

Serum levels of Th2 chemokines, CCL17, CCL22, and CCL27, were the important markers of severity in infantile atopic dermatitis

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Atopic dermatitis (AD) is considered to be Th2 cell-mediated disorder. In most infants with AD, AD may be induced by food allergy. In the early stage of infantile AD, it is unclear whether there are changes in serum Th2 chemokines or in Th2 chemokine production by peripheral blood mononuclear cells (PBMC). Thirty-four patients with AD were examined (mean age, 4.5 months; female:male, 18:16). Ten age-matched infants with no history of allergic disease were used as controls. Thirty of these 34 patients were sensitized with ovalbumin (OVA; radioallergen-solvent score of > 2). Serum levels of CCL17, CCL22, and CCL27 were measured with enzyme-linked immunosorbent assay (ELISA) kits and their correlation with the severity of skin lesions, defined by the scoring atopic dermatitis (SCORAD) index, was analyzed. The amounts of TNF- α , CCL17, CCL22, and CCL27 in the culture supernatants of PBMC from OVA-sensitized AD infants after stimulation with OVA were estimated with ELISA kits. Elevated serum CCL17, CCL22, and CCL27 levels significantly correlated with SCORAD index ($r = 0.7181$, $p < 0.001$; $r = 0.5354$, $p < 0.005$; $r = 0.8312$, $p < 0.0001$, respectively). CCL22 levels produced by PBMC from OVA-sensitized infants with AD reflected serum CCL22 levels. Only six of 30 OVA-sensitized patients in whom the skin signs increased immediately after OVA intake showed markedly high titers of TNF- α produced by PBMC after stimulation with OVA. These high TNF- α titers correlated significantly with serum CCL27 levels ($r = 0.7181$, $p < 0.001$). Serum concentrations of CCL17, CCL22, and CCL27 correlate well with the extent and intensity of AD in infants. Of the three Th2 chemokines examined, serum CCL27 correlated most significantly with the severity of AD. Thus, the peripheral immune responses of infantile AD patients are skewed to a Th2 dominant bias.

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Atopic dermatitis (AD) is a chronically relapsing inflammatory skin disease affecting up to 10% of infants in Japan (1). According to a recent report (2), AD afflicts an increasing number of infants and is likely to develop at younger age.

From the skin lesions of infants with AD, the disease may be considered Th2-dominated

inflammatory reaction in the acute phase (3). This process is highly dependent on chemokines, predominantly Th2 chemokines. These mainly include thymus and activation-regulated chemokine (TARC/CCL17) and macrophage-derived chemokine (MDC/CCL22), which act specifically on the chemokine receptor CCR4, and

cutaneous T cell-attracting chemokine (CTACK/CCL27), which acts specifically on the chemokine receptor CCR10, expressed exclusively on cutaneous lymphocyte-associated antigen (CLA) positive lymphocytes (4, 5). The serum concentrations of these Th2 chemokines correlate with the severity of AD (6, 7). It has also been reported that the peripheral immune responses of AD patients are skewed to a Th2 dominant bias (8). Most of these reported data have been confirmed in adult AD patients with chronic disease.

The question arises whether serum Th2 chemokines correlate with the severity of infantile AD in the early phase, when there are few environmental effects, as it does in adult patients with AD. To address this question, we measured serum titers of CCL17, CCL22, and CCL27 in the early phase of AD in infants. We also examined whether the titers of these Th2 chemokines produced by peripheral blood mononuclear cells (PBMC) reflect those in the serum, to identify the chemokine-producing cells in the peripheral blood. Because it has been suggested that tumor necrosis factor- α (TNF- α) affects CCL27 production in the skin (9), we measured serum TNF- α titers and TNF- α production by peripheral immune competent cells, to analyze the role of TNF- α in AD.

Materials and methods

Study population

Patients aged less than 1 yr with eczema, who were first seen and treated at the pediatric outpatient clinic of Ohashi Hospital, Toho University Medical Center in Tokyo, were eligible. The diagnosis of AD was made at least 2 months after the observation of the clinical course in infants as outpatients, according to the criteria proposed by Hanifin and Rajka and the Committee of the Japanese Dermatological Association (10, 11). The clinical severity of AD was evaluated according to the scoring atopic dermatitis (SCORAD) index (12) when the patients were first seen as outpatients.

Thirty-four patients with AD were examined (mean age, 4.6 months; range, 3–11 months; female:male, 18:16). The clinical and laboratory data and serum samples were obtained when the children were first seen as outpatients. The laboratory data used in this study were total serum IgE levels and ovalbumin (OVA) or milk specific IgE levels. The clinical

Table 1. Clinical characteristics of infantile atopic dermatitis patients

	No. of patients	Percentage (%)
Sex		
Female	18	52.9
Male	16	47.1
Age	3–11 months	Mean = 4.6 months
SCORAD		
<20 (4.8–19.7)	16	47.1
20–40 (23.6–39.8)	11	32.4
>40 (40.9–78.6)	7	20.6
Specific IgE levels (RAST > 2)		
OVA	30	88.8
Milk	4	11.8
OVA + milk	28	82.4

SCORAD, scoring atopic dermatitis; OVA, Ovalbumin.

profiles and the laboratory data of the patients are shown in Table 1. Ten age-matched infants were used as the controls. The healthy controls (mean age, 5.6 months; range, 2–10 months; female:male, 6:4) had no family history of allergic disease, and their serum IgE levels, OVA and milk-specific IgE levels, and eosinophils in the peripheral blood were within the normal ranges. Serum specimens from the infants with AD and the control infants were aliquoted and kept frozen at -40°C until use.

Written informed consent was obtained from the parents of the infants with AD and the control infants.

Peripheral blood mononuclear cells

Peripheral blood mononuclear cells were isolated from heparinized venous blood by gradient centrifugation using a Ficoll-Hypaque method. For this study, we used mononuclear cells with a viability of more than 95% (based on the trypan blue test).

Determination of TNF- α , CCL17, CCL22, and CCL27 production by PBMC

Peripheral blood mononuclear cells (5.0×10^5) in 250 μl of RPMI 1640 medium (Lifetech Science, Tokyo, Japan) containing antibiotics and 5% inactivated AB type human serum (Lifetech Science) were incubated in a humidified 5% CO₂ atmosphere at 37°C for 7 days with or without OVA at a final concentration of 200 $\mu\text{g}/\text{ml}$. The supernatants were gathered by centrifugation on the last day of the culture and stored frozen at -70°C for the later determination of cytokine levels.

Enzyme-linked immunosorbent assay

The amount of TNF- α , CCL17, CCL22, and CCL27 in the culture supernatant was estimated with an ELISA kits (R&D Systems, Minneapolis, MN, USA). Serum levels of TNF- α , CCL17, CCL22, and CCL27 were measured with specific ELISA kits according to the manufacturer's protocols (R&D Systems).

Statistical analysis

The Mann–Whitney *U*-test was used to compare variables between the two groups. Spearman's rank correlation coefficient was used to examine the relationship between two continuous

variables. A *p*-value < 0.05 was considered statistically significant. All data are given as means $\pm$ SD.

Results

Correlation between AD severity and serum CCL17, CCL22, and CCL27 levels

Serum CCL17 levels in infants with AD were significantly higher than those observed in control infants (3572.2 ± 3839.4 pg/ml vs. 768.2 ± 574.7 pg/ml, respectively; $p < 0.01$) (Fig. 1a). As shown in Fig. 1b, serum CCL17 concentrations correlated significantly with SCORAD as an index of the extent and intensity of

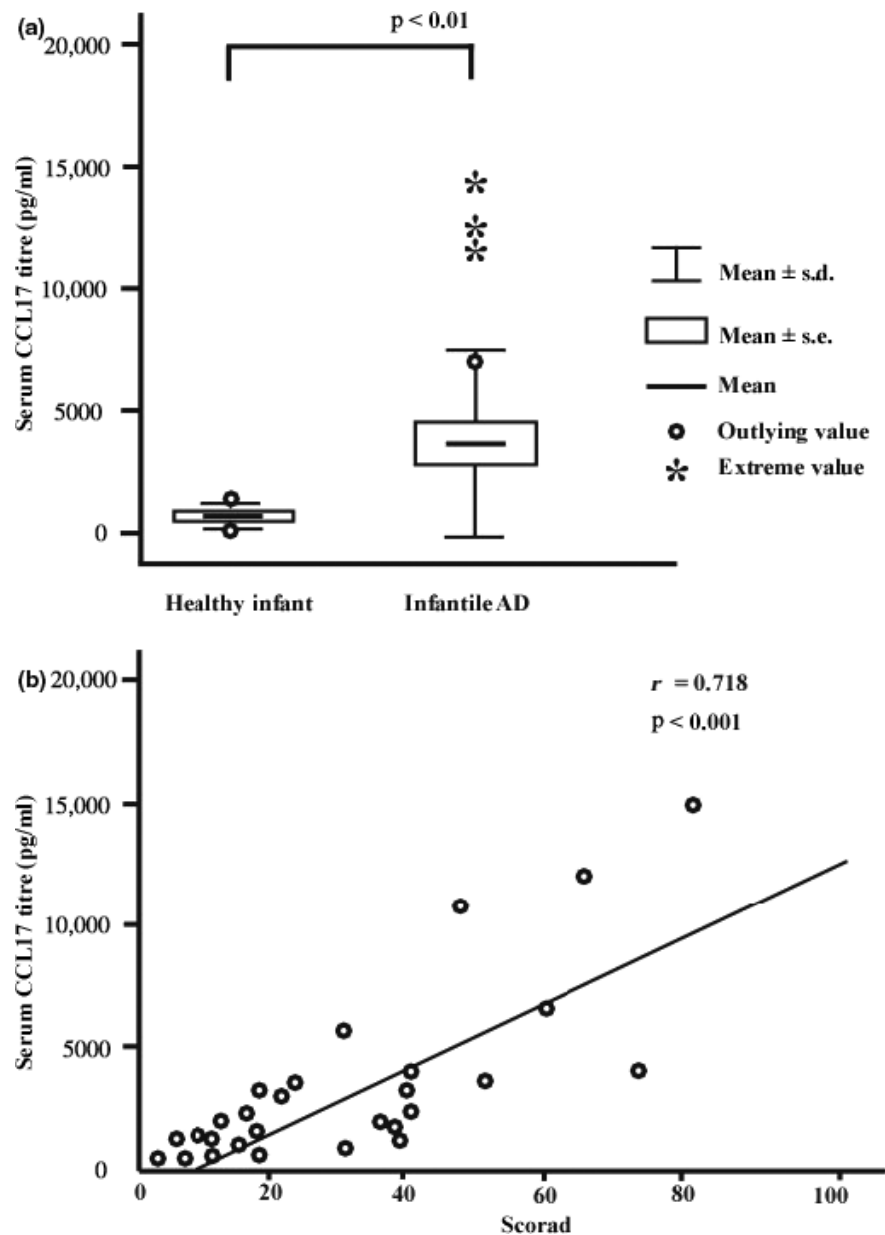


Fig. 1. (a) Serum CCL17 levels in infantile AD patients ($n = 34$) and healthy infants ($n = 10$). (b) The correlation between serum CCL17 levels and the SCORAD index.

skin involvement in AD ($r = 0.7181$, $p < 0.001$). Thus, elevated serum CCL17 levels correlated with the severity of AD. Serum CCL22 levels in infants with AD were significantly higher than those in control infants (2904.9 ± 1886.6 pg/ml vs. 271.4 ± 227.2 pg/ml, respectively; $p < 0.005$) (Fig. 2a). As shown in Fig. 2b, serum CCL22 concentrations correlated significantly with SCORAD ($r = 0.5354$, $p < 0.005$). Thus, elevated serum CCL22 levels also correlated with the severity of AD. Serum CCL27 levels in infants with AD were significantly higher than those in control infants (1080.6 ± 594.5 pg/ml vs. 488.2 ± 316.2 pg/ml, respectively; $p < 0.0001$) (Fig. 3a). As shown in Fig. 3b, serum CCL27 concentration

correlated significantly with SCORAD ($r = 0.8312$, $p < 0.0001$). Thus, of these three Th2 chemoattractants, elevated serum levels of CCL27 correlated most significantly with the severity of AD.

Correlation between serum levels of CCL17, CCL22, and CCL27 and their production by PBMC in patients with infantile AD

Thirty of the 34 patients with AD had a radioallergosorbent test (RAST) score of > 2 for OVA (Table 1). We investigated whether Th2 chemokines were produced by PBMC from these infants sensitized with OVA, to identify the chemokine-producing cells in the peripheral blood. As shown in Fig. 4a, serum levels of

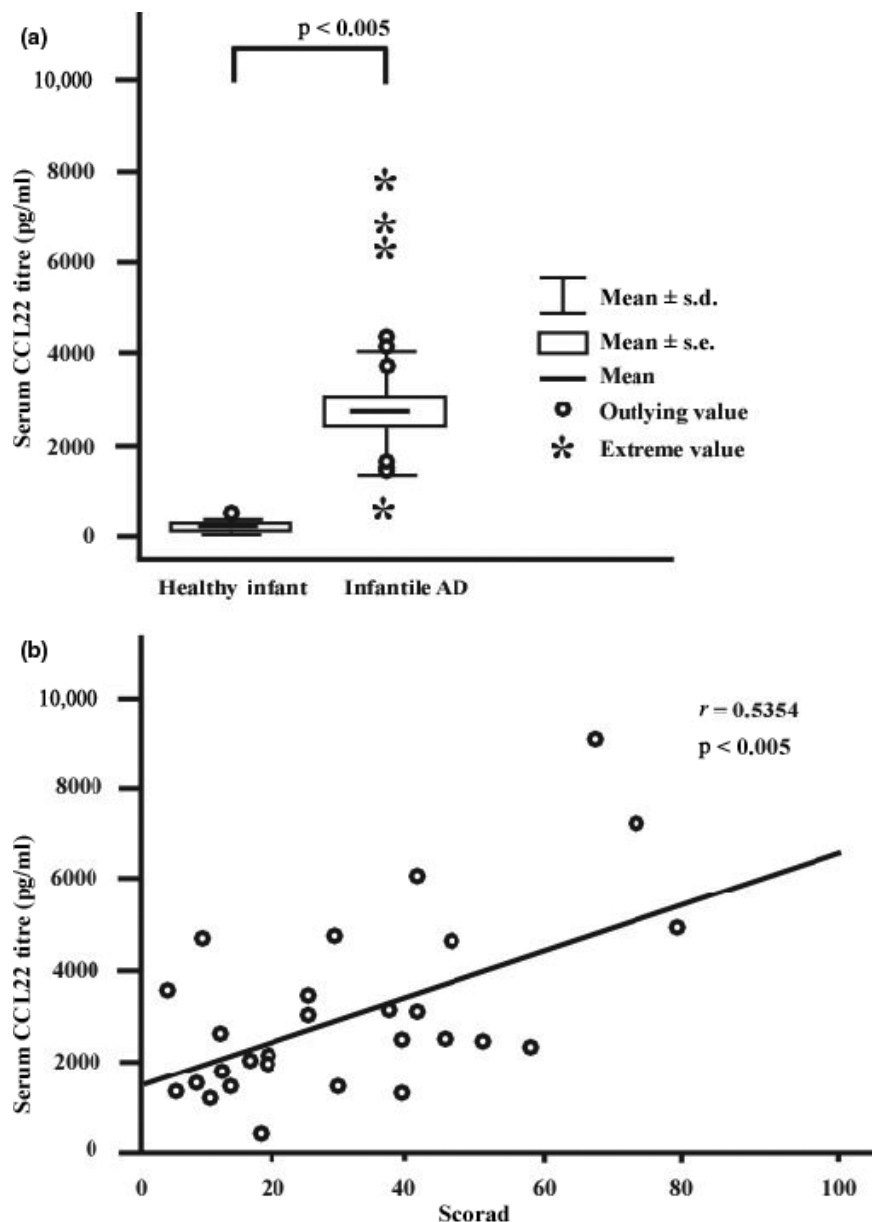


Fig. 2. (a) Serum CCL22 levels in infantile AD patients ($n = 34$) and healthy infants ($n = 10$). (b) The correlation between serum CCL22 levels and the SCORAD index.

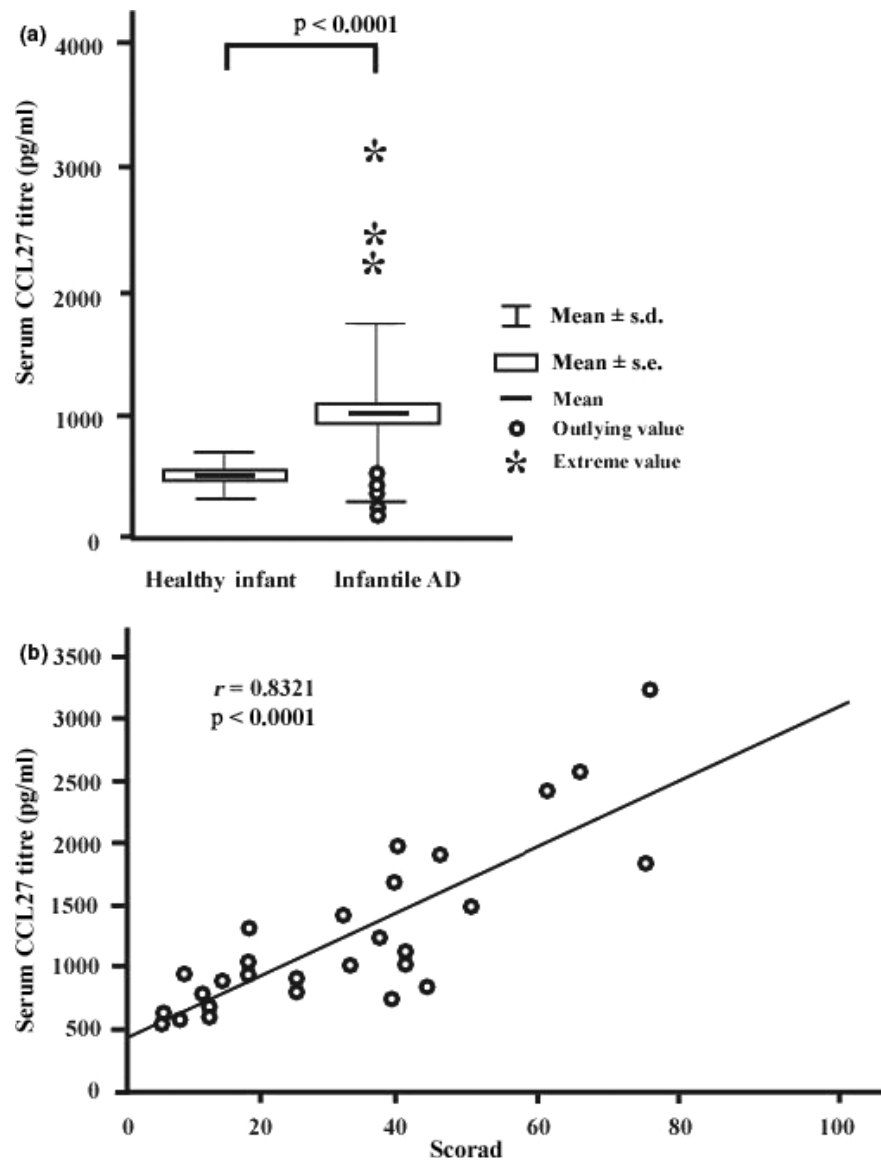


Fig. 3. (a) Serum CCL27 levels in infantile AD patients (n = 34) and healthy infants (n = 10). (b) The correlation between serum CCL27 levels and the SCORAD index.

CCL17 produced by the PBMC of OVA-sensitized AD infants did not correlate with the CCL17 levels in the serum ($r = -0.1317$, $p = 0.496$). However, CCL22 levels produced by the PBMC of OVA-sensitized AD infants correlated significantly with those in the serum ($r = 0.4734$, $p < 0.01$) (Fig. 4b). No patient with AD had measurable CCL27 levels produced by PBMC after stimulation with OVA (data not shown).

Serum TNF- α levels and TNF- α titers released into PBMC culture supernatant

TNF- α is one of the proinflammatory cytokines involved in the early phase of allergic reactions (13). We assayed serum TNF- α levels and the amounts of TNF- α produced by PBMC,

cultured with or without OVA, from infantile AD patients sensitized with OVA or from control infants. Most patients had serum TNF- α levels under the minimal level (5 pg/ml) detectable by the ELISA kit. Serum TNF- α levels were detected in only six patients sensitized with OVA in whom skin signs were augmented immediately after OVA intake. The serum titers of TNF- α in these six patients were 15.7, 5.9, 12.4, 27.1, 8.7, and 15.7 pg/ml. PBMC from OVA-sensitized infants with AD, cultured for 7 days with OVA stimulation, produced high levels of TNF- α , which were higher than those of the controls (503.2 ± 522.2 pg/ml vs. 281.9 ± 102.2 pg/ml, respectively; $p < 0.05$) (Fig. 5). As shown in Fig. 5, six patients in whom the skin signs were augmented within 5 days of OVA intake and

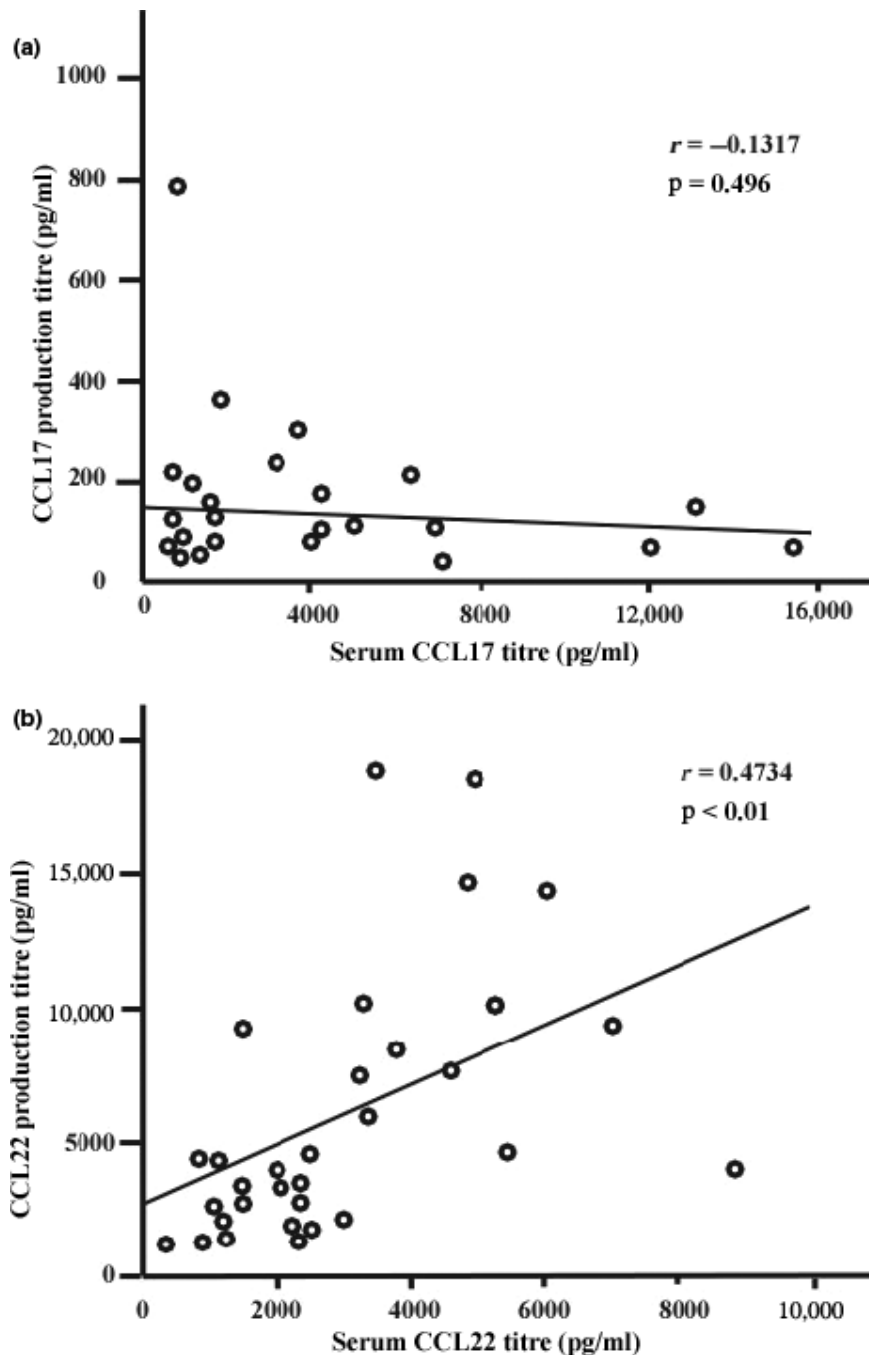


Fig. 4. (a) The correlation between serum concentrations of CCL17 and CCL17 levels produced by PBMC from infantile AD patients. (b) The correlation between serum concentrations of CCL22 and CCL22 levels produced by PBMC from infantile AD.

who had detectable serum TNF- α titers had markedly high TNF- α titers, corresponding markedly high titers produced by PBMC. We infer that TNF- α plays an important role in inducing allergic inflammation in the skin immediately after intestinal mucosal stimulation with food.

Correlation TNF- α produced by PBMC and CCL27 levels

We investigated whether there is a correlation between TNF- α titers produced by PBMC in

infants sensitized with OVA and serum CCL27 levels, to analyze whether CCL27 production in the skin is affected by TNF- α . Levels of TNF- α produced by PBMC from OVA-sensitized AD infants, after stimulation of with OVA, did not correlate significantly with serum CCL27 titers ($r = 0.017$, $p = 0.098$) (Fig. 6). However, as shown in Fig. 6, TNF- α levels produced by PBMC from six patients in whom skin signs were augmented right after OVA intake correlated significantly with serum CCL27 titers ($r = 0.5648$, $p < 0.005$). It can

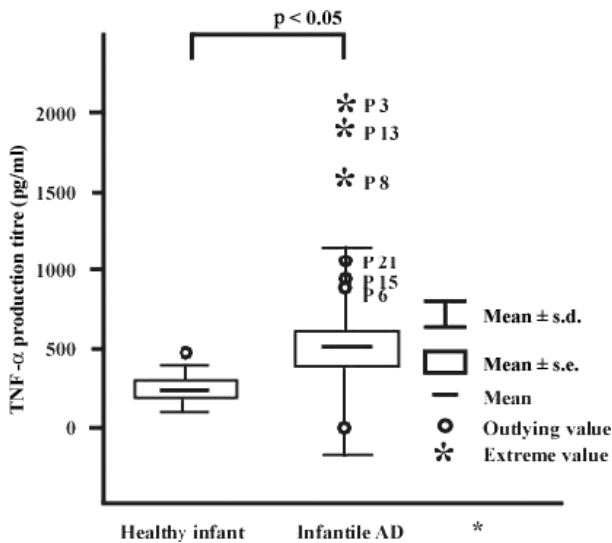


Fig. 5. TNF- α levels produced by PBMC from healthy infants or infantile AD patients. Patients numbers (P); Ovalbumin-sensitized patients immediately after egg intake.

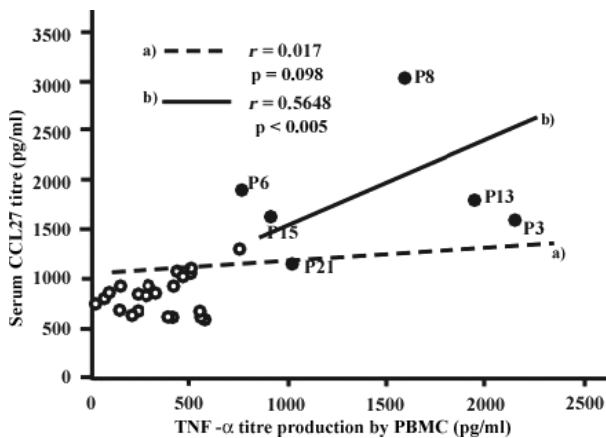


Fig. 6. The correlation between serum CCL27 levels and (a) TNF- α levels produced by PBMC from patients sensitized with ovalbumin (dotted line), or (b) from patients immediately after egg intake (solid line). Patients numbers (P); Ovalbumin-sensitized patients immediately after egg intake. Closed circles; patients immediately after egg intake.

be speculated that TNF- α produced by PBMC is responsible for the upregulated expression of CCL27.

Discussion

Atopic dermatitis is generally considered to be a model of Th2 cell-mediated disorder (14). It has been consistently shown that skin-infiltrating Th2 cytokine producing cells play a pivotal role in the initiation and amplification of atopic skin inflammation, and that the infiltration of inflammatory cells in the skin is regulated by Th2 chemokines (3). Although it has been

suggested that CCL17, CCL22, and CCL27 play pivotal roles in adult AD patients with chronic disease, we have confirmed that the serum levels of CCL17, CCL22, and CCL27 in infantile AD patients, in whom there are few environmental effects, are significantly elevated compared with those of control infants. The serum levels of CCL17, CCL22, and CCL27 correlated significantly with SCORAD in AD. Moreover, the levels of CCL22 produced by PBMC stimulated with OVA correlated significantly with the levels of CCL22 in the sera of infants with AD. This indicates that the peripheral immune responses of infantile AD patients are skewed to Th2 chemokine production. As previously reported by Leung et al. (15), our results indicate that serum concentrations of CCL17 and CCL22 correlate well with the extent and intensity of AD in infants. Furthermore, this study demonstrates that the levels of both CCL17 and CCL22 produced by PBMC are high in infantile AD patients stimulated with OVA. CCL17 and CCL22 are specifically chemoattracted to Th2-type cells through their binding to the CCR4 receptor at local inflammatory sites (16). It has been suggested that CCL17 is predominantly produced by dendritic cells, epidermal keratinocytes, endothelial cells, and fibroblasts, and that CCL22 is predominantly produced by dendritic cells and activated B cells (17, 18). The levels of CCL22 produced by PBMC after stimulation with OVA correlated significantly with serum levels of CCL22 ($r = 0.4734$, $p < 0.01$), whereas levels of CCL17 produced by PBMCs after OVA stimulation did not correlate with serum levels of CCL17 ($r = -0.1317$, $p = 0.496$). In patients with AD, the levels of CCL22 produced by purified monocyte-derived dendritic cells (MoDCs) in the peripheral blood were significantly higher than those of healthy controls (19). This suggests that in infantile AD monocytes from the peripheral blood differentiate into MoDCs and produce CCL22. CCL27 is highly expressed in the basal epidermis of lesioned skin of NC/Nga mice, which are regarded to be a mouse model of human AD, and CCL27 expression in the basal epidermis disappears as the lesion heals (20). These data suggest that the predominant production of CCL27 occurs at local inflammatory sites in the skin. CCL27 is pivotal in mediating the migration of CLA-positive lymphocytes into the skin. In several previous reports of adults and children with AD (3, 21), serum levels of CCL27 in the AD infants were higher than those in the control. In our study, levels of

CCL27 correlated more significantly with the severity of the disease than did those of CCL17 or CCL22. No CCL27 production by PBMC was detected in AD infants with or without OVA stimulation (data not shown). CCL27 is produced constitutively by keratinocytes and is strongly upregulated in inflammatory skin conditions such as AD (22). Food challenge studies have demonstrated that food allergens can induce eczematoid skin rashes in infants with AD. In this study, most infants with AD were sensitized to OVA and OVA intake contributed to the induction of the skin immune response. It has been suggested that, in AD patients sensitized to food allergens, memory T cells expressing the skin homing receptor CLA play an important role in the pathogenesis of skin lesions (23). CCL27 plays an important role in attracting CLA-positive T cells to skin lesions. In this study, distinctly elevated TNF- α production by PBMC stimulated with OVA was detected in OVA-sensitized infants immediately after egg intake. The PBMC from these patients produced markedly high TNF- α after stimulation with OVA. Because it has been reported that TNF- α affects on CCL27 production by keratinocytes (24), it is possible that the upregulation of CCL27 production by TNF- α is one of the factors involved in the development of skin lesions. In conclusion, serum concentrations of CCL17, CCL22, and CCL27 correlated well with the extent and intensity of the disease in infants with AD. CCL27 concentrations were more significantly correlated with the severity of AD than was the CCL17 or CCL22 concentrations. The peripheral immune responses of infantile AD patients are skewed to a Th2 dominant bias.

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