different groups, we used Mann-Whitney U test. The relation between the various numerical parameters was studied by the Pearson correlation coefficient (r) test with graphic representation using linear regression line; r value was considered weak if  $< 0.5$ , moderate if ranged between 0.5-0.75 and strong if  $> 0.75$ . p values below 0.05 for all tests were considered significant.

## RESULTS

### *Serum CCL28 levels of the studied groups (table 1; figs 1 & 2):*

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

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

Within the group of AD, 14 subjects had concomitant asthma as well. Their serum CCL28 levels ranged from 832 to 2059 pg/ml (median = 1425, mean  $\pm$  SD = 1601  $\pm$  428.3 pg/ml). These values were statistically comparable to the values of the AD only group whether during flare ( $Z = 0.7$ ;  $p > 0.05$ ) or quiescence ( $Z = 0.9$ ;  $p > 0.05$ ), but were significantly higher when compared to the values of subjects with BA only ( $Z = 2.9$ ;  $p < 0.0001$ ) (Fig.2) and the control group ( $Z = 3.9$ ;  $p < 0.0001$ ).

### *Serum CCL28 levels in relation to other clinical and laboratory variables (figs 3-6):*

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

increased CCL28 expression with increased severity.

A positive correlation could be elicited between the CCL28 serum levels during AD exacerbation and the corresponding values during remission meaning that the higher the level got during acute attacks the higher it remained after remission ( $r = 0.91$ ;  $p < 0.0001$ ) (Fig.5).

The mean ( $\pm$ SD) absolute eosinophil count values were statistically comparable among subjects with AD, BA, and AD associated with BA (650  $\pm$  235, 590  $\pm$  197, and 660  $\pm$  301 cells/mm<sup>3</sup> respectively). However, each value was significantly higher when compared to the control group (220  $\pm$  90 cells / mm<sup>3</sup>;  $p < 0.01$  for all groups). Serum CCL28 levels correlated positively with the absolute eosinophil count in all groups during flare ( $r = 0.73$ ;  $0.42$  and  $0.65$  respectively,  $p < 0.01$ ). Similar correlation was found in the AD group during quiescence ( $r = 0.87$ ;  $p < 0.01$ ). Another positive correlation could be elicited between the SCORAD index 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  $\pm$  385.6, 655  $\pm$  492.5, and 627.9  $\pm$  336.6 IU/ml respectively) and each mean value was significantly higher than that of the healthy control subjects (140.6  $\pm$  19.7 IU/ml;  $p < 0.001$ ). Serum CCL28 or SCORAD index values could not be related by correlation coefficient to IgE concentrations or IgE percent 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  $\pm$  486.2 U/L). These values were significantly higher than the control values (range = 40-110 U/L; mean  $\pm$  SD = 70  $\pm$  25 U/L,  $t = 1.2$ ;  $p < 0.05$ ). LDH values correlated positively with each of the serum CCL28 levels and the SCORAD indices (Fig.6).

Serum CCL28 levels could not be correlated to age, weight centiles, and 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 patients.

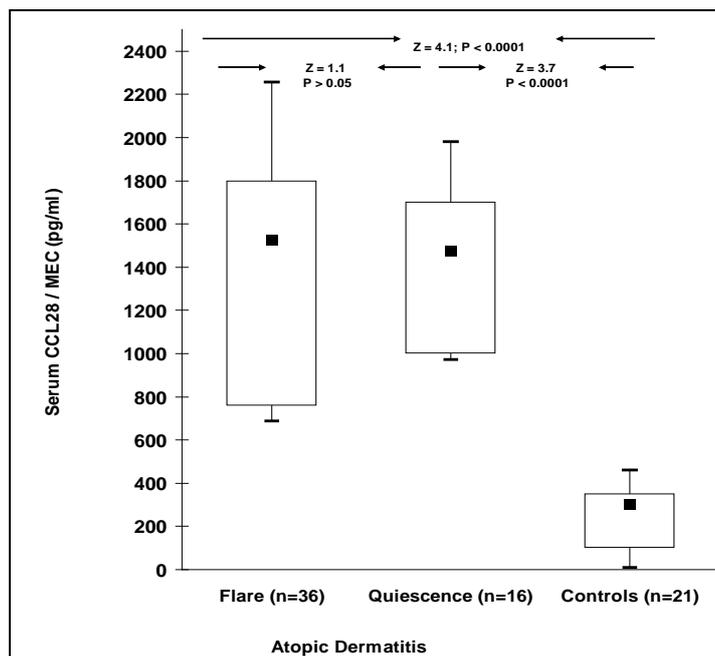
**Table 1.** Serum mucosa-associated epithelial chemokine (MEC/CCL28) in the studied groups

Serum MEC/CCL28 (pg/ml)	Atopic Dermatitis (AD)		BA (Flare)	AD + BA (Flare)	Control group
	Flare	Quiescence			
Range	687-2256	970-1980	153-620	832-2059	10-460
Median	1530	1477	340	1425	301
IQR	1032	640	240	785	220
Mean	1590.4	1575.2	201.6	1601	189.6
SD	724.3	522.1	109.5	428.3	92.8
Z1		1.1			
Z2	3.1 *	2.9 *			
Z3	0.7	0.9			
Z4			2.9 *		
Z5	4.1 *	3.7 *	0.4	3.9 *	

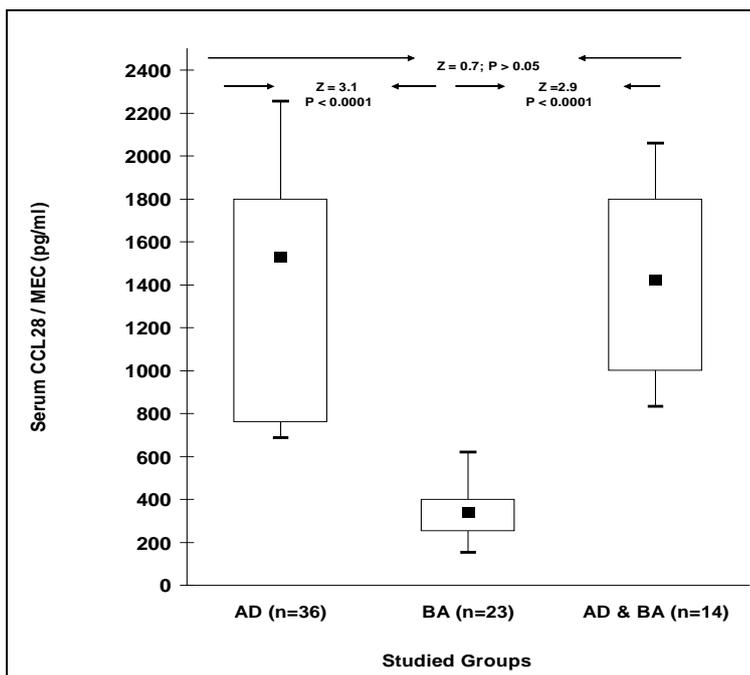
AD: Atopic dermatitis; BA: Bronchial asthma; IQR: Interquartile range; MEC: Mucosa-associated epithelial chemokine; SD: Standard deviation

Mann-Whitney test results: Z1 = AD flare versus quiescence; Z2 = AD versus BA; Z3 = AD versus (both AD & BA); Z4 = BA versus (both AD & BA); Z5 = patients versus controls

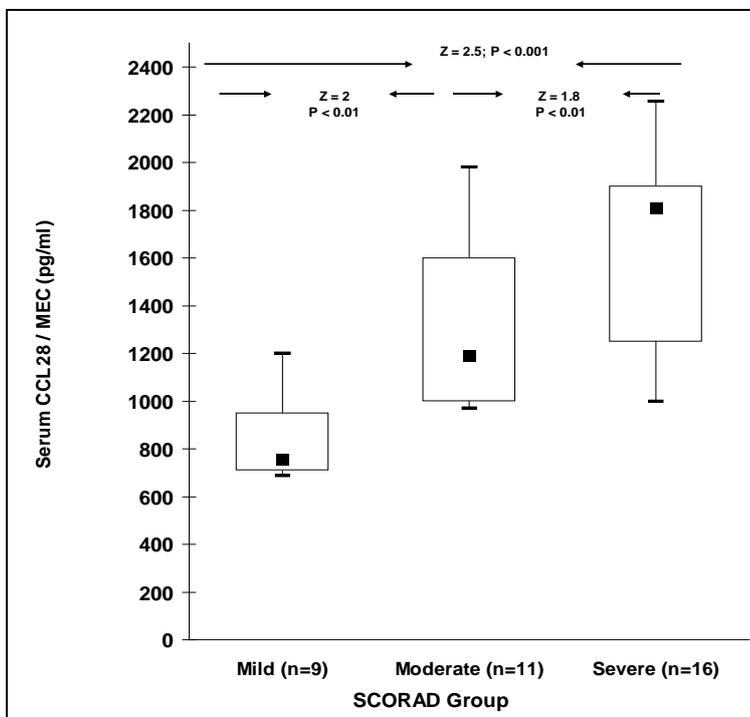
\*: Highly significant ( $p < 0.0001$ )



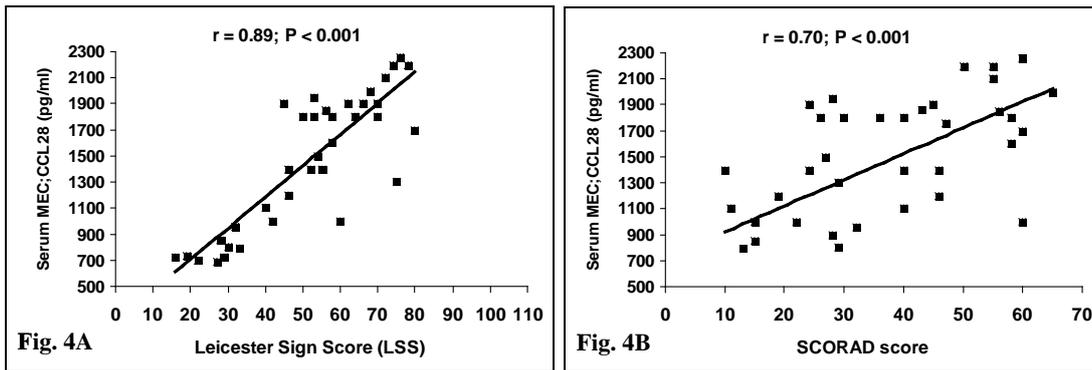
**Figure 1.** Box-plot summary of serum mucosa-associated epithelial chemokine (MEC/CCL28) levels (pg/ml) in atopic dermatitis (AD) and control subjects. Mann-Whitney test was used in the comparisons. Black squares represent the median and the boxes encompass the interquartiles (25<sup>th</sup> and 75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



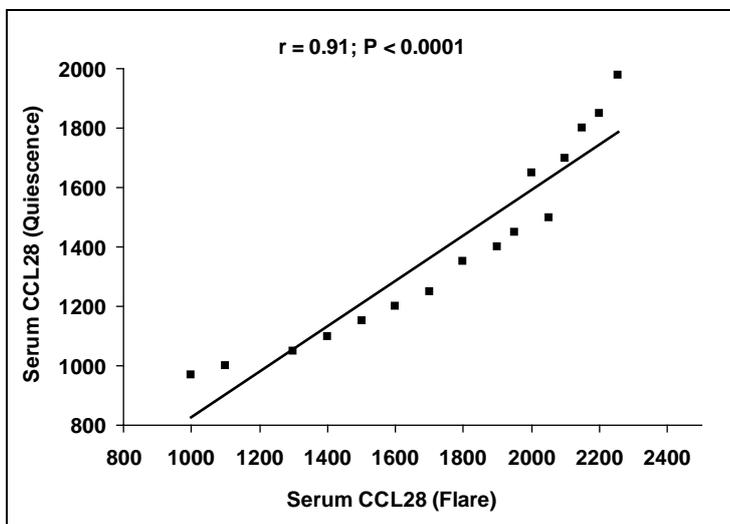
**Figure 2.** Box-plot summary of serum mucosa-associated epithelial chemokine (MEC/CCL28) levels (pg/ml) in the studied groups. Mann–Whitney test was used in the comparisons.



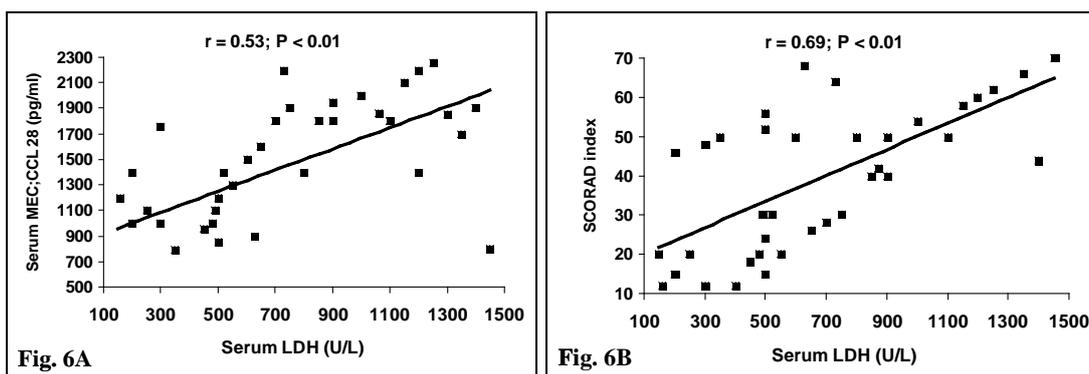
**Figure 3.** Box-plot summary of serum mucosa-associated epithelial chemokine (MEC/CCL28) levels (pg/ml) variation according to atopic dermatitis SCORAD index of severity. Mann–Whitney test was used in the comparisons.



**Figure 4.** Serum mucosa-associated epithelial chemokine (MEC/CCL28) levels (pg/ml) in relation to atopic dermatitis severity scores; the LSS (Fig. 4A), and the SCORAD index (Fig. 4B).



**Figure 5.** Positive correlation between the atopic dermatitis flare and remission levels of serum CCL28 (pg/ml).



**Figure 6.** Positive correlations between serum total lactate dehydrogenase (LDH) (U/L) and serum CCL28 (pg/ml) (Fig. 6A), and the SCORAD index (Fig. 6B), in atopic dermatitis during flare.

## DISCUSSION

MEC or CCL28 has been implicated in the homing of lymphocytes to the inflammatory sites. It was assumed to play an important role in T-cell migration to skin in adult AD, psoriasis vulgaris

and bullous pemphigoid<sup>15</sup>; however, the expression of CCL28 in both normal children and children with atopic diseases has not been extensively studied. 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 to a group of healthy non-atopic 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 the control (Fig.1) and asthmatic groups (Fig.2). Our results which conform with those of Kagami et al.<sup>15</sup> 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 significant reduction in serum CCL28 levels after remission of the flare could be due to an ongoing process of subclinical allergic cutaneous inflammation. Also, it could be indicative of the need for more intensive non-conventional anti-inflammatory therapy. Therefore, we suggest that serum CCL28 levels be used as a marker of the ongoing inflammatory activity in the skin.

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

CCL28 is expressed in a variety of human tissues, and it appears to be predominantly produced by epithelial cells.<sup>5</sup> It is strongly expressed constitutively in lesional epidermal keratinocytes of patients with AD and psoriasis vulgaris.<sup>15</sup> In vitro, CCL28 displays chemotactic activity for resting CD4<sup>+</sup> CD8<sup>+</sup> T cells.<sup>4</sup> These cells can be detected in a significant proportion in peripheral blood of AD children.<sup>1</sup> This may suggest a faulty maturation of the T-lymphocyte system as a basic pathophysiological change in AD, leading to skin inflammation with CD4<sup>+</sup> CD8<sup>+</sup> T lymphocytes resembling immature T cells.<sup>21</sup> This is likely to lead

to skewing of many immune reactions in those 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 the CCR10 particularly CLA<sup>+</sup>CCR10<sup>+</sup> memory T cells. Chronic cutaneous inflammation induces CD4<sup>+</sup> T cells expressing E-selectin binding activity in draining lymph node. CLA is a ligand for E-selectin, an adhesion molecule, which is critical in recruiting T cells. These E-selectin ligand<sup>+</sup> T cells migrate efficiently to CCL28 and to CCL27 as well.<sup>22, 23</sup> 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 CCL28 response in AD, we sought to investigate its expression in a group of asthmatic subjects during acute exacerbations. The serum CCL28 levels of the asthmatic patients were statistically comparable to the values of controls, but were significantly lower as compared to the values of children with AD whether during flare or quiescence. The normal values of serum CCL28 in our asthmatic patients indicates that the results of studies that use bronchial cell lines and mouse models<sup>24, 25</sup> 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 who presented with concomitant cutaneous and respiratory allergy (BA & AD), the over expression of CCL28 can be ascribed to the presence of AD, providing further evidence for the implication of CCL28 in atopic cutaneous responses. 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 as severe AD cases were significantly higher as compared to the moderate and mild groups with a positive correlation to the LSS and the SCORAD severity scores (Fig.3&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<sup>26, 27</sup> have been linked to disease activity and severity in

AD, however most of them lack specificity. The high levels of IgE in AD have not been satisfactorily explained but can be ascribed to a deficiency of IgE isotype-specific "suppressor" T-cell function. It is not established that 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.<sup>28, 29</sup> Moreover, typical lesions of AD may occur in individuals with X-linked agammaglobulinemia, who have virtually no IgE.<sup>7</sup>

Serum CCL28 levels and the SCORAD score results in the current study could not be correlated to the serum total IgE concentrations or % values from normal during AD flare. This was not concordant with some reports<sup>27, 28</sup>. The discrepancy can be ascribed to the small number of cases with elevated IgE (6 children only). We also recruited subjects at different stages of disease activity and serum total IgE levels might fluctuate throughout the disease stages.

On the other hand, serum CCL28 concentrations and the SCORAD indices correlated positively with the absolute eosinophil counts both during AD flare and quiescence. Previous studies had identified eosinophil chemoattractant properties of CCL28 using human cells.<sup>4, 24</sup> It is not clear 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 confirmed that CCL28 was a chemoattractant for murine eosinophils. There appears to be a temporal relationship of CCL28 production with eotaxin and other CCR3 ligands in animal models.<sup>24</sup> Perhaps they play an important role for the localization of eosinophils around the inflammatory sites. Interestingly, anti-CCL28-treated animals showed also reduced eosinophil accumulation.<sup>30</sup> Therefore, CCL28, a CCR3 ligand, may be important for various stages of eosinophil migration and/or activation within tissues. It might be possible one day to target tissue eosinophilia in severe allergic diseases by manipulating CCL28/CCR3 interactions.

Serum total LDH levels of our AD patients during flare were significantly higher as compared to controls. Moreover, significant positive correlations could be elicited between serum LDH levels during flare and serum CCL28 concentrations and SCORAD indices (Fig.6).

Elevated levels 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 was previously reported as a useful yet non specific marker for severity of skin eruption being elevated in many other diseases.<sup>31, 32</sup>

In conclusion, it seems that serum CCL28 expression is up regulated in children with AD irrespective of allergic respiratory comorbidity. It might be one of the important chemokines in the lesional formation of AD and might 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 the lesional AD skin and other cutaneous hypersensitivities are warranted as well as studies addressing the relations 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, like monoclonal antibodies on the CCL28 kinetics is worth evaluation. CCL28 antagonism may provide a conceptual basis for the development of new therapeutic strategies in severe AD.

## REFERENCES

1. **OLESEN AB, ANDERSEN G, JEPPESEN DL, BENN CS, JUUL S, THESTRUP-PEDERSEN K.** Thymus is enlarged in children with current atopic dermatitis. A cross-sectional study. *Acta Derm Venereol* 2005; 85(3): 240-3.
2. **VESTERGAARD G, JOHANSEN G, OTKJAER K, DELEURAN M, IVERSEN L.** Tumor necrosis factor-alpha-induced CTACK/CCL27 (cutaneous T-cell-attracting chemokine) production in keratinocytes is controlled by nuclear factor kappaB. *Cytokine* 2005; 29(2): 49-55.
3. **HIESHIMA K, OHTANI H, SHIBANO M, IZAWA D, NAKAYAMA T, KAWABAKI Y, ET AL.** CCL28 has dual roles in mucosal immunity as a chemokine with broad-spectrum antimicrobial activity. *J Immunol* 2003; 170: 1452-61.
4. **WANG W, SOTO H, OLDHAM ER, BUCHANAN ME, HOMEY B, CATRON D, ET AL.** Identification of a novel chemokine (CCL28), which binds CCR10 (GPR2). *J Biol Chem* 2000; 275(29): 22313-23.

5. **WILSON E, BUTCHER EC.** CCL28 controls immunoglobulin (Ig)A plasma cell accumulation in the lactating mammary gland and IgA antibody transfer to the neonate. *J Exp Med* 2004; 200(6): 805-9.
6. **HIESHIMA K, KAWABAKI Y, HANAMOTO H, NAKAYAMA T, NAGAKUBO D, KANAMARU A, ET AL.** CC chemokine ligands 25 and 28 play essential roles in intestinal extravasation of IgA antibody-secreting cells. *J Immunol* 2004; 173(6): 3668-75.
7. **LEUNG DY.** Atopic dermatitis. In: Behrman RE, Kleigman RM, Jenson HB, editors. *Nelson Textbook of Pediatrics*, 17<sup>th</sup>ed. Philadelphia: WB Saunders Company; 2004: 774-78.
8. **SOWDEN JM, BERTH-JONES J, ROSS JS, MOTLEY RJ, MARKS R, FINLAY AY, ET AL.** Double-blind, controlled, crossover study of cyclosporin in adults with severe refractory atopic dermatitis. *Lancet* 1991; 338(8760): 137-40.
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(1): 10-9.
10. **PUGGI N, NOVEMBRE E, GAMMARATA MG, BERNARDINI R, MONACO MG, CALOGERO C, ET AL.** Scoring atopic dermatitis in infants and young children: distinctive features of the SCORAD index. *Allergy* 2005; 60(1): 113-6.
11. **SPRIKKELMAN AB, TUPKER RA, BURGERHOF H, SCHOUTEN JP, BRAND PL, HEYMANS HS, ET AL.** Severity scoring of atopic dermatitis: a comparison of three scoring systems. *Allergy* 1997; 52(9): 944-9.
12. **LIU AH, SPAHN JD, LEUNG DY.** Childhood Asthma. In: Behrman RE, Kleigman RM, Jenson HB, editors. *Nelson textbook of pediatrics*. 17<sup>th</sup>ed. Philadelphia: WB Saunders; 2004. p. 760-73.
13. **NATIONAL HEART, LUNG, AND BLOOD INSTITUTE (NHLBI).** National Asthma Education and Prevention Program. Expert Panel Report 2: Guidelines for the Diagnosis and Management of Asthma. In: NIH Publication No. 97-4051A. Bethesda, MD, US Department of Health and Human Services, National Institute of Health, 1997.
14. **GEAGHAN SM.** Normal blood values: selected reference values for neonatal, pediatric and adult populations. In: Hoffman R, Benz EJ, Shattil SJ, Furie B, Cohen HJ, Silberstein LE, McGlave P, editors. *Hematology basic principles and practice*. 3<sup>rd</sup>ed. New York: Churchill Livingstone; 2000. p. 2520-8.
15. **KAGAMI S, KAKINUMA T, SAEKI H, TSUNEMI Y, FUJITA H, SASAKI K, ET AL.** Increased serum CCL28 levels in patients with atopic dermatitis, psoriasis vulgaris and bullous pemphigoid. *J Invest Dermatol* 2005; 124(5): 1088-90.
16. **HOMEY B, ALENIUS H, MULLER A, SOTO H, BOWMAN EP, YUAN W, ET AL.** CCL27-CCR10 interactions regulate T cell-mediated skin inflammation. *Nat Med* 2002; 8: 157-65.
17. **MORALES J, HOMEY B, VICARI AP, HUDAK S, OLDHAM E, HEDRICK J, ET AL.** CTACK, a skin-associated chemokine that preferentially attracts skin-homing memory T cells. *Proc Natl Acad Sci USA* 1999; 96: 14470-5.
18. **ROMAGNANI S.** Cytokines and chemoattractants in allergic inflammation. *Mol Immunol* 2002; 38(12-13): 881-5.
19. **HIJNEN D, DE BRUIN-WELLER M, OOSTING B, LEBRE C, DE JONG E, BRUIJNZEEL-KOOMEN C, ET AL.** Serum thymus and activation-regulated chemokine (TARC) and cutaneous T cell-attracting chemokine (CTACK) levels in allergic diseases: TARC and CTACK are disease-specific markers for atopic dermatitis. *J Allergy Clin Immunol* 2004; 113(2): 334-40.
20. **KAKINUMA T, SAEKI H, TSUNEMI Y, FUJITA H, ASANO N, MITSUI H, ET AL.** Increased serum cutaneous T cell-attracting chemokine (CCL27) levels in patients with atopic dermatitis and psoriasis vulgaris. *J Allergy Clin Immunol* 2003; 111(3): 592-7.
21. **BANG K, LUND M, WU K, MOGENSEN SG, THESTRUP-PEDERSEN K.** CD4+ CD8+ (thymocyte-like) T lymphocytes present in blood and skin from patients with atopic dermatitis suggest immune dysregulation. *Br J Dermatol* 2001; 144(6): 1140-7.
22. **GIUSTIZIERI ML, MASCIA F, FREZZOLINI A, DE PITA O, CHINNI LM, GIANNETTI A, ET AL.** Keratinocytes from patients with atopic dermatitis and psoriasis vulgaris show a distinct chemokine production profile in response to T cell-derived cytokines. *J Allergy Clin Immunol* 2001; 107(5): 871-7.
23. **REISS Y, PROUDFOOT AE, POWER CA, CAMPBELL JJ, BUTCHER EC.** CC chemokine receptor (CCR) 4 and the CCR10 ligand cutaneous T cell-attracting chemokine (CTACK) in lymphocyte trafficking to inflamed skin. *J Exp Med*. 2001; 194(10): 1541-7.
24. **JOHN AE, THOMAS MS, BERLIN AA, LUKACS NW.** Temporal production of CCL28 corresponds to eosinophil accumulation and airway hyperreactivity in allergic airway inflammation. *Am J Pathol* 2005; 166(2): 345-53.

25. **HUMBLES AA, LU B, FRIEND DS, OKINAGA S, LORA J, AL-GARAWI A, ET AL.** The murine CCR3 receptor regulates both the role of eosinophils and mast cells in allergen-induced airway inflammation and hyperresponsiveness. *Proc Natl Acad Sci USA* 2002; 99: 1479-84.
26. **CHARMAN C, CHAMBERS C, WILLIAMS H.** Measuring atopic dermatitis severity in randomized controlled clinical trials: what exactly are we measuring? *J Invest Dermatol* 2003; 120(6): 932-41.
27. **LASKE N, NIGGEMANN B.** Does the severity of atopic dermatitis correlate with serum IgE levels? *Pediatr Allergy Immunol* 2004; 15(1): 86-8.
28. **ROKAITER, LABANAUSKAS L, VAIDELIENE L.** Role of the skin patch test in diagnosing food allergy in children with atopic dermatitis. *Medicina (Kaunas)* 2004; 40(11): 1081-87.
29. **SEIDENARI S, GUISTI F, BERTONI L, MANTOVANI L.** Combined skin prick and patch testing enhances identification of peanut allergic patients with atopic dermatitis. *Allergy* 2003; 58(6): 495.
30. **MA W, BRYCE PJ, HUMBLES AA, LAQUINI D, YALCINDAG A, ALENIUS H, ET AL.** CCR3 is essential for skin eosinophilia and airway hyperresponsiveness in a murine model of allergic skin inflammation. *J Clin Invest* 2002; 109(5): 621-28.
31. **MUKAI H, NOGUCHI T, KAMIMURA K, NISHIOKA K, NISHIYAMA S.** Significance of elevated serum LDH (lactate dehydrogenase) activity in atopic dermatitis. *J Dermatol* 1990; 17(8): 477-81.
32. **JACYK WK, UNGERER JP.** Serum lactate dehydrogenase activity in exfoliative dermatitis. *J Dermatol* 1991; 18(12): 743.