

Original article

Serum thymus and activation-regulated chemokine, macrophage-derived chemokine and eotaxin as markers of severity of atopic dermatitis

Background: Expression of CCR4 ligands, such as thymus and activation-regulated chemokine (TARC) and macrophage-derived chemokine (MDC), leads to preferential influx of T-helper (Th) 2-type lymphocytes to the lesional skin in atopic dermatitis (AD). Eotaxin, like the CCR3 ligand, is an important contributor of eosinophils recruitment in the course of AD. These chemokines are assumed to play an important role in the pathomechanism of AD.

Methods: In this study, the serum concentration of TARC, MDC, eotaxin and total immunoglobulin E (IgE) in AD patients and healthy people were compared. Correlation between the studied indices and activity of AD was established. Severity of AD was assessed according to the SCORAD score. The study comprised 44 healthy people and 43 patients with AD. The serum concentrations of TARC, MDC, eotaxin and IgE were measured with the use of enzyme-linked immunosorbent assay kits.

Results: The serum levels of TARC, MDC, eotaxin and IgE appeared to be significantly higher in patients with AD than in healthy people. A strong positive correlation was revealed between the levels of TARC, MDC, total IgE in serum of patients with AD and SCORAD. In contrast, no significant relationship was found for the serum eotaxin concentration and TARC, MDC, IgE or disease severity.

Conclusion: Our findings indicate that TARC and MDC are actively involved in the pathogenesis of AD and their expression, opposite to that of eotaxin, is strongly associated with clinical picture of atopic dermatitis.

**K. Jahnz-Rozyk¹, T. Targowski¹,
E. Paluchowska², W. Owczarek²,
A. Kucharczyk¹**

¹Department of Internal Medicine, Pneumology and Allergology; ²Department of Dermatology, Central Hospital of Military Institute of Medicine, Warsaw, Poland

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K. Jahnz-Rozyk
Department of Internal Medicine, Pneumology and Allergology
Military Institute of Medicine
128 Szaserów Street
00-909 Warsaw
Poland

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Atopic dermatitis (AD) is a chronically relapsing skin disease characterized by the chemokine-mediated infiltration of numerous mononuclear cells in lesional skin (1, 2). It has been established that T-helper (Th) 2-type lymphocytes play a pivotal role in the pathogenesis of AD because of the increased expression of Th2-related cytokines, such as IL-4 and IL-5, in lesional skin (3). Recently, a preferential expression of CC chemokine receptor (CCR)4 in the subpopulation of Th2-type cells was demonstrated, so thymus and activation-regulated chemokine (TARC) and macrophage-derived chemokine (MDC) – as CCR4 ligands – can induce recruitment of Th2-type lymphocytes to the site of allergic inflammation in the course of AD (4–6). Moreover, eosinophils are thought to play an effector role in the skin lesion associated with AD (7–9). One of the strongest selective contributors of eosinophils recruitment is a specific ligand for CC chemokine receptor (CCR)3 – eotaxin (10–12). We measured and compared the serum TARC, MDC, eotaxin and total immunoglobulin E (IgE) concentrations

in AD patients and healthy people. We also established the relationship between the chemokines, IgE and clinical picture of AD.

Methods

The study group comprised 43 people with atopic dermatitis. Atopic dermatitis was diagnosed with Hanifin's and Rajka's criteria (1). People with other allergic diseases (i.e. rhinoconjunctivitis, bronchial asthma), ever suffering from immunodeficiencies, malignancies or psychiatric diseases, having any infection during the 3 months preceding the study were not recruited. Treatment with topical corticosteroids was allowed before inclusion into the study. Severity of AD was assessed according to the SCORAD score (ranging from 0 to 103 points) with assessment of extensity and localization of the skin lesions and the intensity of objective and subjective symptoms (13). The control group consisted of 44 healthy people with a negative history of allergic diseases, confirmed by negative results of skin prick tests (less than class ++; Allergopharma, Reinbek, Germany) and the normal level of total serum IgE (Allergopharma).

Table 1. Serum levels of the studied indices

	Control group [mean (SD); median (25–75%)]*	Atopic dermatitis [mean (SD); median (25–75%)]	P
TARC (pg/ml)	437.9 (292.0); 377.7 (224.4–581.1)	755.0 (804.7); 493.7 (223.0–758.1)	0.005
MDC (pg/ml)	516.9 (287.1); 500.0 (290.5–619.7)	1001.0 (479.4); 877.4 (675.1–1195.0)	0.0000001
Total IgE (IU/ml)	25.2 (8.4); 25.0 (17.0–32.0)	831.5 (1796.3); 94.0 (36.0–587.0)	0.0000001
Eotaxin (pg/ml)	120.7 (40.8)	154.7 (95.6)	0.03

The mean value of SCORAD in patients with atopic dermatitis was 46.0 (SD 23.8). A strong positive correlation was revealed between the activity of the disease, serum levels of TARC, MDC and total IgE (Figs 1–3). In contrast, no significant relationship between eotaxin and TARC, MDC, IgE or SCORAD was found (Table 2).

*Except serum eotaxin value.

Table 2. Pearson correlation coefficients between the measured indices

	MDC	IgE	SCORAD	EOTAXIN
TARC	0.85*	0.84*	0.82*	0.18
MDC	–	0.68*	0.76*	0.18
IgE	–	–	0.62*	0.04
SCORAD	–	–	–	0.13

*Marked factors are statistically significant with $P < 0.05$.

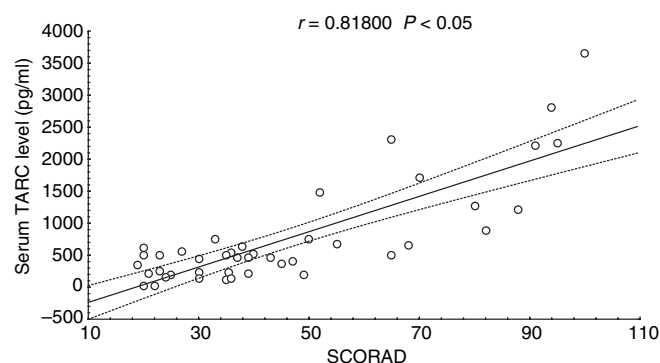


Figure 1. Relationship between serum thymus and activation-regulated chemokine level and SCORAD score.

Venous blood samples were obtained from each subject. Blood drawing, handling and storage were performed by the same researcher. Thirty minutes after drawing the blood, samples were centrifuged at 1300 g at 4°C for 10 min, next, the separated serum samples were kept frozen at –70°C until measurements. The serum concentrations of TARC and MDC were measured using commercially available enzyme-linked immunosorbent assay (ELISA) kits (pg/ml; R&D Systems Inc., Minneapolis, MN, USA; detection thresholds – 7 and 62.5 pg/ml, respectively). Enzyme-linked immunosorbent assay method was also applied for measurement of the serum eotaxin content (Quantikine, Human Eotaxin/CCL11 Immunoassay, pg/ml; R&D kits, Minneapolis, MN, USA; detection threshold – 5.0 pg/ml) and the serum concentration of total IgE (IU/ml, Allergopharma; detection threshold – 1.0 IU/ml). The data were analysed with the Mann–Whitney

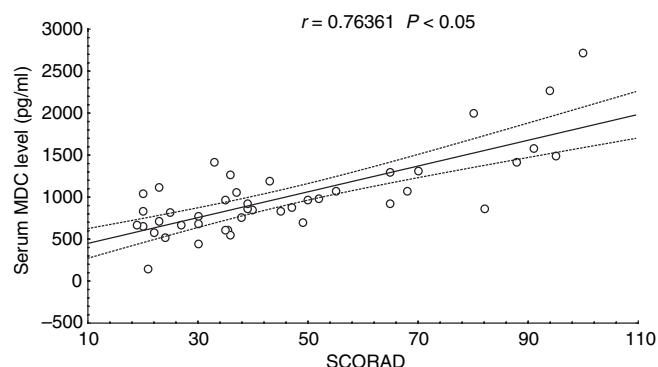


Figure 2. Relationship between serum macrophage-derived chemokine level and SCORAD score.

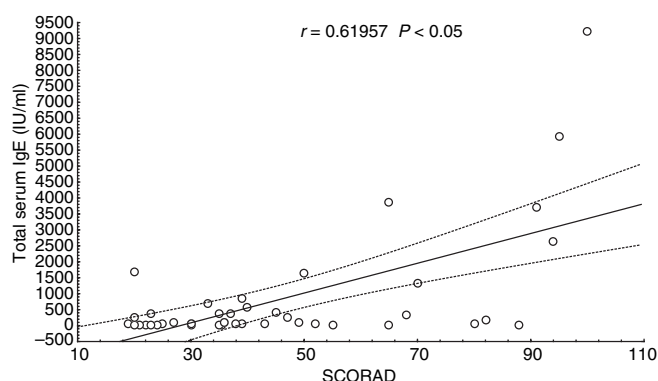


Figure 3. Relationship between serum immunoglobulin E level and SCORAD score.

U-test. In case of normally distributed data, *t*-test for nonpaired changes was used. The correlation coefficients were determined with the Pearson correlation test. A P -value < 0.05 was considered to be statistically significant.

The study protocol was approved by the Ethics Committee of the Military University School of Medicine, Warsaw, Poland and a written informed consent was obtained from each subject.

Results

The study group consisted of 29 females and 14 men with mean age of 24.7 years (SD 10.7), while the control group comprised 28 women and 16 men with mean age of 23.8 years (SD 9.1). Difference in mean age between the two groups was not statistically significant ($P = 0.7$).

In the subjects with AD, the serum levels of TARC, MDC and eotaxin were significantly higher than in the healthy subjects (Table 1).

Discussion

Atopic dermatitis is characterized by the predominant infiltration of mononuclear cells, especially T cells,

eosinophils and macrophages in lesional skin and is associated with a high serum level of IgE (2, 8, 14). The most prominent cells of allergic inflammation, Th2 cells and eosinophils selectively express CC chemokine receptor (CCR)4 and CCR3, respectively. The chemokine ligands of those receptors, such as TARC, MDC (for CCR4) and eotaxin (for CCR3) implicate the mechanism of inflammation in allergic diseases (4, 15, 16). As participation of CCR3/eotaxin pathway in Th2-lymphocytes recruitment to the site of allergic inflammation was revealed, it has been presumed that ligands of both receptors have a supplementary effect on migration of Th2 cells (17). It has also been reported that anti-TARC antibodies strongly attenuate the accumulation of eosinophils as well as lymphocytes in the lung of laboratory mice with asthma (18). So far, the role of TARC and MDC has been discovered in generation of eosinophilic pneumonia and AD (16, 19). An increased level of TARC and MDC was found in bronchoalveolar fluids of asthmatics and serum of patients with allergic bronchial asthma or AD (4, 6, 8, 20). Eotaxin, as an eosinophil selective chemoattractant, is a significant contributor of allergic response (10–12, 21). Its increased mucosal output and serum concentration was demon-

strated in patients with allergic seasonal rhinitis and bronchial asthma (15, 22). It was also shown that an elevated plasma eotaxin content in active AD and acute urticaria and exacerbations did not correlate with the serum concentration of total IgE (23). The relationship between the serum concentration of eotaxin and TARC (but not MDC) was found in asthmatics (4). Other studies prove the existence of correlation between TARC and MDC on the one side and eotaxin-3, but not eotaxin-2, on the other, in people with AD (9). In this study, we revealed a significantly higher serum level of TARC, MDC and eotaxin in patients with atopic dermatitis. We also found a significant positive correlation between TARC, MDC, total IgE and clinical picture of AD. Furthermore, we observed a significantly elevated serum eotaxin concentration in the AD group. No significant correlation was noted between eotaxin on the one side and TARC, MDC or IgE on the other. These findings indicate that TARC and MDC are actively involved in the pathogenesis of atopic dermatitis and their serum concentrations, opposite to that of eotaxin, are strongly associated with activity of atopic dermatitis.

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