

Chemokine RANTES in Atopic Dermatitis

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Abstract. Chemokines play a key role in inflammatory diseases. The aim of this study was to estimate chemokine RANTES in the sera of patients with atopic dermatitis (AD) and to analyze the correlation between RANTES serum level and the immunological and clinical parameters of the disease. Serum levels of RANTES (ELISA; R&D Systems), total IgE and specific IgE (FEIA; Pharmacia CAP System) were estimated in 24 patients with AD, 28 patients with pollinosis (PL) and 22 healthy nonatopic subjects (HC). The division of the AD group into a pure AD (pAD) subgroup, without a coexisting respiratory allergy, and a subgroup of patients with AD and a respiratory allergy (AD+AO) was done according to Wütrich. Levels of RANTES were higher in the AD group than in the HC group and the PL group. RANTES levels did not differ among subgroups with various clinical scores and between the pAD and AD+AO subgroups. There were no correlations between levels of RANTES and total IgE. Significant positive correlations between serum levels of RANTES and *Dermatophagoides farinae* and cat dander-specific IgE were found in the AD group. We conclude that the serum level of chemokine RANTES differs patients with AD from patients with PL. The increase of RANTES concentration in the serum of patients with AD depends neither on a clinical picture nor an IgE system.

Key words: allergic inflammation; atopic dermatitis; chemokine RANTES; IgE.

Introduction

Atopic dermatitis (AD) is a chronic inflammatory skin disease. Its characteristic features are a typical distribution of skin changes of a typical morphology, pruritus, a chronic or relapsing course of the disease and a positive personal and/or familial history of other allergic disorders². High serum IgE levels are observed in about 80% of patients with AD and allergisation

towards air-borne and food allergens is found in 40–80% of them². AD is frequently associated with peripheral blood eosinophilia, but intact eosinophils are rarely seen in the atopic dermatitis skin⁷. However, dermal deposition of eosinophil-granule major basic protein has been detected⁸.

Chemokines, a new group of cytokines that are able to selectively attract leukocytes and activate them, have been discovered recently. Their role in allergic inflam-

Abbreviations used: as-IgE – allergen specific immunoglobulin E, AD – atopic dermatitis, AO – respiratory allergy, CD – cluster of differentiation, d1 – allergen *Dermatophagoides pteronyssinus*, d2 – allergen *Dermatophagoides farinae*, e1 – cat dander allergen, e5 – dog dander allergen, ELISA – enzyme-linked immunosorbent assay, FEIA – fluoroimmunoassay, g5 – allergen *Lolium perenne*, g6 – allergen *Phleum pratense*, HC – healthy control, IgE – immunoglobulin E, IgG – immunoglobulin G, IL – interleukin, pAD – pure form of atopic dermatitis, PL – pollinosis, RANTES – regulated upon activation, normal T cell expressed and presumably secreted, Th – T helper cell.

mation has taken a central place in much scientific research.

RANTES belongs to a C-C chemokine family, whose characteristic feature is the presence of a structural motif containing two adjacent cysteines. RANTES is chemotactic for various cells and potentially recruits these cells from the circulation to an inflamed focus. It functions also in haptotaxis^{1, 6, 11}. Furthermore, it activates some of these cells. RANTES' role in chemotaxis and activation of eosinophils seems to be the most important one. It has also been reported that RANTES selectively enhances IgE and IgG4 production by human B cells⁵. To extend our understanding of AD pathogenesis, we performed this study, the aim of which was to quantify RANTES levels in the sera of patients with AD to further elucidate the role of this chemokine in this inflammatory skin disease.

Materials and Methods

Subjects. The study involved 24 patients (12 males, 12 females) with atopic dermatitis. All patients fulfilled the diagnostic criteria of HANIFIN and RAJKA². Their ages ranged from 16 to 46 (mean 26.8 ± 7.52) years. Most of the patients periodically used topical steroids on limited (up to 10%) body surface. None had used systemic corticosteroids or other immunosuppressive agents in last 3 months. The patients also used antihistamines and emollients to control symptoms of the disease.

Twenty eight patients (17 males, 11 females) in age from 20 to 46 (mean 28.5 ± 7.74) years suffering from pollinosis served as a comparison group (PL group). These patients were examined during natural exposition to allergens; they used antihistamines and/or topical corticosteroids. None had been treated with immunotherapy.

Twenty two healthy subjects (20 male, 2 female) in age from 18 to 44 (mean 31.9 ± 7.63) years with negative personal and familial histories of atopy and normal IgE serum levels (below 100 kU/l) were also studied as a control group (HC group). The 3 groups were comparable in terms of age ($p < 0.05$, Kruskal-Wallis test).

The group of AD patients was stratified into 2 subgroups according to the proposal of WÜTRICH¹⁴ regarding the presence of symptoms of an inhalant allergy (AD+AO group) or their absence, the latter representing the pure form of the disease (pAD group). The pure form of AD was observed in 14 subjects (7 males, 7 females) in age from 16 to 44 (mean 25.64 ± 6.95) years. A concomitant inhalant allergy was found in 10 subjects (5 males, 5 females) in age from 21 to 46

(mean 28.5 ± 8.33) years. Three patients were found to have AD and atopic bronchial asthma, one patient AD, atopic bronchial asthma and pollinosis, one patients AD, atopic bronchial asthma and perennial allergic rhinitis, 2 patients AD and perennial allergic rhinitis, 2 patients AD and pollinosis, and one patient was found to have AD, pollinosis and perennial allergic rhinitis. In addition, within the pure form group, the intrinsic type of AD (negative skin prick test results, normal total IgE serum level, low or undetectable specific IgE levels) was diagnosed in 3 patients. The clinical severity of the disease was determined according to an established score system described by RAJKA and LANGELAND¹⁰ with the parameters of extent, course and intensity of the disease.

Measurement of RANTES, total and specific IgE. Ten millilitres of peripheral blood was collected from all subjects by venepuncture. Centrifugation was performed at 2500 rpm and the serum was aspirated and frozen at -70°C until testing.

The level of RANTES was measured using a commercially available ELISA kit (R&D Systems Europe Ltd). Briefly, samples were pipetted into the wells coated with monoclonal antibodies specific for RANTES. After washing away any unbound proteins, enzyme-linked (horseradish peroxidase) polyclonal antibodies specific for RANTES were added to the wells to sandwich RANTES immobilised during the first incubation. Then a substrate solution for enzymatic reactions was added, and color developed in proportion to the amount of RANTES bound in the initial step. The absorbency of the color was determined at 450/540 nm with a spectrophotometer. A calibration curve was generated with the standard solution, and the RANTES concentration in each sample was determined from this curve. Sensitivity was 2.5 pg/ml.

Total IgE and allergen specific IgE (as-IgE) serum levels were determined with a commercially available fluoroimmunoassay kit from Pharmacia CAP System according to the manufacturer's instructions. Specific IgE against *Dermatophagoides pteronyssinus* (d1) and *Dermatophagoides farinae* (d2), *Tymothy* (g6) and *Ray-grass* (g5), cat dander (e1) and dog dander (e5) allergens were estimated in all AD patients and some PL patients. The levels of specific IgE were stratified into classes from 0 to 6 according to the manufacturer's instructions. Classes 2–6 (from 0.7 to 100 kU_A/l) were found to be clinically significant.

Statistical analysis of results. Results were expressed as median, quartile and total range. Nonparametric statistical tests were used because of the non-normality of the data. The Mann-Whitney U test or the

Kruskall-Wallis analysis of variance by ranks were used to compare results between the examined groups. Spearman's rank correlation was used to evaluate correlations. The criterion for statistical significance was set at $p < 0.05$.

Results

RANTES serum levels

Levels of RANTES were significantly higher in the AD group than in the PL group (500 pg/ml, 440–670, 320–1000 vs. 430, 330–570, 200–860, $p < 0.05$, Mann-Whitney U test) and in the HC group (500 pg/ml 440–670, 320–1000 vs. 380, 330–405, 220–650, $p < 0.00005$, Mann-Whitney U test) (Fig. 1). The median, quartile and total range of serum RANTES levels were in the pAD and AD+AO groups: 477.5 pg/ml, 450–620, 320–1000 and 535, 430–700, 390–1000, respectively, and did not differ between the 2 groups ($p > 0.05$, Mann-Whitney U test). However, in both groups serum RANTES levels were significantly higher than in the HC group ($p < 0.005$ and $p < 0.0005$, respectively). Three patients were diagnosed to have the intrinsic type of AD. Their RANTES levels were 430, 500 and 620 pg/ml and were statistically significantly higher than in the HC group ($p < 0.03$, Mann-Whitney test), but did not

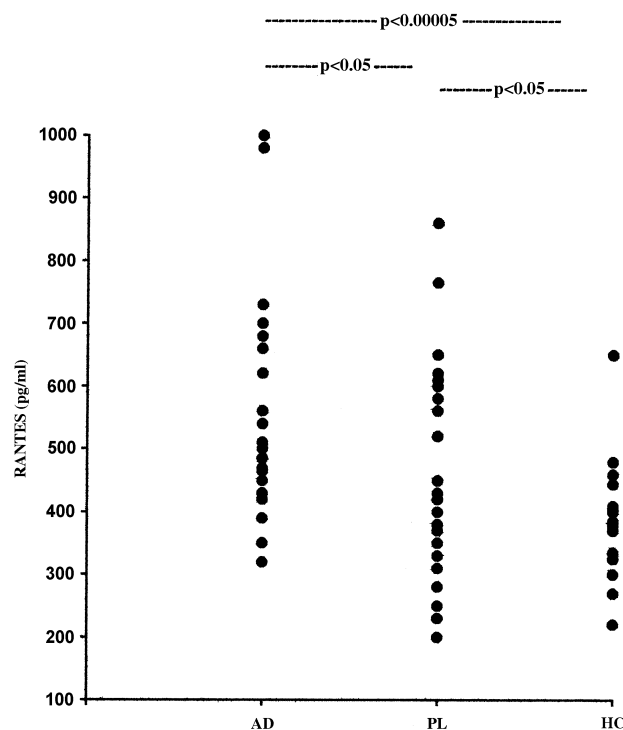


Fig. 1. RANTES serum levels in atopic dermatitis (AD), pollinosis (PL) and healthy subjects (HC)

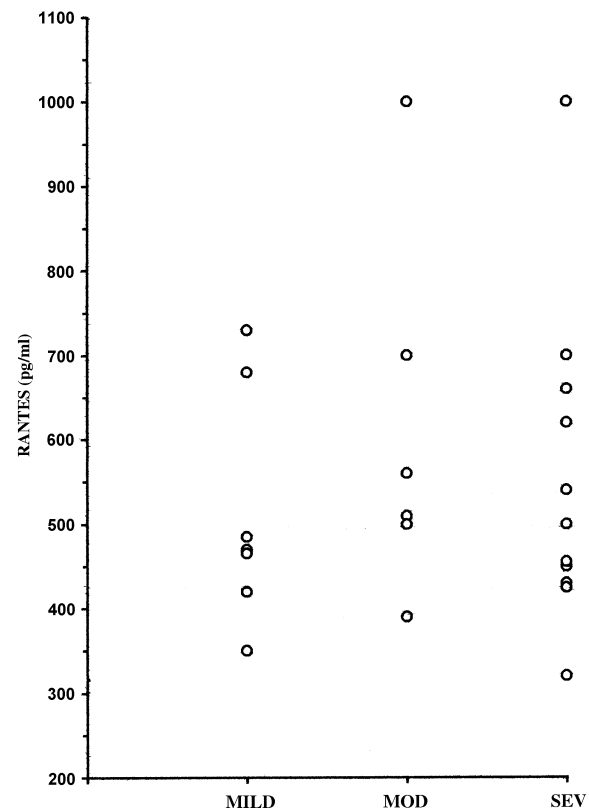


Fig. 2. RANTES serum levels in patients with mild (MILD), moderate (MOD) and severe (SEV) AD. Values did not differ significantly among the groups ($p > 0.05$, Kruskal-Wallis test)

differ from values found in other patients with pAD (extrinsic type).

Mild AD (3–4 points) was found in 7 patients (29.2%), moderate AD (4.5–7.5 points) in 6 patients (25%) and severe AD (8–9 points) was found in 11 patients (45.8%).

RANTES serum levels of patients with mild, moderate and severe AD were 470, 420–680, 350–730 vs. 535, 390–630, 390–1000 vs. 500, 430–660, 320–1000, respectively, and did not differ among these subgroups ($p > 0.05$, Kruskal-Wallis analysis of variance) (Fig. 2).

Total IgE serum levels

Serum total IgE levels were significantly higher in the AD group than in the PL group (338 kU/l, 202–978, 25.2–2000 vs. 129.5, 76.35–255, 9.42–1774, $p < 0.002$, Mann-Whitney U test). Serum total IgE levels did not significantly differ statistically between the pAD and AD+AO subgroups (322, 86.4–978, 25.2–2000 vs. 348.5, 206–541, 181–2000, $p > 0.05$, Mann-Whitney U test).

Table 1. Correlations coefficients (r_s) between RANTES and allergen-specific IgE levels, size of groups (n), significance levels (p) in all examined groups and subgroups

	AD			pAD			AD + AO			PL		
	r_s	n	p	r_s	n	p	r_s	n	p	r_s	n	p
d1	0.38	17	0.14	-0.18	9	0.64	0.59	8	0.12	n.c.	3	
d2	0.54	18	0.019	0.30	9	0.43	0.87	9	0.002	n.c.	5	
g5	0.25	15	0.37	-0.17	8	0.69	0.64	7	0.12	0.16	16	0.55
g6	0.19	15	0.50	-0.45	8	0.26	0.57	7	0.18	0.04	15	0.87
e1	0.81	6	0.049	n.c.	4		n. c.	2		n.c.	1	
e5	0.16	11	0.63	n.c.	5		0.02	6	0.96	n.c.	4	

d1 – *Dermatophagoides pteronyssinus*, d2 – *D. farinae*, g5 – *Raygrass*, g6 – *Tymothy grass*, e1 – cat dander, e5 – dog dander, n.c. – not calculated because of too small size of the group.

Total IgE serum levels in the HC group were 12.5, 6.43–29.45, 2–87.

Total IgE serum levels of patients with mild, moderate and severe AD were: 296 kU/l, 242–452, 198–662 vs. 239, 111–300, 111–410 vs. 1294, 86.4–2000, 25.2–2000 and did not differ among these groups ($p > 0.05$, Kruskal-Wallis analysis of variance).

Correlations between RANTES serum levels and total IgE serum levels were in the AD group $r_s = 0.001$, $p > 0.05$, in the PL group $r_s = -0.14$, $p > 0.05$, in the pAD subgroup $r_s = -0.04$, $p > 0.05$ and in the AD+AO subgroup $r_s = 0.18$, $p > 0.05$. We failed to observe any significant correlations between RANTES and total IgE serum levels in the subgroups of patients with different clinical activity of the disease (mild AD: $r_s = -0.11$, $p > 0.05$; moderate AD: $r_s = -0.29$, $p > 0.05$; severe AD $r_s = 0.67$, $p > 0.05$), as well.

Allergen-specific IgE serum levels

In the AD group clinically significant d1-as-IgE were found in 17 subjects (70.8%), d2-as-IgE in 18 subjects (75%), g5-as-IgE in 15 subjects (68.2%), g6-as-IgE in 15 subjects (68.2%), e1-as-IgE in 6 subjects (28.6%), e5-as-IgE in 11 subjects (52.4%). In the PL group clinically significant d1-as-IgE were observed in 3 subjects (15.8%), d2-as-IgE in 5 subjects (26.3%), g5-as-IgE in 16 subjects (84.2%), g6-as-IgE in 15 subjects (78.9%), e1-as-IgE in 1 subjects (5.3%), e5-as-IgE in 4 subjects (21.1%). Correlations between RANTES and as-IgE were calculated only in those subjects whose as-IgE levels were clinically significant. A statistically significant correlation was found in the AD group between RANTES levels and d2-as-IgE: $r_s = 0.54$, $n=18$, $p < 0.02$. A similar correlation was observed in the AD+AO subgroup: $r_s = 0.87$, $n=9$, $p < 0.005$. Moreover, a significant correlation was found between RANTES and e1-as-IgE serum levels in the AD group and was $r_s = 0.81$; $n=6$; $p < 0.05$. No other

correlations between RANTES and other as-IgE were found in the AD group. No correlations were found in the PL group. All correlations coefficients, p values and number of subjects are presented in Table 1.

Discussion

The present study shows that the serum levels of RANTES were significantly elevated in patients with AD.

Data concerning the role of RANTES in the pathogenesis of AD are few. SCHRÖDER et al.¹², using high performance liquid chromatography (HPLC) analysis of pooled lesional scale extracts of patients with AD, showed fractions containing only weak heparin-binding eosinophils-chemotactic activity, which showed RANTES immunoreactivity. To elucidate the putative cellular origin of RANTES in AD skin, the same group of investigators⁹ examined supernatants of skin-derived T lymphocytes and stimulated with TNF- α or IL-1 β for 48 h dermal fibroblasts. They failed to find any heparin-bound Eo attractants in supernatants of stimulated cultured atopic skin-derived T lymphocytes clones, whereas they showed by preparative RP-8 HPLC that fibroblasts produced eosinophils-chemotactic activity. The activity corresponded well to RANTES when tested with a solid-phase ELISA. YAMADA et al.¹⁶ examined the expression of RANTES mRNA in dermal and colonic tissue in AD patients by the reverse transcription polymerase chain reaction method. RANTES mRNA was detected in most AD patients, both in clinically involved and uninvolved sites.

Apart from the report⁴ on elevated plasma level of IL-8 in AD children and the decrease in this C-X-C chemokine plasma level after treatment, no other chemokines were studied in AD. However, the biological activity of RANTES suggests its role in the pathogenesis of AD.

In our study highly statistically significant elevated

serum RANTES levels were found in atopic dermatitis patients in comparison to healthy controls. High statistical significance of differences between RANTES serum levels compared to healthy people was observed both in the whole AD group and in the subgroups pAD and AD+AO. Furthermore, serum RANTES levels were significantly higher in the AD group than in the PL group. So it may confirm our hypothesis of a pathogenic role of this chemokine in AD.

Within pAD, the intrinsic form of the disease may be distinguished by analogy with asthma, according to WÜTRICH¹⁴. This form, also called nonatopic or nonallergic atopic dermatitis, is characterised by a negative history of an inhalant allergy, negative skin prick tests, low total IgE and low or undetectable specific IgE levels. It should be noted that the clinical picture of the disease does not differ from the other form of AD. Frequency of the intrinsic form is estimated to be up to 20–40% of all AD cases¹⁴. Other features of the intrinsic form of AD are the absence of IgE on Langerhans dermal cells, low CD23 expression on lymphocytes in peripheral blood and different cytokine profiles of peripheral blood and skin biopsies T cells (increased levels of IL-5, low levels of IL-4)^{3, 15}. The interest concerning this subgroup is understandable, as therapeutic strategies should differ from those applied in other AD patients, especially as anti-allergen treatment is pointless.

In our study, the intrinsic form of AD was found in 3 subjects. The RANTES levels were comparable to other patients with AD and were higher than RANTES levels found in healthy controls. However, because of the small size of the intrinsic form subgroup our conclusions are not clear, and should be extended by further studies.

It has been recently indicated⁵, both *in vivo* and *in vitro*, that RANTES enhances IgE production by directly stimulated with immunological factors (IL-4 and monoclonal antibodies anti-CD40 or anti-CD58) sIgE⁺ B cells. The presence of receptors specific for RANTES has also been shown. Unexpectedly, we failed to find significant correlations between RANTES and total IgE serum levels in the examined groups. We did not find it even among patients with extremely high serum total IgE levels, i. e. exceeding 1000 kU/l.

Most patients with AD (40–60%) have symptoms of sensitisation towards house dust mites, and the frequency increases with the age¹³. We found statistically significant correlations between RANTES and *D. farinae* as-IgE levels in the AD and AD+AO groups. This observation may confirm the pathogenic role of dust mite allergens in AD.

The role of cat and other pet allergens in inducing allergy symptoms is well-known. The correlation between RANTES and cat dander as-IgE was found in our study. However, the significance of that correlation was not strong, so this finding is of minor importance. In light of our observations, the potential relationship between RANTES and allergisation towards common air-borne allergens is equivocal.

Concluding, it is impossible that a single chemokine plays a crucial role in the pathogenesis of the disease. However, a better understanding of the inflammatory reactions in atopic diseases may lead to new treatment strategies.

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