Chemokine RANTES in Atopic Dermatitis

JOANNA GLÜCK and BARBARA ROGALA

Department and Clinic of Internal Diseases, Allergology and Clinical Immunology, Silesian University Medical School in Katowice, 3 Maja 13, 41-800 Zabrze, Poland

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 disorders\(^2\). 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 them\(^2\). AD is frequently associated with peripheral blood eosinophilia, but intact eosinophils are rarely seen in the atopic dermatitis skin\(^7\). However, dermal deposition of eosinophil-granule major basic protein has been detected\(^8\).

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-
tion has taken a central place in much scientific re-

RANTES belongs to a C-C chemokine family, 
whose characteristic feature is the presence of a struc-
tural 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. Furthermore, it activ-
ates some of these cells. RANTES’ role in chemotaxis 
and activation of eosinophils seems to be the most im-
portant one. It has also been reported that RANTES 
selectively enhances IgE and IgG4 production by 
human B cells. 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 Rajka. 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 antihis-
tamines 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 immu-
notherapy.

Twenty two healthy subjects (20 male, 2 female) in 
age from 18 to 44 (mean 31.9 ± 7.63) years with nega-
tive 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 compara-
tible in terms of age (p<0.05, Kruskall-Wallis test).

The group of AD patients was stratified into 2 sub-
groups according to the proposal of Wütrich regarding 
the presence of symptoms of an inhalant allergy 
(AD+AO group) or their absence, the latter repre-
senting 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 se-
verity of the disease was determined according to an 
established score system described by RAJA and 
LANGELAND 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 per-
formed at 2500 rpm and the serum was aspirated and 
frozen at −70°C until testing.

The level of RANTES was measured using a com-
mercially available ELISA kit (R&D Systems Europe 
Ltd). Briefly, samples were pipetted into the wells 
coated with monoclonal antibodies specific for RAN-
TES. 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 enzy-
matic reactions was added, and color developed in pro-
portion 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, ant 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) aller-
gens 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 in-
structions. Classes 2–6 (from 0.7 to 100 kU/l) were 
found to be clinically significant.

Statistical analysis of results. Results were ex-
pressed as median, quartile and total range. Nonpar-
arametric statistical tests were used because of the non-
normality of the data. The Mann-Whitney U test or the
Kruskal-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 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, Kruskall-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).

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

<table>
<thead>
<tr>
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<th>pAD</th>
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<th>PL</th>
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<tr>
<td>r</td>
<td>n</td>
<td>p</td>
<td>r</td>
<td>n</td>
</tr>
<tr>
<td>d1</td>
<td>0.38</td>
<td>0.14</td>
<td>-0.18</td>
<td>0.64</td>
</tr>
<tr>
<td>d2</td>
<td>0.54</td>
<td>0.019</td>
<td>0.30</td>
<td>0.43</td>
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<tr>
<td>g5</td>
<td>0.25</td>
<td>0.37</td>
<td>-0.17</td>
<td>0.69</td>
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<tr>
<td>g6</td>
<td>0.19</td>
<td>0.50</td>
<td>-0.45</td>
<td>0.26</td>
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<tr>
<td>e1</td>
<td>0.81</td>
<td>0.049</td>
<td>n.c.</td>
<td>4</td>
</tr>
<tr>
<td>e5</td>
<td>0.16</td>
<td>0.63</td>
<td>n.c.</td>
<td>5</td>
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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, Kruskall-Wallis analysis of variance).

Correlations between RANTES serum levels and total IgE serum levels were in the AD group r = 0.001, p>0.05, in the PL group r = -0.14, p>0.05, in the pAD subgroup r = -0.04, p>0.05 and in the AD+AO subgroup r = 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 = -0.11, p>0.05; moderate AD: r = -0.29, p>0.05; severe AD r = 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 = 0.54, n=18, p<0.02. A similar correlation was observed in the AD+AO subgroup: r = 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 = 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. SCHRODER et al., using high performance liquid chromatography (HLPC) 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 investigators examined supernatants of skin-derived T lymphocytes and stimulated with TNF-α or IL-1β 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., 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 report 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 WUTRICH. 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 cases. 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). 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 indicated, 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) all IgE+ 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 age. 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 dermatitis may lead to new treatment strategies.

References

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