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Serum eotaxin levels in patients with chronic spontaneous urticaria

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Summary
Eotaxin is a potent agonist for CC chemokine receptor 3 that can attract eosinophils at sites of inflammation. Given the potential role of eosinophils in chronic spontaneous urticaria (CU), we measured serum eotaxin levels together with C-reactive protein in 100 CU patients who were characterized according to autologous serum skin test (ASST) and disease severity. Serum eotaxin concentration was significantly higher in CU patients (median 140.1 pg/ml, range 33.7-718.7 pg/ml) than in 45 healthy controls (median 108.9 pg/ml, range 45.5-409.4 pg/ml) (p=0.032) Serum eotaxin concentration was not significantly different in ASST-positive and ASST-negative patients as well as in patients with different urticaria activity scores. However, eotaxin levels tended to be higher in patiens with intense symptoms. In the 7 patients observed during CU exacerbation and during remission, eotaxin serum levels tended to decrease during remission, although statistical significance was not reached (median concentration decreased from 170.0 pg/ml to 123.8 pg/ml). CRP levels were not significantly different in CU patients and healthy subjects, although there was a trend towards higher levels in the former population. Furthermore, in the 7 patients observed during CU exacerbation and during remission, CRP levels decreased significantly during remission (median concentration dropped from 4.1 µg/ml to 0.7 µg/ml, p=0.015). No significant correlation was found between eotaxin and CRP serum levels. These findings indicate that serum eotaxin levels are increased in CU patients, although they do not reflect strictly disease activity. A role for eotaxin in eosinophil attraction and activation in CU can be envisaged.

Introduction

The pathophysiology of chronic spontaneous urticaria (CU) is linked to the recurrent degranulation of dermal mast cells that are generally regarded as the main effector cells of this disease. However, the events leading to mast cell degranulation and histamine release in CU are only partially defined. Histamine-releasing autoantibodies directed against the alpha subunit of the high affinity IgE receptor or against IgE have been detected in less than 50% of CU patients, and the mechanism operating in the other cases still has to be defined (1-4). Some recent experimental findings support the concept that CU is an inflammatory disorder independently of the presence of histamine-releasing autoantibodies. In

fact, increased levels of inflammatory markers as C-reactive protein and matrix-metalloproteinase-9 have been detected in CU patients either with or without circulating histamine-releasing autoantibodies (5-7). Furthermore, the histological picture of the skin lesion is more or less the same in all patients with CU irrespective of the presence or absence of autoantibodies (8). A perivascular infiltrate of CD4+ lymphocytes is always present and increased numbers of intradermal CD3, CD4, CD8-positive T cells have been detected with a TH0 cytokine profile; neutrophils and a variable degree of eosinophils are also present (9,10). Interestingly, the eosinophil-derived major basic protein has been identified in skin biopsies performed at sites of autologous serum injection, along with eosinophil infiltration (11,12). Although eosinophils are not recognized as key effector cells in CU and are not detected in increased amounts in peripheral blood, they are often present in skin lesions and they can release potent inflammatory mediators, including major basic protein, eosinophil cationic protein, leukotriene C4, platelet-activating factor, and vascular endothelial growth factor (8,13,14). So far, the chemotactic factors causing eosinophil accumulation in the skin lesions of CU have not been determined. Eotaxin is a potent and highly specific agonist for CC chemokine receptor 3 that can attract eosinophils at sites of inflammation (15,16). Increased levels of plasma eotaxin have been found in children with acute urticaria, with a marked decrease following disease remission, and in children with atopic dermatitis (17). In contrast, in one study by Caproni et al. (18), eotaxin has been detected in sera from CU patients but the levels have not been found different from those of normal controls. Given the renewed recent interest for the potential role of eosinophils in CU, we measured serum eotaxin levels together with C-reactive protein in CU patients who were characterized according to skin reactivity to autologous serum and disease severity; in a group of patients, eotaxin levels were measured during disease activity and during remission.

Subjects and methods

Subjects

One-hundred patients with chronic spontaneous urticaria (CU) (30 males and 70 females, median age 42.6 years, range 16-77 years) were evaluated at the allergy centre of Maggiore Policlinico Hospital of Milan and selected for the study. CU was diagnosed on the basis of the recurrence of spontaneous weals with or without angioedema for more than 6 weeks. Physical urticarias as well as other possible causes of urticaria (i.e. food and drug allergy and parasitoses) were excluded by proper investigations. Only patients with chronic spontaneous urticaria were selected for the study, whereas patients with physical urticaria and urticaria vasculitis were excluded. All patients had active urticaria at the time of the study. At the first visit, based on patients' recent history and according to the number of wheals and pruritus present, disease activity was estimated following the urticaria activity score (UAS) recommended by the recent EAACI guidelines (19); in particular, urticaria was classified as mild (score 1), moderate (score 2) or intense (score 3). All the patients were under treatment with H1 antihistamines, which were suspended at least 5 days before office visit, in order to perform autologous serum skin test (ASST). No patient was taking steroids, leukotriene receptor antagonists or immunosuppressive drugs at the time of the visit. At the time of blood sampling, none of the patients had associated chronic inflammatory disorders, hepatitis C or story of recent acute infectious disease. In 7 patients blood samples were taken during active disease and during remission.

Forty-five healthy subjects (17 males and 28 females, median age 48 years, range 24-69 years) were considered as control group. All subjects participating in the study gave their informed consent to ASST and peripheral blood collection for eotaxin and C-reactive protein (CRP) determinations.

Autologous serum skin test

An intradermal test with 0.05 ml of fresh autologous serum (ASST) was carried out at least five days after stopping anti-histamine therapy (cetirizine, levocetirizine, desloratadine, rupatadine, ebastine or fexofenadine in all cases). ASST was performed following the method by Sabroe et al. (20), reading the weal and flare reaction at 30 min. Intradermal injection of saline solution (0.9% weight/volume NaCl) was performed as negative control and skin prick test with 10 mg/ml histamine as positive control. Patients showing a red weal with a diameter at least 1.5 mm greater than the control saline solution were considered positive.

Eotaxin determination

Eotaxin concentration was measured in serum samples taken from patients and controls using a sandwich enzyme immunoassay manufactured by R & D Systems (Minneapolis, MN, USA) employing a solid-phase bound

monoclonal antibody and an enzyme-linked polyclonal antibody specific for eotaxin. The sensitivity of the assay is less than 5 pg/ml. The intra- and inter-assay variability was lower than 12%.

C-reactive protein determination

C-reactive protein (CRP) serum concentration was measured using a sandwich enzyme immunoassay (Zymutest CRP, Hyphen BioMed, Neuville-sur-Oise, France). Intra- and inter-assay variability was lower than 11%.

Statistical analysis

Results were expressed as median and range. Differences were analysed by Mann-Whitney U test for unpaired values and Kruskal Wallis test (nonparametric ANOVA) with Dunn's multiple comparison test. Correlation was assessed by Spearman rank test. P values lower than 0.05 were considered significant.

Results

Serum eotaxin concentration was significantly higher in CU patients (median 140.1 pg/ml, range 33.7-718.7 pg/ml) than in healthy controls (median 108.9 pg/ml, range 45.5-409.4 pg/ml) (p=0.032) (Figure 1). ASST was positive in 65 CU patients and negative in 35. No significant difference was found between the two patient populations regarding eotaxin levels (median 145.6 pg/ml, range 48.5-648.3 pg/ml in ASST-positive patients; median 139.9 pg/ml, range 33.7-718.7 pg/ml in ASST-negative patients) (Table 1). Urticaria activity score was classified as mild (score 1) in 65 patients, moderate (score 2) in 26 patients and intense (score 3) in 9 patients. No significant difference was found among the three patient groups regarding eotaxin serum concentration, although the highest concentration was found in patients with intense CU (median 202.42 pg/ml, range 118.6-547.5 pg/ml vs. median 137.9 pg/ml, range 48.5-718.7 pg/ml in patients with mild CU). In the 7 patients observed during CU exacerbation and during remission, serum eotaxin levels

Figure 1 - Serum eotaxin levels in 45 normal subjects and 100 patients with chronic spontaneous urticaria. Individual values are reported and results are espressed as pg/ml. Horizontal bars are median values. Eotaxin levels were significantly higher in patients with chronic spontaneous urticaria than in controls.

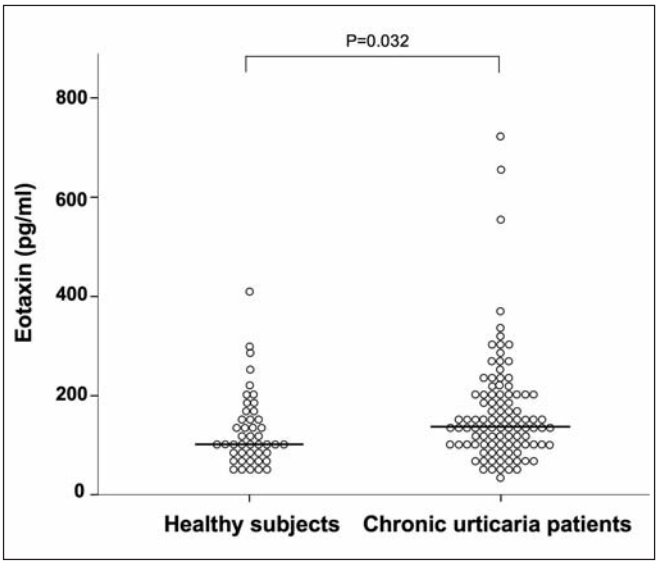
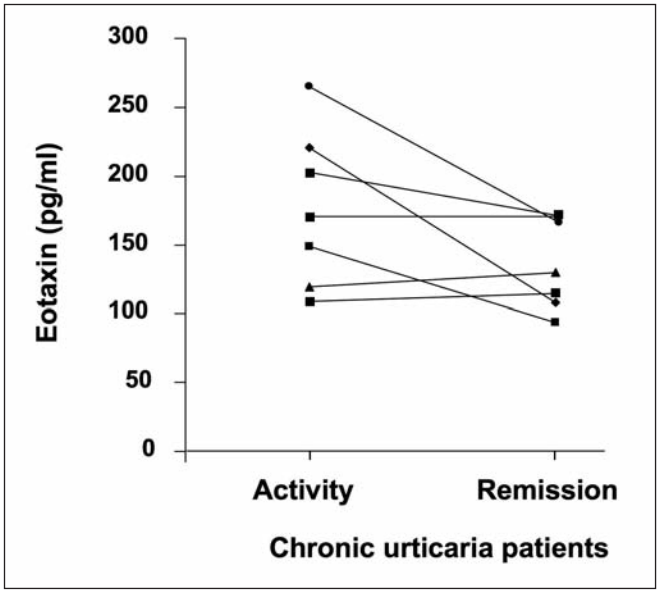


Table 1 - Eotaxin and CRP serum levels in CU patients divided according to the results of autologous serum skin test (ASST). Results are expressed as median and (range) and are not significantly different in the patient groups.

Patient category	N	Eotaxin (pg/ml)	CRP (μg/ml)
ASST-positive	65	139.9 (33.7-718.7)	2.0 (0.21-17.5)
ASST-negative	35	145.6 (48.5-648.3)	1.0 (0.19-18.66)

Figure 2 - Eotaxin levels in serum samples taken during active disease and during remission in 7 chronic urticaria patients.

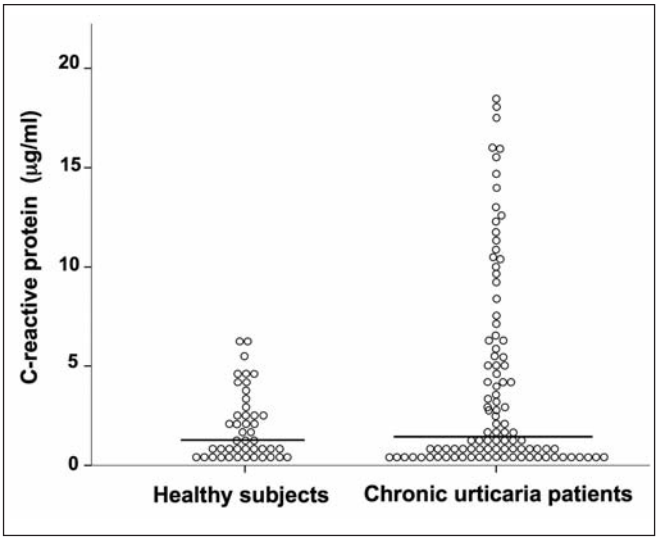


tended to decrease during remission, although statistical significance was not reached (median concentration decreased from 170.0 pg/ml, range 109.3-265.2 pg/ml, to 123.8 pg/ml, range 93.9-170.9 pg/ml) (Figure 2).
CRP levels were not significantly different in CU patients and healthy subjects, although there was a trend towards higher levels in the former population (median 1.35 μg/ml, range 0.19-18.7 μg/ml vs. median 1.15 μg/ml, range 0.19-13.5 μg/ml) (Figure 3). Moreover, it is noteworthy that 26 out of 100 CU patients had CRP levels above the 95th percentile of normal controls. CRP levels tended to be higher in ASST-positive than in ASST-negative patients (median 2.0 μg/ml, range 0.21-17.5 μg/ml vs. median 1.04 μg/ml, range 0.19-18.66 μg/ml). In the 7 patients observed during CU exacerbation and during remission, CRP levels significantly decreased during remission (median concentration from 4.1 μg/ml, range 0.3-17.8 μg/ml to 0.7 μg/ml, range 0.2-4.9 , p=0.015). No significant correlation was found between eotaxin and CRP serum levels.

Discussion

Our study shows that serum eotaxin levels are slightly higher in CU patients than in healthy controls suggesting its participation in the pathophysiology of CU. This disease is now increasingly recognized as an inflammatory disorder (6,7) and a variable degree of eosinophil infiltration has been detected in lesional skin together with lymphocytes and neutrophils (9,10). Eosinophils have been supposed to play a role in CU pathophysiology since they can release potent inflammatory mediators like major basic protein, eosinophil cationic protein, leukotriene C4 and platelet-activating factor (14), and can express tissue factor, the major initiator of blood coagulation (22,23). The activation of the blood coagulation cascade that has been found in CU patients seems mainly related to tissue factor-expressing eosinophils and contributes to amplify local inflammation and increase in permeability (24). Eotaxin is a potent chemoattractant for eosinophils (15,16) and could play a role in their recruitment and activation in the skin of CU patients (25,26).
The difference in serum eotaxin levels between CU patients and healthy controls was rather small, but this may be explained by the fact that eotaxin was measured in peripheral blood where it is diluted, and not in the skin microenvironment where eosinophil infiltration occurs. Serum eotaxin concentrations were not significantly different in patients with different urticaria activity scores, although they tended to be higher in patiens with intense symptoms. Furthermore, in the 7 patients observed during CU exacerbation and during remission, eotaxin serum levels tended to decrease during remission. These findings are in agreement with Hossny et al., who found increased eotaxin levels in children with acute urticaria with a marked decline during urticaria remission (17). In contrast, Caproni et al. failed to find any increase in plasma eotaxin in patients with CU (18).The discrepancy with our data may be explained by the selection of patients with different degrees of urticaria activity. In our case series, the increase of eotaxin concentration was modest but significant, probably reflecting a systemic inflammatory response occurring during active CU. It must be noted, however, that eotaxin levels did not show a good correlation with urticaria activity score and with serum levels of CRP, an inflammatory biomarker which correlates well with urticaria activity (6,7). In the 7 patients observed during CU exacerbation and during remission, CRP levels showed a significant drop during remission whereas eotaxin levels only tended to reduction. Therefore, CRP reflects urticaria activity more faithfully than eotaxin. No significant difference was found between ASST-positive and ASST-negative patients regarding serum eotaxin levels, not surprisingly because an inflammatory process un-

Figure 3 - C-reactive protein levels in serum samples from 45 normal subjects and 100 patients with chronic spontaneous urticaria. Individual values are reported and results are espressed as μg/ml. Horizontal bars are median values. C-reactive protein levels tended to be higher in patients than in controls although the difference did not reach statistical significance.



derlies CU either with or without detectable circulating histamine-releasing factors. In fact, increased levels of inflammatory markers as CRP and matrix-metalloproteinase-9 have been detected in CU patients independently of the presence of circulating histamine-releasing autoantibodies (5-7) and the cutaneous inflammatory cell infiltrate is moreless the same in patients with and without anti-FcεRIα or anti-IgE autoantibodies (8).

In sum, the results of this study show that serum eotaxin levels are increased in patients with CU, with a tendency to higher levels in patients with active disease. This suggests that eotaxin may be involved in CU pathophysiology by recruitment of eosinophils at sites of skin inflammation.

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