

Elevation of the plasma level of RANTES during asthma attacks

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Background: RANTES is considered to play an important role in various immune and allergic disorders since it is a potent chemoattractant for inflammatory cells such as eosinophils, memory T cells, and monocytes.

Objective: To investigate the possible role of RANTES in the pathogenesis of bronchial asthma.

Methods: The plasma level of RANTES was measured in 12 asthma patients and 15 normal controls by a sandwich enzyme-linked immunosorbent assay.

Results: In the patients with asthma, the plasma RANTES level was significantly elevated during acute attacks compared to that in the controls. In addition, it was higher than that during the asymptomatic state in the same patients. Plasma β -thromboglobulin levels were also elevated in asthma patients during acute attacks and showed a correlation with the RANTES level.

Conclusion: These findings suggest a role for RANTES in the pathogenesis of asthma and a possible role for platelets in RANTES release in asthma. (*J Allergy Clin Immunol* 1997; 100:S52-S55.)

Key words: Bronchial asthma, chemokine, eosinophils, platelets, RANTES, platelet factor 4, β -thromboglobulin

Much attention has recently been focused on the role of allergic inflammatory reaction in asthma. Many types of cells, such as eosinophils, macrophages, lymphocytes, and platelets participate in inflammatory reactions in the respiratory tract. Eosinophils are considered to be the major type of inflammatory cells in bronchial asthma¹ because eosinophil specific granule proteins can damage bronchial mucosal cells.² Platelets also have been implicated as mediators of inflammatory reactions, because they are an excellent source of a wide range of biologically active materials of relevance to the inflammatory process.^{3,4} Chemokines related to the beta subfamily, the so-called platelet factor 4 superfamily,⁵ have been shown to stimulate human eosinophils or basophils and are considered to be important mediators of inflammation.⁵ RANTES, which might be released from activated

Abbreviations used

ELISA:	Enzyme-linked immunosorbent assay
RANTES:	Regulated on activation, normal T-expressed and secreted
CR3:	Complement receptor type 3
ECP:	Eosinophil cationic protein
kD:	Kilodalton
PF4:	Platelet factor 4
β -TG:	β -thromboglobulin

platelets, is considered to play an important role in various immune and allergic disorders.^{5,6} RANTES is a potent chemoattractant for various inflammatory cells such as eosinophils⁷ and memory T cells and monocytes.⁵ RANTES potentially recruits these cells from the circulation to an inflamed focus. Involvement of eosinophils and T cells in bronchial asthma also has been reported.¹ In the present study, to extend our understanding of the participation of eosinophils, T cells, and RANTES in the pathogenesis of bronchial asthma, we examined the plasma level of RANTES in asthma patients by sandwich enzyme-linked immunosorbent assay (ELISA).

METHODS

The study involved 15 healthy persons who served as normal controls (21 to 55 years of age; average, 35.8 years; eight women) and 12 patients with bronchial asthma as defined by the American Thoracic Society⁸ (16 to 75 years of age; average, 37.0 years; seven women). Eight of the 12 patients with asymptomatic asthma also participated. Seven patients had allergic asthma, that is, a concentration of serum IgE greater than 400 IU/ml and IgE-RAST higher than class 3 against at least one of the common airborne allergens, such as house dust mite, pollens, and fungi. Medications during the 6 days preceding blood collection for the asymptomatic period or preceding an attack were the same for each patient. No patient took medication containing antipyretics or antiplatelet agents that suppressed platelet function during the 2 weeks before blood collection. None of the participants took steroids within 2 weeks before blood collection, although they were taking theophylline preparations and β_2 agonists. An acute asthma attack was defined as the period during which a patient had dyspnea and audible expiratory wheeze accompanied by a 20% reduction in FEV₁ compared with asymptomatic periods.

Plasma was obtained as previously reported,⁹ with platelet activation avoided during blood collection. Three milliliters of

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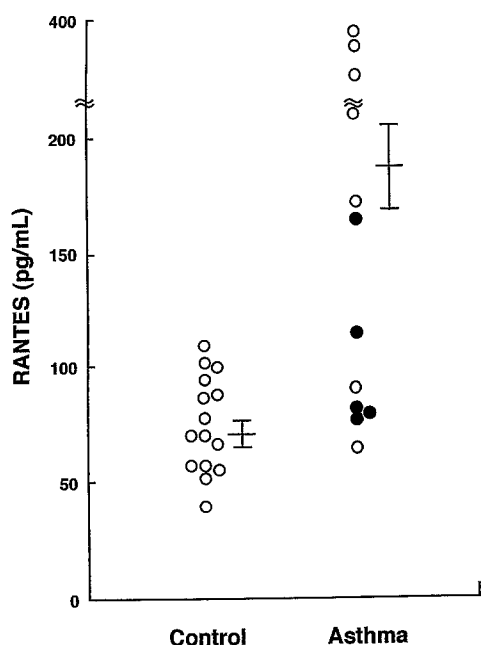


FIG. 1. Plasma level of RANTES among patients with asthma during acute attack was significantly elevated compared with that of controls ($p < 0.01$). Among patients with asthma, there was no difference in plasma level of RANTES between atopic patients (open circles) and nonatopic asthma (closed circles) ($p > 0.1$).

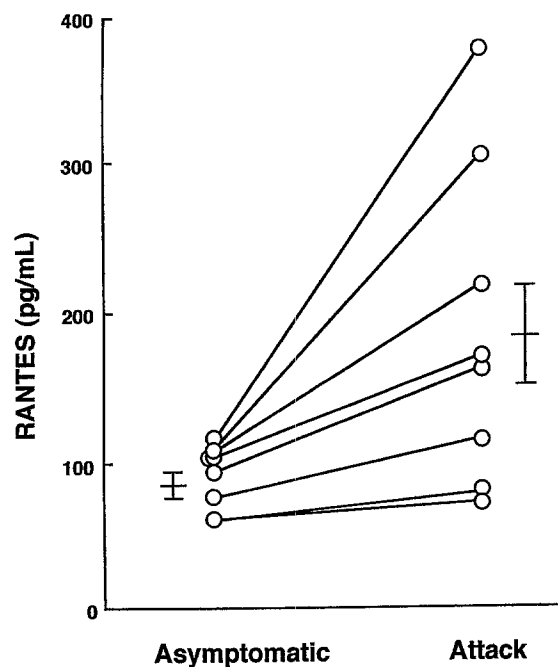


FIG. 2. Plasma level of RANTES among symptomatic and asymptomatic asthma patients with asthma during acute attack (*Attack*) was higher than that during the asymptomatic condition (*Asymptomatic*) in the same patient ($p < 0.05$).

blood was drawn from the median cubital vein through a 21-gauge butterfly needle into a plastic syringe containing 1/10 volume of 3.2% sodium citrate. Blood was collected between 11 A.M. and 3 P.M. to avoid the daily variation in platelet function that occurs early in the morning.¹⁰ Great care was taken not to pull the syringe out too forcibly, not to use a tourniquet, and not to reinsert the needle to avoid platelet activation during blood collection. Immediately after collection, 2.5 ml of the blood sample was gently transferred to a pre-cooled tube containing a solution of 1.08 mg theophylline, dipyridamole, and sodium citrate (provided by Amersham, Tokyo, Japan). The tube was inverted gently three times and put into crushed ice within 2 minutes of blood collection to minimize platelet activation during handling. Fifty minutes later, the tube was centrifuged at 2000g, 4°C for 30 minutes, and platelet-poor plasma was carefully collected and stored at -20°C until assay.

RANTES in plasma was measured by means of ELISA as previously reported.¹¹ In brief, an ELISA kit (R & D Systems, Minnesota) with two antibodies to differentiate epitopes of RANTES was used for the assay. A 96-well plate was treated with anti-RANTES monoclonal antibody, and 100 μ l of the standard solution or the test sample was added followed by 50 μ l of horseradish peroxidase-labeled anti-RANTES antibody. Absorbance was determined at 450/540 nm with a Titertek Multiskan MCC/340 spectrophotometer (ICN, LabSystems Oy., Helsinki, Finland). A calibration curve was generated with the standard solution, and the RANTES concentration in each sample was determined from this curve (sensitivity 15.6 pg/ml or more). All determinations were performed in duplicate or triplicate. β -Thromboglobulin in plasma was assessed according to a method described in a previous study.⁹

Data were expressed as mean $\pm$ SE. The Student *t* test was used for comparison of means. A *p* value of 0.05 or less was considered statistically significant.

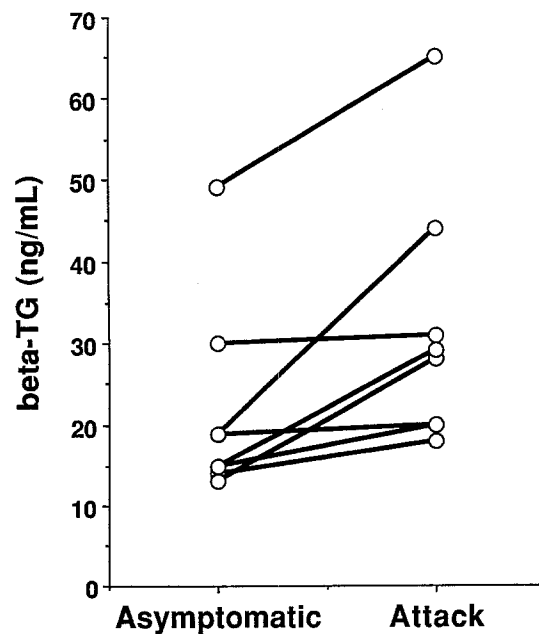


FIG. 3. Plasma level of β -thromboglobulin among patients with asthma during an acute attack (*Attack*) was higher than that during the asymptomatic condition (*Asymptomatic*).

RESULTS

Plasma levels of RANTES among patients with asthma during an attack was significantly higher than those of controls (171.58 ± 32.68 pg/ml versus $71.71 \pm$

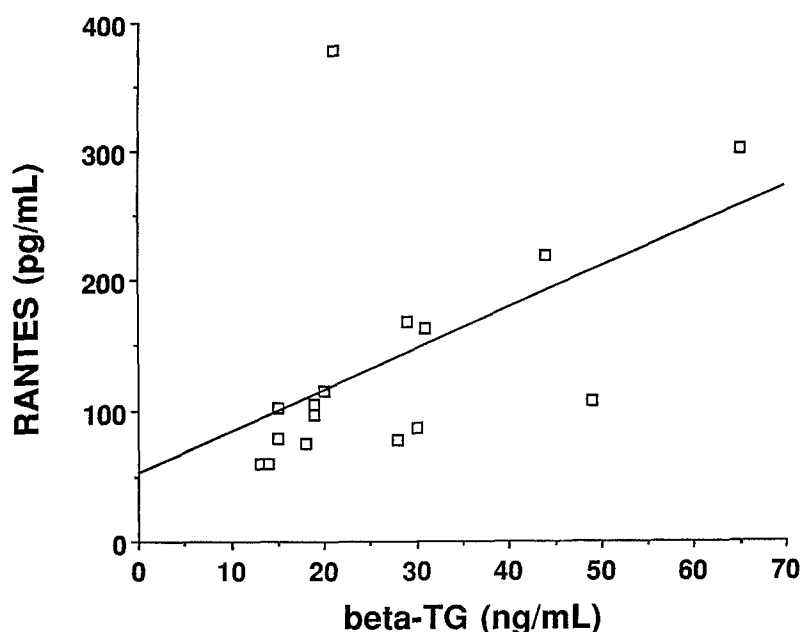


FIG. 4. Correlation between plasma β -TG and RANTES levels in patients with asthma.

5.22, $p < 0.01$; Fig. 1). There was no significant difference in plasma level of RANTES between patients with asthma in the asymptomatic state (87.68 ± 6.86 pg/ml) and controls ($p > 0.1$; Figs. 1 and 2). The seven patients with atopic asthma showed no difference in plasma level of RANTES from the five nonatopic patients (221.49 ± 47.15 pg/ml versus 101.72 ± 17.01 ; Fig. 1). Plasma level of RANTES in patients with asthma during an attack was higher than that during the asymptomatic condition among the same patients (187.43 ± 38.14 versus 87.68 ± 6.86 , $p < 0.05$; Fig. 2). In addition, levels of β -thromboglobulin (β -TG) were elevated among patients with asthma during an attack in comparison with those during the asymptomatic condition (32.25 ± 5.53 ng/ml versus 21.7 ± 4.34 ng/ml, $p < 0.05$; Fig. 3). Moreover, a significant correlation between β -TG level and RANTES level was observed ($r = 0.504$, $p < 0.05$; Fig. 4).

DISCUSSION

The presence of elevated levels of RANTES in the plasma of patients with bronchial asthma suggest that RANTES might play a key role in the pathogenesis of bronchial asthma. RANTES, which is released from not only activated T lymphocytes but also thrombin-stimulated platelets, is a member of the 8 kilodalton cytokine family that has been shown to possess chemotactic activity for eosinophils. In another study, we examined the effect of RANTES on radical oxygen products from eosinophils.¹² RANTES treatment resulted in enhancement of the peak value and integrated value of productivity of eosinophil-mediated radical oxygen products determined by means of luminol-dependent chemiluminescence studies.¹² We concluded that RANTES might

play an important role in the pathogenesis of allergic inflammation through involvement in selective eosinophil infiltration and eosinophil activation.

That RANTES, which might be released from activated platelets,⁷ enhances the capability of radical oxygen products from eosinophils can be considered evidence for the participation of platelets in allergic inflammation.^{3, 4, 13} To clarify whether platelet activation was related to RANTES release, we examined the plasma level of β -TG in the present study. We found an elevated β -TG level in the asthma patients during acute attacks as compared to that during the asymptomatic period, and it showed a correlation with the RANTES level. In this context of platelet involvement in allergic inflammation, we also have observed that platelet factor 4 and RANTES¹⁴ may be involved in eosinophil radical oxygen products as well as in eosinophil chemotaxis,¹⁵ eosinophil Fc receptor expression,^{15, 16} hypodense eosinophils,¹⁷ and adhesion molecule expression such as of complement receptor type 3 (CD11b/CD18).^{18, 19} These findings may suggest a role of platelet activation through the release of RANTES in the pathogenesis of asthma, although it is only one of several possible sources of RANTES. Besides platelets, several sources of RANTES production or release, such as bronchial epithelium or nasal epithelium, have been described.²⁰⁻²² It should be clarified whether RANTES in the circulation is capable of activating eosinophils, because many studies have suggested that the Duffy antigen found on red blood cells,²³ which is a receptor for chemokines, may play a role in decreasing the inflammatory effects of intravascular chemokines.^{23, 24}

In conclusion, our findings of elevated levels of

RANTES in the plasma of patients with asthma during an attack suggest the role of RANTES in the pathogenesis of asthma. The precise mechanism of involvement of RANTES and the source of RANTES production require investigation.

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