

CCL5/RANTES, sVCAM-1, and sICAM-1 in Chronic Spontaneous Urticaria

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Key Words

Chronic urticaria · RANTES · Interleukin-8 · Adhesion molecules · Soluble VCAM-1 · Soluble ICAM-1

Abstract

Background: Chronic urticaria (CU) is a common disease characterized by recurrent itchy wheals and/or angioedema for more than 6 weeks. We aimed to investigate the potential involvement of chemotactic mediators and soluble adhesion molecules as markers of endothelial dysfunction in the pathogenesis of chronic spontaneous urticaria (CSU). The potential relevance of these soluble mediators in the evaluation of disease activity was also investigated. **Methods:** We measured the levels of CCL5/RANTES, CXCL8/IL-8, sVCAM-1, and sICAM-1 in the sera of 87 patients with CSU and 61 normal healthy subjects (NHS) using ELISA assays. According to the results of autologous serum skin tests (ASST), CSU patients were classified into ASST-positive and ASST-negative subgroups. Furthermore, we investigated in 4 patients whether H₁-antihistamine therapy decreases sVCAM-1 and sICAM-1 levels. **Results:** We detected a significantly higher concentration of CCL5/RANTES ($p < 0.0001$) but not of CXCL8/IL-8 in CSU patients compared to NHS. The serum levels of sICAM-1 and sVCAM-1 were significantly increased

in CSU patients compared to NHS ($p = 0.0121$ and $p = 0.0043$, respectively). No difference in chemokine or soluble adhesion molecule levels was detected between the ASST-positive and ASST-negative subgroups. A positive correlation was found between sICAM-1 and sVCAM-1 ($p = 0.0022$) but not between these and CCL5/RANTES. After H₁-antihistamine therapy, sVCAM-1 and sICAM-1 levels did not decrease in the 4 CSU patients tested. **Conclusions:** Our study suggests that CCL5/RANTES, sICAM-1, and sVCAM-1 play a potential role in the pathogenesis of CSU but they do not parallel disease activity and are not predictive of the response to H₁-antihistamine therapy.

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Introduction

Chronic urticaria (CU) is a common and disabling disease characterized by recurrent itchy wheals and/or angioedema for more than 6 weeks [1]. These symptoms are the consequence of skin mast cell degranulation with the release of histamine and other vasoactive mediators. Chronic spontaneous urticaria (CSU) is frequently characterized by autoreactivity as intradermal injection of autologous serum causes wheal and flare reactions in 30–

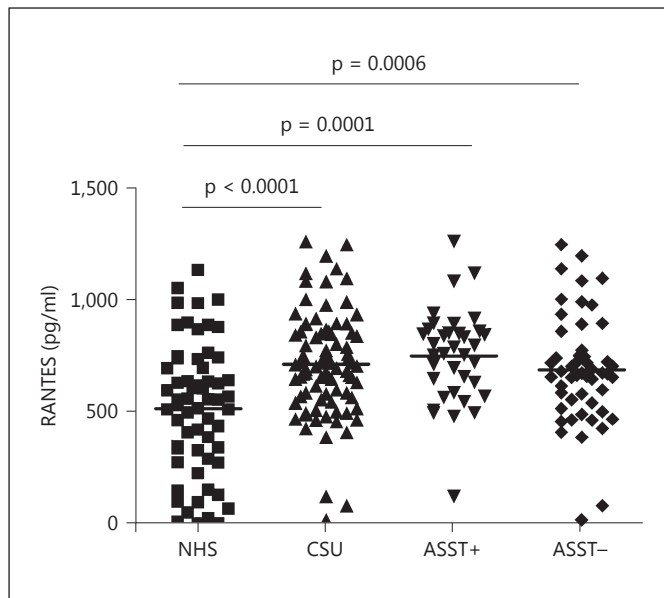


Fig. 1. Levels of CCL5/RANTES in the sera of CSU patients and in the ASST-positive (ASST+) and ASST-negative (ASST-) subgroups vs. the control group (NHS).

60% of patients [2]. Autoantibodies (anti-IgE, anti-FcεRI, and anti-TPO) [3] are detected in only one third of cases, suggesting that other circulating mediators released by immune cells and/or structural cells may be involved in the pathogenesis of CSU. Chemokines are a large family of small structurally homologous cytokines that stimulate leukocyte movement and regulate the migration of leukocytes from the blood to the tissue. Among chemokines, CCL5/RANTES is a potent chemoattractant protein for T cells, eosinophils, and monocytes [4] and CXCL8/IL-8 is the main chemoattractant factor for neutrophils [5]. An involvement of chemokines in the pathogenesis of CSU has been suggested by immunological studies conducted on skin biopsies [6, 7], sera [8–11], and purified peripheral blood mononuclear cells [10]. In addition, an important role for CCL5/RANTES, CCL2/MCP-1, and CXCL8/IL-8 in histamine and serotonin release by mast cells has been described [12]. Parallel to the chemokine system, the endothelium plays a critical role in controlling the passage of fluid into the tissue and influences cellular trafficking [13]. In the skin, dysfunction of the endothelium might increase vasopermeability, with a consequent proinflammatory response. The soluble forms of adhesion molecules such as VCAM-1 and ICAM-1 are widely used as biomarkers of endothelial dysfunction, and their increase in the circulation seems to reflect a proinflamma-

tory endothelium phenotype in several skin diseases, including CSU [6, 7, 14]. Since a link between the chemokine system and biomarkers of endothelial dysfunction in CSU has been hypothesized [15], the aim of our study was to investigate, in a large group of CSU patients, the circulating levels of CCL5/RANTES, CXCL8/IL-8, sICAM-1, and sVCAM-1 and their potential clinical relevance in the setting of the disease.

Materials and Methods

We studied 87 CSU patients (53 females and 34 males, age 46 ± 16 years, range 14–90 years) and 61 sex- and age-matched normal healthy subjects (NHS) as controls. CSU was diagnosed according to EAACI/GA²LEN/EDF guidelines [1]. All of the patients were being treated with nonsedating oral H₁-antihistamines (40% of the patients were treated with desloratadine 5 mg/day, 30% levocetirizine 5 mg/day, 10% rupatadine 10 mg/day, and 20% ebastine 10 mg), and the treatment was stopped 5 days before serum samples were taken for autologous serum skin tests (ASST) and for the detection of chemokines and soluble adhesion molecules. The study protocol conformed to the ethical guidelines of the Declaration of Helsinki. All of the subjects studied gave their informed consent before enrollment. Before starting H₁-antihistamine therapy, disease activity was calculated according to the recent EAACI/GA²LEN/EDF guidelines [1] using the urticaria activity score of 7 consecutive days (UAS7). ASST was performed as reported [16]. CCL5/RANTES, CXCL8/IL-8, sVCAM-1, and sICAM-1 concentrations were measured in the sera via commercial ELISA (R&D Systems, Inc., Minneapolis, Minn., USA) according to the manufacturer's instructions. The minimum detectable doses were: sICAM-1 0.096 ng/ml, VCAM-1 0.6 ng/ml, IL-8 30 pg/ml, and RANTES 15.6 pg/ml. Results are expressed as means $\pm$ SD. The Mann-Whitney U test was used to compare data between the CSU patients and NHS. Correlation analysis was performed using Pearson's test. $p < 0.05$ was considered statistically significant.

Results

Among the CSU patients, 36 (41.4%) had positive ASST and 51 (58.6%) had negative ASST. In 44 patients, the activity of the urticaria was scored. According to the UAS classification, urticaria was mild in 15 patients, moderate in 26, and severe in 3. As shown in figure 1, the levels of CCL5/RANTES were significantly higher in CSU patients compared to NHS (710.9 ± 25.58 vs. 512.7 ± 36.93 ng/ml; $p < 0.0001$). On the contrary, no differences in CXCL8/IL-8 levels were observed between the two groups (276.8 ± 85.54 vs. 165.6 ± 94.85 ng/ml). Quantification of soluble adhesion molecules showed that both sVCAM-1 (fig. 2a) and sICAM-1 (fig. 2b) were significantly increased in the sera of CSU patients compared to

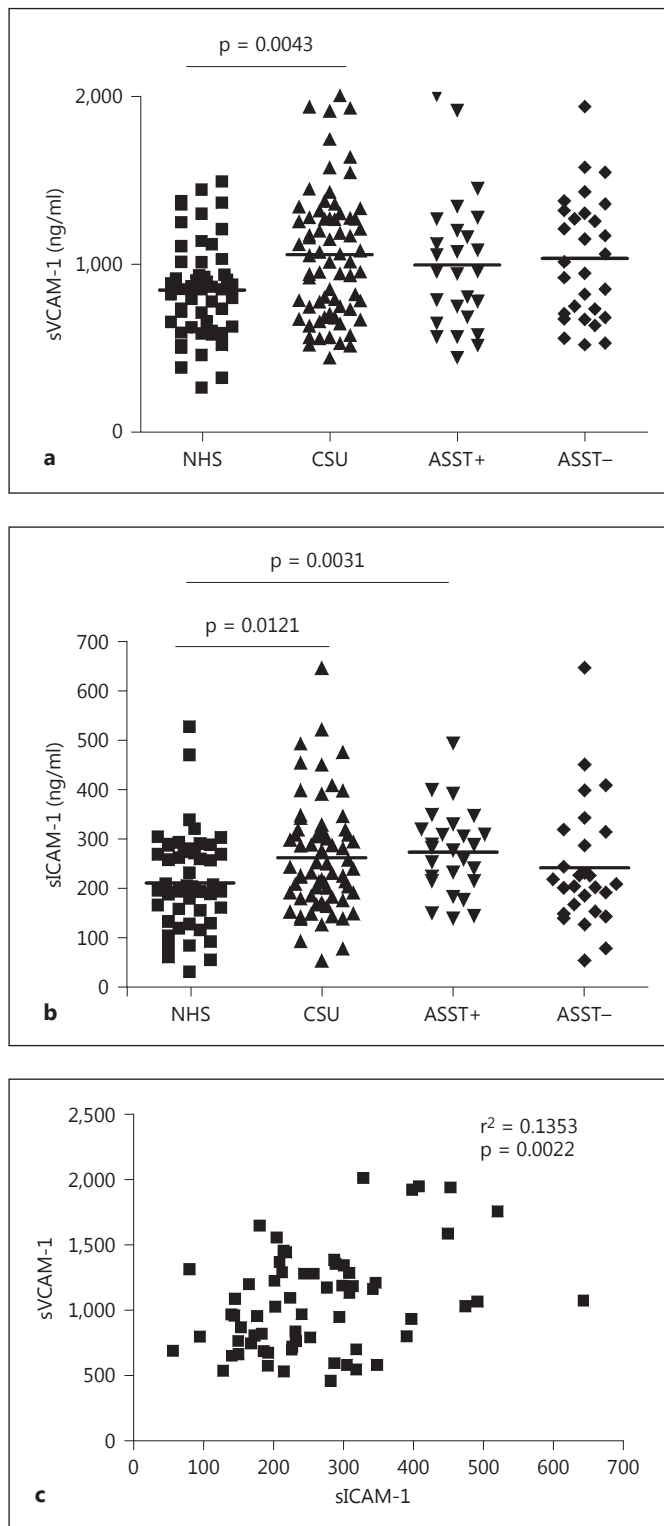


Fig. 2. Levels of sVCAM-1 (a) and sICAM-1 (b) in the sera of CSU patients and in the ASST-positive (ASST+) and ASST-negative (ASST-) subgroups vs. the control group (NHS). Correlation between levels of sVCAM-1 and sICAM-1 in the sera of CSU patients (c).

the control group (sVCAM-1: $1,049 \pm 376.9$ vs. 843 ± 286.5 ng/ml, $p = 0.0043$; sICAM-1: 263.2 ± 110 vs. 213 ± 92.67 ng/ml, $p = 0.0121$), with no differences between the ASST-positive and ASST-negative subgroups. A significant positive correlation was observed between the serum levels of sVCAM-1 and sICAM-1 ($r^2 = 0.1353$, $p = 0.0022$) (fig. 2c), but not between soluble adhesion molecules and CCL5/RANTES. When the potential relevance of CCL5/RANTES, sVCAM-1, and sICAM-1 in the clinical setting of the disease was investigated, no correlation between these soluble mediators and UAS7 was detected. In order to evaluate whether H₁-antihistamine therapy might affect the levels of sVCAM-1 and sICAM-1, four CSU patients with the highest levels of adhesion molecules were retested after 6 weeks of nonsedating H₁-antihistamine therapy. For comparison, we included another 4 CSU patients with normal levels of adhesion molecules in the analysis. After 6 weeks of H₁-antihistamine therapy with desloratadine 5 mg/day (2 patients) or levocetirizine 5 mg/day (1 patient) or ebastine 10 mg/day (1 patient), the serum levels of sVCAM-1 and sICAM-1 were unchanged in all of the CSU patients tested.

Discussion

In this study we showed that CCL5/RANTES, but not CXCL8/IL-8, and the biomarkers of endothelium dysfunction sVCAM-1 and sICAM-1 were increased in a large number of patients with CSU. Chemokines and their receptors have a fundamental role in the regulation of leukocyte trafficking, forming a highly redundant and complex network deeply involved in the expression of different inflammatory skin diseases [17]. Their pattern of expression shows overlapping features but also important differences. This seems to be due to the different sources and types of proinflammatory signals involved in their induction, but also to the inherent capacity of resident skin cells to produce chemokines. The increased levels of CCL5/RANTES, but not of CXCL8/IL-8, in the sera of CSU patients might explain the cellular profile detected in skin biopsies from urticaria, characterized by eosinophils, monocytes, and T cells. CCL5/RANTES might contribute not only to the recruitment of inflammatory cells to the skin [6] but also to the promotion of mast cell progenitor migration, differentiation, and activation by CCR5 binding [12]. Therefore, peripheral blood mononuclear cells and structural cells such as epithelial cells, keratinocytes, endothelial cells, and fibroblasts, through their production of CCL5/RANTES, may contribute to

the amplification and perpetuation of the inflammatory process during the late phase of urticaria. Parallel to chemokines, adhesion molecules play an important role in the migration of inflammatory cells to the site of inflammation. The serum increase in their soluble forms is strictly related to what occurs at the site of inflammation, leading to the definition of 'biomarkers' of endothelial functional status [13]. Augmented levels of ICAM-1 and VCAM-1 have been detected in skin biopsies from patients with CU [6], reflecting a proinflammatory phenotype of endothelium in this disease. However, in the literature the levels of ICAM-1 and VCAM-1 were not always significantly elevated in CU sera. [18]. On the contrary, our study, which included a large numbers of CSU patients, showed a significant increase in both sICAM-1 and sVCAM-1 levels in sera. These findings may suggest a potential epiphenomenon during CSU; however, an increase in these mediators may also represent a consequence of an inflammatory response driven by other factors. In fact, given the variety of all of the stimuli that can induce and produce the soluble factors we measured, it is difficult to depict the cellular circuits involved in CSU on the basis of increased levels of CCL5/RANTES and sVCAM-1/sICAM-1.

Parallel to soluble adhesion molecules, other markers of endothelial destruction such as factor VIII and von Willebrand factor have been detected in the plasma of CSU [19].

Due to the parallel role of chemokines and adhesion molecules in the inflammatory milieu, we also hypoth-

esized that CCL5/RANTES might exert their activity together with sVCAM-1 and sICAM-1 in order to promote cellular infiltration in the skin. The lack of correlation between CCL5/RANTES and soluble adhesion molecules suggests that these two systems act independently during CSU. Since some H₁-antihistamines are capable of reducing the expression of adhesion molecules in the skin of CU patients [18], we also investigated whether a new generation of H₁-antihistamines might induce any decrease in serum levels of sICAM-1 and sVCAM-1. Our data do not demonstrate any effects of H₁-antihistamine therapy on sera levels of these soluble adhesion molecules. These results are in line with those previously reported by Caproni et al. [18], showing that 6 weeks of treatments with levocetirizine 5 mg/day decreases the levels of ELAM-1 and P-selectin in the sera of patients with CU, but not those of sICAM-1 and sVCAM-1.

Taken together, our results indicate that RANTES/CCL5 and soluble ICAM-1 and VCAM-1 play a potential role in the pathogenesis of CSU but do not parallel disease activity and are not predictive of the response to H₁-antihistamine therapy.

Acknowledgements

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