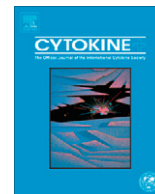


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Short Communication

Free IL-18 and IL-33 cytokines in chronic spontaneous urticaria

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ABSTRACT

Overproduction of IL-18 has been described in chronic urticaria. To evaluate free IL-18 and IL-33 in chronic spontaneous urticaria (CSU). IL-18, its inhibitor IL-18BP, IL-33 and its soluble receptor ST2 (sST2) were measured (ELISA) in the sera of 73 CSU patients. Free IL-18 was calculated (law of mass action). Autologous serum skin test (ASST) was performed in all patients. Total IL-18, IL-18BP and free IL-18 serum levels were significantly higher in CSU than in controls. IL-18 and IL-18BP increased significantly in both ASST-positive and negative subgroups. Free IL-18 resulted significantly higher in the ASST-negative, but not in the ASST-positive subgroup. No differences in IL-33/sST2 levels were detected between CSU and controls. Increased levels of free IL-18 and IL-18BP, but not IL-33, was detected in CSU. Whether IL-18 up-regulation is a consequence of inflammation or one of the causes of the pathology needs to be addressed.

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1. Introduction

Chronic spontaneous urticaria (CSU) is a common and disabling disease characterized by recurrent itchy wheals with or without angioedema for more than 6 weeks [1]. These symptoms are the consequence of skin mast cells degranulation with release of histamine and other vasoactive mediators. CSU is frequently characterized by autoreactivity as intradermal injection of autologous serum causes a wheal and flare reaction in 30–60% of patients [2]. Auto-antibodies (anti-IgE, anti-FcεRI, anti-TPO) are detected in only one third of the cases, suggesting that other circulating mediators, including cytokines, may be involved in the pathophysiology of CSU.

Cytokines of the interleukin 1 (IL-1) family play a key role in mediating the activation of innate and acquired immune responses. A member of the IL-1 family is the pro-inflammatory cytokine IL-18, initially identified as a major inducer of IFN- γ in Th1 and NK cells, with a key role in Th1 activation in synergy with IL-12, although also capable of inducing Th2 cytokines [3]. IL-18 activity is regulated by IL-18BP, a soluble molecule that binds mature IL-18 with high affinity and prevents its interaction with cell surface receptors. In fact, IL-18 can be bound and neutralised by its soluble inhibitor IL-18BP and only the fraction of IL-18, that is not bound to IL-18BP, is actually free

of interacting with the membrane receptors on target cells/organs and is biologically active. The interaction of IL-18 with IL-18BP has been described in detail, and is a stoichiometric 1:1 interaction with a K_d of 400 pM [4]. Thus, the level of free IL-18 depends on the absolute concentrations of both IL-18 and IL-18BP, and on their affinity of interaction, according to the law of mass action.

IL-33, constitutively expressed in endothelial and epithelial cells, is an intracellular protein that is also released in the extracellular space following cell injury. IL-33, similarly to IL-1 α , may function both as a pro-inflammatory cytokine and as an intracellular nuclear factor involved in transcriptional regulation. Its receptor ST2 is an IL-1R-related protein expressed on Th2 cells, mast cells, basophils and eosinophils. Consistent with the expression of ST2, IL-33 induces or amplifies Th2-type responses, but also displays pro-inflammatory effects in different disease models [5]. A soluble form of ST2 (sST2) is generated by alternative gene splicing, which functions as a soluble decoy that binds IL-33 and prevents its binding to the membrane receptor [6].

The role of IL-33 in skin diseases is still unknown, but it has been shown that IL-33 mRNA levels are increased almost 10-fold in the skin of atopic dermatitis patients compared to healthy subjects [7]. Elevated levels of sST2 are associated to a wide range of diseases, from food allergy to Dengue virus infection to sepsis, and sST2 is being considered as a prognostic biomarker in heart failure [8].

In order to have a comprehensive view of the functional role of IL-18 and IL-33 in CSU, we evaluated the free IL-18 levels in sera of CSU patients, by analyzing IL-18 and IL-18BP concentrations, and

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measured the levels of IL-33 and its soluble receptor sST2. The results were correlated with the clinical features of the disease.

2. Methods

2.1. Subjects

We studied 73 CSU patients (46 female and 27 male; age 41 ± 16 , range 14–89) and 40 sex-matched and age-matched healthy subjects (HS) (30 female and 10 male; age 50 ± 5 , range 25–89) as control. CSU was diagnosed according to the EAACI/GA²-LEN/EDF guidelines [1]. All the patients were being treated with non-sedating oral H1 anti-histamines, stopped 5 days before serum samples were taken for detection of Autologous Serum Skin Test (ASST), IL-18, IL-18BP, IL-33 and sST2. The study protocol conformed to the ethical guidelines of the Declaration of Helsinki. All the subjects studied gave their informed consent before enrollment. Disease activity in all CSU patients was determined by use of the UAS7, the urticaria activity score of seven consecutive days as previously reported [9]. ASST was performed as reported [10].

2.2. Detection of IL-18 and IL-33 cytokines

IL-18 was measured by ELISA with commercially available kits (human IL-18 ELISA kit; MBL, Woburn, MA, USA). Detection of IL-18BP was performed with the commercial IL-18BP ELISA (R&D Systems, Minneapolis, MN, USA). After measurement of the concentrations of both IL-18 and IL-18BP in each sample, the law of mass action was used to calculate free IL-18 (i.e., the fraction of cytokine not bound to its inhibitor IL-18BP), as described by Novick et al. [4] and modified by Migliorini et al. [11]. IL-33 and its soluble receptor sST2 were measured by ELISA with available commercially kits (Duo-set; R&D Systems, Minneapolis, MN, USA).

2.3. Statistical analysis

Results are expressed as mean $\pm$ SD. The Mann–Whitney *U*-test was used to compare data between the patient groups and the HS. A *P* value less than 0.05 is considered statistically significant.

3. Results and discussion

Among the CSU patients, 32 (43.8%) had positive and 41 (56.2%) negative ASST. The levels of IL-18 and IL-18BP were significantly higher in CSU patients compared to HS ($P < 0.0003$ and $P < 0.0001$, respectively) (Fig. 1A and B), without differences between the ASST-positive ($P < 0.0027$ and $P < 0.0021$ for IL-18 and IL-18BP, respectively) and negative ($P < 0.0013$ and $P < 0.0005$ for IL-18 and IL-18BP, respectively) sub-groups. As shown in Fig. 1C, serum levels of free IL-18 were significantly higher in CSU patients than in controls ($P < 0.0001$), with significant differences between the ASST-negative subgroup and HS ($P = 0.022$), but not between the ASST-positive subgroup and HS. In order to investigate the potential role of free IL-18 in the clinical activity of CSU patients, we scored urticaria activity of seven consecutive days (UAS7) in 58 patients and correlated the concentrations of total IL-18 and IL-18BP, and of free IL-18, to the UAS7 in these subjects. According to the UAS classification, urticaria was mild in 20, moderate in 31 and severe in 5 patients. Total or free IL-18 levels and IL-18BP levels were not correlated with UAS7; mean levels of the cytokines were not significantly different in the three groups of patients.

No differences in IL-33 and its soluble receptor sST2 levels have been observed between CSU and HS groups (575.3 ± 105.6 vs. 1189 ± 271.5 and 397.1 ± 78.3 vs. 404.1 ± 117.3 , respectively) (data

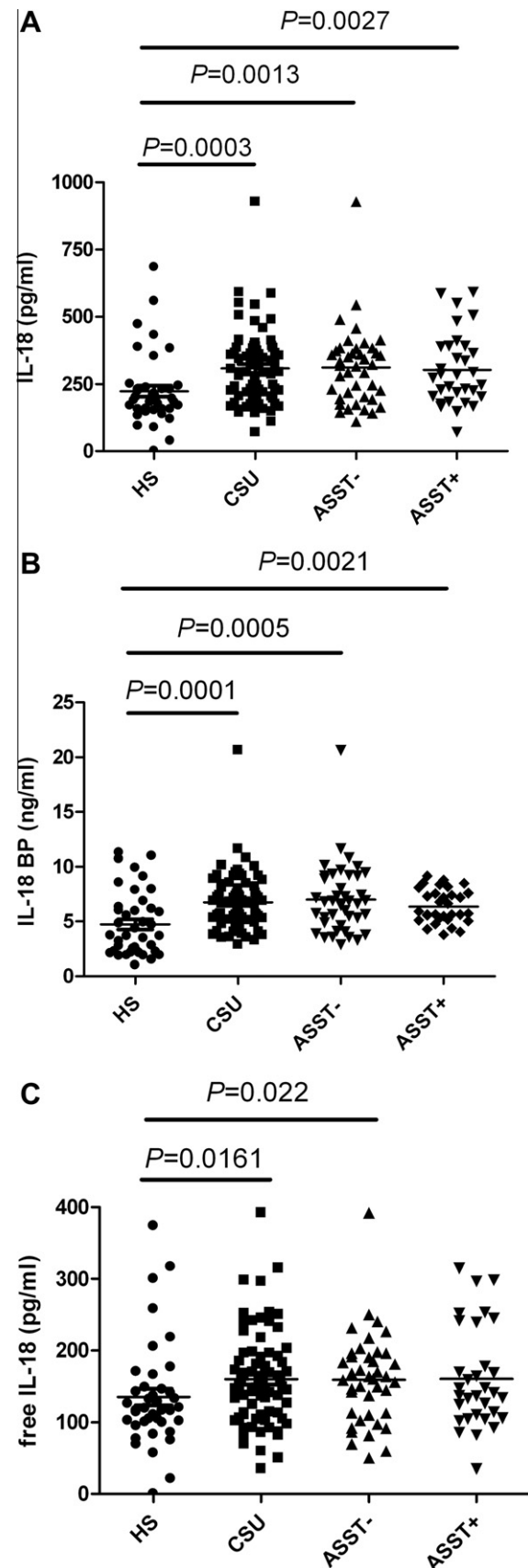


Fig. 1. Levels of IL-18, IL-18BP and free IL-18 in the sera of CSU patients. Levels of IL-18 (A), IL-18BP (B) and free IL-18 (C) in the sera of CSU patients and in the ASST-negative (ASST-) and ASST-positive (ASST+) subgroups vs. control group (HS).

not shown). IL-33 and sST2 levels were not different in the ASST-positive or negative subgroups and were not correlated with UAS7.

These data indicate that in CSU, like in other inflammatory conditions characterized by high levels of IL-18, IL-18BP levels are also increased, in the attempt to counteract the inflammatory effects of IL-18 [11]. This is possibly due to the fact that IL-18 indirectly increases the production of its own inhibitor in a feedback loop, through up-regulation of the major IL-18BP inducer IFN- γ [3]. On the contrary, our data do not support a role of IL-33 in CSU confirming that IL-33 may function mainly as a nuclear alarmin. Conflicting results have been recently reported on IL-18 levels in CSU. In a small group of patients, Tedeschi et al. did not find any difference in IL-18 serum levels between CSU patients and healthy controls but, interestingly, in the ASST-positive group the IL-18 level correlated with the clinical activity of urticaria assessed by the clinical severity score [12]. On the contrary, a correlation between IL-18 levels and ASST-positive chronic urticaria has been detected by Kurt et al. [13]. In both studies, however, only total IL-18 levels were analyzed, and thus the functional consequences of the increased cytokine levels are unclear.

Our data suggest that increased levels of free IL-18 may contribute to the recruitment and activation of cells involved in the pathogenesis of CSU. A mixed inflammatory perivascular infiltrate of neutrophils, eosinophils, macrophages and T cells, producing Th2 and Th1 cytokines, has been described in the skin of CSU patients [1]. Eosinophils stimulated by IL-18 up-regulate IL-8 expression [14], suggesting that increased levels of functional active IL-18 may stimulate skin eosinophils to release IL-8 with consequent recruitment of neutrophils in inflamed tissue.

The role of IL-18, and its balance with other cytokines of the IL-1 family, in pathological skin inflammation needs further studies. Urticaria and skin rash are common symptoms in several inflammatory diseases, including auto-inflammatory diseases and drug-induced inflammation. In all these circumstances the excessive production of IL-18 has been described, but it remains to be

understood whether IL-18 up-regulation is a consequence of the inflammatory reaction or one of the causes of the pathology.

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