



LETTER TO THE EDITOR

Serological detection of eotaxin, IL-4, IL-13, IFN- γ , MIP-1 α , TARC and IP-10 in chronic autoimmune urticaria and chronic idiopathic urticaria

Dear Sir,

Chronic idiopathic urticaria (CIU) includes a heterogeneous patients' subset in which the autologous serum skin test (ASST) is able to induce in vivo a wheal-and-flare response. ASST+ positive(+) patients' serum contains different autoantibody activities leading to histamine release and supposed to play a main pathogenetic role.

Autoantibody detection allows to speculate about an autoimmune pathogenesis [the so called "chronic autoimmune urticaria" (CAIU)], that is further suggested by the increased incidence of immunological disorders in the same subjects [1].

It has been previously demonstrated that infiltrating cell amount and type, expression of cytokines, chemokines and cellular adhesion molecules are partially overlapping in lesions from serum+ and negative(–) patients. Furthermore, no significant differences have been noted overall between lesional and unaffected skin from CIU patients [2].

This last finding has suggested the possibility of a wide immunological activation in chronic urticaria involving not only lesional tissues, but also extending to the whole skin's immune system [2]. This "latent urticarial status" could explain the extreme spread that characterises CIU wheals.

To further validate this hypothesis, in this study representative Th1 and Th2 inflammatory markers (IFN- γ , MIP-1 α , IP-10; IL-4, IL-13, eotaxin, TARC) were investigated in patients affected by CIU with either positive or negative ASST, and in control subjects.

Sixty-eight patients suffering from CIU (46 female, 22 male, mean 43 years, range 19–81) were enrolled. Antihistamines were discontinued one week before examination and none of the patients was taking any other drug at that moment.

Physical urticaria, allergic urticaria, urticaria vasculitis, C₁-esterase-inhibitor deficiency, drug or alcohol abuse, pregnancy and lactation were considered as exclusion criteria.

As control, 20 healthy volunteers (12 female, 8 male, mean 39 years, range 21–74) were tested.

Clinical evaluation of urticaria activity was performed according to the Breneman scale score [3]. We investigated the presence of angioedema, gastrointestinal symptoms, flushing, associated autoimmune thyroid disease, familiar or personal history of atopy (IgE > 100 KU/L, atopic dermatitis, asthma, hay fever or rhinitis).

Peripheral blood was collected from patients during a whealing episode, and from controls. Patients' sera were used to perform both ASST and histamine release assay (HRA) as previously described [4].

Moreover, a solid phase enzyme-linked immunosorbent assay (ELISA) was used to determine IL-4, IFN- γ (ICN Biomedicals, Aurora, Ohio), IL-13 (Technogenetics, Milan, Italy), eotaxin, MIP-1 α , TARC and IP-10 (R&D, Minneapolis, USA) according to manufacturer's instructions. Cytokine concentrations were presented as mean value $\pm$ standard deviation (S.D.) (Table 1).

Statistical evaluation was carried out using the χ^2 test for clinical data, and non-parametric Kruskal–Wallis test for serological findings. Statistical significance was considered for $p \leq 0.05$.

ASST was positive in 23/68 (34%) patients (9 male, 14 female, mean 45 years), while 45 subjects (66%) gave a negative response (13 male, 32 female, mean 41 years). These findings agree with previous investigations, who assessed a positive response to 27–50% of CIU subjects [1]. By contrast, HRA was positive in 18/68 patients (26%), all belonging to the serum+ group. The discrepancy between in vivo and in vitro tests (23 versus 18 patients) had already been noted [5] and it suggests that autoantibody activity could be more easily initiated in vivo than in vitro, where histamine-releasing cofactors (complement or others) may be absent or fail to have effect.

Clinically, ASST+ subjects showed higher wheal number and more frequent urticaria episodes than the ASST– group (data not shown, $p < 0.0001$). Patients with autoantibodies had also higher itch scores [5], whereas there was no difference between ASST+ and ASST– group in the incidence

Table 1 IgE, cytokine and chemokine serum levels in patients with positive and negative ASST, and in healthy controls

	Normal range	CIU ASST+ (mean $\pm$ S.D.)	CIU ASST- (mean $\pm$ S.D.)	p^1	HC (mean $\pm$ S.D.)	p^2
IgE	<100**	48.3 $\pm$ 23.0	57.7 $\pm$ 30.4	n.s.	53.0 $\pm$ 18.6	n.s.
IL-4	0–31.2*	0.03 $\pm$ 0.13	1.49 $\pm$ 4.4	n.s.	0.01 $\pm$ 0.06	n.s.
IL-13	0–44.4*	14.5 $\pm$ 25.6	5.8 $\pm$ 9.7	0.006	2.4 $\pm$ 2.2	0.0001
IFN- γ	0–36.3*	13.8 $\pm$ 18.4	29.5 $\pm$ 40.9	n.s.	10.7 $\pm$ 12.8	n.s.
Eotaxin	50–642*	119.1 $\pm$ 59.2	97.6 $\pm$ 40.9	n.s.	132.8 $\pm$ 52.4	n.s.
MIP-1 α	< 46.9*	15.5 $\pm$ 31.4	29.8 $\pm$ 74.9	n.s.	6.5 $\pm$ 10.0	n.s.
TARC	71–848*	306 $\pm$ 301	375 $\pm$ 469	n.s.	258 $\pm$ 123	n.s.
IP-10	38–361*	93.5 $\pm$ 43.7	78.6 $\pm$ 36.9	n.s.	72.1 $\pm$ 20	n.s.

CIU: chronic idiopathic urticaria patients; ASST: autologous serum skin test, +: positive /–: negative; HC: healthy controls; S.D.: standard deviation; p^1 : statistical comparison between ASST+ and ASST– subjects; p^2 : statistical comparison between ASST+ subjects and healthy controls; n.s.: not significant.

* pg mL $^{-1}$.

** KU/L.

of angioedema, gastrointestinal symptoms, flushing and atopy. Autoimmune thyroid disease was more frequent in serum+ patients (data not shown, $p = 0.06$) [5]. These data all seem to confirm that a more severe form of urticaria with higher frequency of acute attacks, higher lesion number, and higher itch scores, characterizes the serum+ group, as previously reported [1].

Concerning cytokine and chemokine evaluation, in each group (ASST+/ASST–/HC) mean concentrations resulted within the normal range, although increased IL-13 levels (Fig. 1) and MIP-1 α levels were found in some ASST+ and ASST– subjects, respectively. Besides no significant differences could be detected between urticaria subjects and healthy controls, nor between serum+ and serum–. The only exception was represented by IL-13, whose expression was augmented in CIU patients compared to controls ($p = 0.0001$), the highest concentration being detected in serum+ individuals ($p = 0.006$).

To date, only few studies [6–8] deal with the determination of the serological profile in CIU, and

they overall observe neither a dominant Th1 nor Th2 cytokine pattern. Our data partially overlap those mentioned, reproducing normal IFN- γ levels in urticaria and higher MIP-1 α levels in serum– subjects. As previously shown, we also demonstrated that IL-4 concentrations were comparable to healthy controls [6], without any difference between ASST+ and ASST– patients.

However, since IL-4 expression has been reported in lesional urticaria skin by immunohistochemistry [2], suggesting a role of the cytokine in mast-cell degranulation and wheal development, it could be supposed that low circulating levels might depend on rapid cytokine consumption in lesional skin, as it happens in other autoimmune disorders.

In fact, a slightly prevalent Th2-polarized activity also emerged from our data in virtue of increased IL-13 expression in CAIU patients, renewing the trend previously observed by Irinyi et al. [7]. As is known, pleiotropic cytokine IL-13 shares biological properties with IL-4, such as isotype switching to IgE and expression of endothelial cell adhesion molecules [9]. Based on these similarities, it could be supposed that in CIU IL-13 might increase mast-cell Fc ϵ RI expression and mediator release [10], leading to hive development and cutaneous inflammation enhancement. Accordingly, we found higher cytokine levels in patients showing more frequent and diffuse exacerbations with severe itch (Fig. 1), thus suggesting the preliminary hypothesis to regard IL-13 as a serological marker of disease activity.

Taken together, our data support an inflammatory etiopathogenesis in chronic urticaria, with increased IL-13 levels mainly characterizing CAIU patients. Cytokine and chemokine short half-life and consumption in lesional tissues, due to autocrine and paracrine activity, may explain why patients and

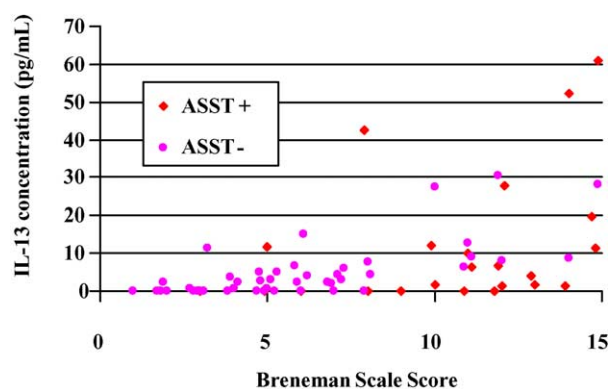


Fig. 1 IL-13 concentrations and disease activity (according to Breneman scale score) in ASST+ and ASST– subjects.

controls show comparable concentrations of other molecules analyzed.

Overall, serological immune profile of our series highlighted a mixed Th1/Th2 pattern with a slight Th2 predominance.

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