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Serum interleukin-18 in patients with chronic ordinary urticaria: association with disease activity

A. Tedeschi, M. Lorini, C. Suli and R. Asero*

*Allergy and Clinical Immunology Unit, Hospital Maggiore Policlinico, Mangiagalli and Regina Elena, Fondazione IRCCS, Milan, Italy; and *Allergy Unit, San Carlo Clinic, Paderno Dugnano, Milan, Italy*

Summary

Interleukin (IL)-18 is a pleiotropic cytokine, which may play a role in autoimmune and allergic disorders. Serum IL-18 levels were measured in 34 patients with chronic ordinary urticaria (COU) and 17 normal subjects. *In vivo* and *in vitro* assays for histamine-releasing factors, an autologous serum skin test (ASST) and a basophil histamine release assay were also performed for all patients with COU. Serum IL-18 concentration was not significantly different between patients with COU and normal subjects (mean ± standard error of the mean 246.47 ± 18.40 pg/mL vs. 213.88 ± 22.24 pg/mL), and no significant difference was found between ASST-positive and ASST-negative patients. However, in ASST-positive patients, IL-18 levels paralleled clinical severity scores and showed a tendency to correlate with *in vitro* histamine release. The increased IL-18 levels in the ASST-positive patients with most active chronic urticaria may reflect stronger immune system activation and possibly an involvement of IL-18 as a direct histamine-releasing factor.

Interleukin (IL)-18 is a pleiotropic cytokine produced by monocytes/macrophages, dendritic cells, keratinocytes, Langerhans' cells and B cells, which can activate T cells and induce either T-helper (Th)1 or Th2 responses, depending on the cytokine environment.[1] It has been suggested that IL-18 may play an important role either in autoimmune disorders, characterized by a predominant Th1 response, or in allergic diseases, characterized by a Th2 response.[2] In recent years, chronic ordinary urticaria (COU), formerly considered an idiopathic disorder, has been shown to be often an autoimmune disease due to histamine-releasing autoantibodies directed against the α subunit of the high-affinity IgE receptor or against IgE.[3] The autologous serum skin test (ASST) is an *in vivo* screening test for histamine-releasing autoantibodies and other vasoactive factors, and allows discrimination between chronic autoimmune and chronic idiopathic urticaria.[4,5] However, the pathomechanism of COU is complex, and T lymphocyte-mediated mast-cell activation has also been suggested, because a perivascular infiltration of T lymphocytes, which are predominantly CD4-positive, together with monocytes, neutrophils and eosinophils, has been found at sites of urticarial lesions.[6] The possible involvement of IL-18 in COU has not yet been explored. In the present study, we measured serum IL-18 levels in patients with COU and evaluated the possible association of this cytokine with circulating histamine-releasing factors and clinical severity.

Correspondence: Dr A. Tedeschi, UO Allergologia e Immunologia Clinica, Ospedale Maggiore Policlinico, Mangiagalli e Regina Elena, Fondazione IRCCS, Via Pace, 920122 Milano, Italy.
E-mail: albited@alice.it

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Report

In total, 24 adult patients (6 men, 28 women; mean ± SEM age 45.9 ± 2.6 years), diagnosed with COU at the allergy centres of the Hospital Maggiore Policlinico and the San Carlo Clinic (both Milan, Italy) during the first quarter of 2005 were selected for the study. COU was

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diagnosed on the basis of the recurrence of hives with or without angio-oedema for >6 weeks. In all cases, known causes of chronic or recurrent urticaria were ruled out by appropriate investigations. Patients with physical urticaria were also excluded. Two patients had autoimmune thyroiditis; no other autoimmune or chronic inflammatory disorders were reported. All patients had active urticaria at the time of the study and were being treated with H1 antihistamines. These drugs (cetirizine, levocetirizine, loratadine, desloratadine or fexofenadine in all cases) were stopped 5 days before serum samples for *in vivo* (ASST) and *in vitro* [basophil histamine release (BHR) and IL-18] assays were taken. Disease activity was graded from 1 to 4 (slight, moderate, severe and very severe) according to the number of weals present at the time when blood samples were collected, as previously described by Sabroe *et al.*[7] Sera from 17 gender-matched and age-matched healthy subjects (3 men, 14 women; mean ± SEM age 43.9 ± 3.3 years) formed the control group. All subjects participating in the study gave their informed consent.

ASST was performed by intradermal injection of 0.05 mL of fresh autologous serum and read at 30 min, following the method by Sabroe *et al.*[4] Intradermal injection of saline solution (0.9% w/v NaCl) was used as negative control and skin-prick test with 1 mg/mL histamine as positive control. Patients showing a weal with a diameter at least 1.5 mm greater than that produced by the control saline solution were considered positive. BHR was performed using leucocyte suspensions obtained from three normal blood donors, showing a 30% net release on challenge with an optimum dose (10 μg/mL) of goat polyclonal antihuman IgE (Sigma Chemical Co., St. Louis, MO, USA), as described previously.[8] Histamine concentration was measured by an automated fluorometric method, and a 5% net release cut-off value was used. One assay per serum was performed to detect histamine-releasing activity. Serum IL-18 concentration was measured by a sandwich enzyme immunoassay with a sensitivity of 12.5 pg/mL (MBL Medical & Biological Laboratories, Nagoya, Japan).

Results were expressed as mean ± SEM. Mann–Whitney *U*-test, Kruskal–Wallis test with Dunn's multiple comparisons test, Fisher's exact test and Spearman's rank correlation test were used for statistical analysis. *P*-values < 0.05 were considered significant.

Mean ± SEM IL-18 concentration was 246.67 ± 18.40 pg/mL in COU patients and 213.88 ± 22.24 pg/mL in normal subjects (NS; Table 1). In total, 17 patients were positive on ASST (50%) and 17 patients were negative. IL-18 concentration was not significantly different between ASST-positive and ASST-negative patients (241.29 ± 27.95 pg/mL vs. 251.65 ± 24.73 pg/mL, respectively; NS). Two ASST-negative patients had associated autoimmune thyroiditis, and their IL-18 concentrations were 310 and 220 pg/mL, respectively. BHR was positive in 9 of 17 ASST-positive patients and in 2 of 17 ASST-negative patients (52.9% vs. 11.1%, $P = 0.02$) with a mean ± SEM percentage release of 14.2 ± 2.4% and 6.1% ± 0.4%, respectively. A trend towards a positive correlation was found between percentage histamine release and serum IL-18 concentration in ASST-positive patients with a positive BHR assay ($r = 0.37$, NS). When patients with COU were grouped according to ASST and BHR results, no difference in serum IL-18 concentration was observed between patients who tested positive or negative on ASST or BHR (Table 2). However, when patients were classified according to disease severity, those with severe disease showed the highest IL-18 levels. This was very clear in ASST-positive patients (Fig. 1a), whereas it was not evident in ASST-negative patients (Fig. 1b).

In conclusion, serum IL-18 concentrations did not differ significantly between patients with COU and normal subjects, nor did they differ in patients with COU according to ASST and BHR assay results. Nevertheless, interestingly, IL-18 levels paralleled disease severity in the ASST-positive group and showed a tendency to correlate with *in vitro* BHR. This suggests that in ASST-positive patients there may be some role for IL-18 as a direct histamine-releasing factor, in

Table 1 Serum IL-18 levels (pg/mL) in patients with COU and control healthy subjects.

Patient category	*n*	Mean ± SEM	Median (range)
Patients with COU	34	246.47 ± 18.4	220 (74–520)
Control subjects	17	213.88 ± 22.24	230 (72–410)

Table 2 Serum IL-18 levels (pg/mL) in patients with COU divided in subsets according to results of ASST and BHR assays.

Patient category	*n*	Mean ± SEM	Median (range)
ASST-positive	17	241.29 ± 27.95	196 (74–520)
ASST -negative	17	251.65 ± 24.73	220 (120–480)
ASST-positive/BHR-positive	9	243.78 ± 39.10	196 (74–400)
ASST-positive/BHR-negative	8	238.50 ± 42.73	208 (140–520)
ASST-negative/BHR-negative	15	258.53 ± 27.56	240 (120–480)
ASST-negative/BHR-positive	2	200.00 ± 28.28	200 (180–220)

ASST, autologous serum skin test; BHR, basophil histamine release.

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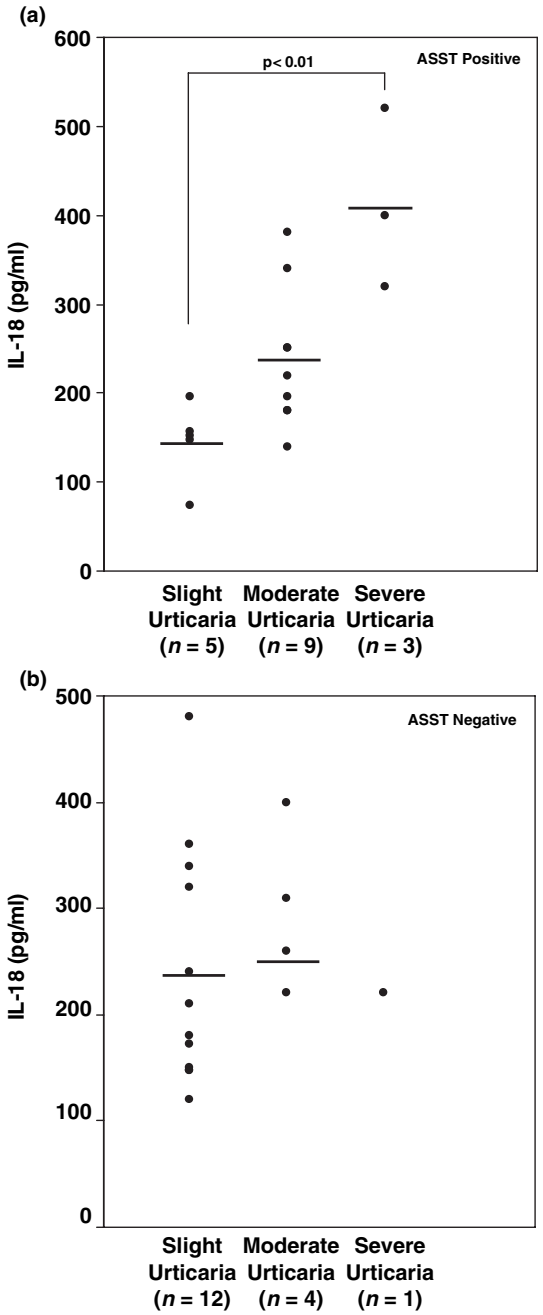

Figure 1 (a) Serum IL-18 levels in ASST-positive patients classified according to clinical severity score. Statistical analysis by Kruskal–Wallis test showed that there was a significant difference between the three groups ($P = 0.01$). Post tests were performed by Dunn's multiple comparisons test. (b) Serum IL-18 levels in ASST-negative patients classified according to clinical severity score. No significant difference was found between the patient groups.

agreement with previous observations on its capacity to stimulate IL-4 production and histamine release by basophils.[9] In contrast, the possible role of IL-18 as a direct releasing factor is not evident in ASST-negative patients, suggesting that different disease pathomechanisms may operate in the two patient groups. An autoantibody-mediated mast cell activation has been demonstrated in most ASST-positive patients; in the remaining patients, mast-cell activation might be due to other mechanisms, perhaps involving T-lymphocytes or coagulation factors such as thrombin, which might act as amplifiers/inducers of inflammation.[10]

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