

Infiltrating cells and related cytokines in lesional skin of patients with chronic idiopathic urticaria and positive autologous serum skin test

Caproni M, Volpi W, Macchia D, Giomi B, Manfredi M, Campi P, Cardinali C, D'Agata A, Fabbri P. Infiltrating cells and related cytokines in lesional skin of patients with chronic idiopathic urticaria and positive autologous serum skin test
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Abstract: In approximately one-third of patients with chronic idiopathic urticaria (CIU), autoantibodies against the high-affinity IgE receptor and/or against IgE can be detected and a wheal-and-flare response can be provoked by the intradermal injection of autologous serum (ASST). In this study we aimed to further characterize the inflammatory response observed in the subgroup of CIU patients with positive ASST and serum-evoked histamine-release *in vitro* from basophils in comparison with unaffected skin and healthy donors. An immunohistochemical analysis of infiltrating cells (CD4, MPO, EG1, EG2, tryptase), cytokines (IL-4, IL-5, IFN- γ), chemokines and chemokine receptors (IL-8, CCR3, CXCR3), and adhesion molecules (ICAM-1, VCAM-1, ELAM-1) was performed on seven selected patients (four males and three females; median age: 45 years; range: 22–57) and five healthy donors. Cytokine evaluation was also performed in five psoriatic patients to obtain an additional control. In spontaneous wheals we observed an increased number of CD4+ T lymphocytes when compared with the controls, and an increased number of neutrophils and eosinophils, whereas mast cells did not show a significant variation. A significant expression for IL-4 and IL-5 could only be observed in lesional skin, while IFN- γ showed a slight expression in the same site. Chemokine receptors CCR3 and CXCR3 did not show a defined polarized response in either lesional or unaffected skin. An increased expression of all cellular adhesion molecules (CAMs) studied was detected in spontaneous wheals. The lack of a significant difference in the expression of tryptase + mast cells, T lymphocytes, IL-8, CXCR3 and CCR3, a few CAMs between the lesional and unaffected skin of CIU patients suggests a wide immunological activation that involves not only lesional tissues, but possibly extends to the whole of the skin's immune system.

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Introduction

Chronic idiopathic urticaria (CIU) is a common skin disorder defined by the daily or almost daily occurrence of widespread pruritic wheals for at least 8 weeks, without an identified and specific disease aetiology (1). Greaves and coworkers (2) have demonstrated that in approximately one-third of CIU patients, skin testing with autologous serum is able to elicit an immediate wheal-and-flare response during disease activity. On this

basis, further studies (3–7) have repeatedly confirmed *in vitro* such histamine-releasing activity of CIU sera and have attributed it to the presence of functional circulating antibodies (Abs) against IgE or against the high-affinity IgE receptor (Fc ϵ RI), finally suggesting an autoimmune basis for the pathogenesis of the disease.

Until now, the existence of chronic autoimmune urticaria (CAIU) seems to be supported by the increased frequency of thyroid autoimmune

disorders in this subset of CIU patients (8) and by the clinical improvement after plasmapheresis (9). Although Maurer et al. developed an ELISA system for the measurement of anti-FcεRIα autoAbs in non-fractionated serum samples (10) no routine laboratory assay for the identification of autoantibodies is yet available. The autologous serum skin test (ASST) remains the only specific marker of CAIU, together with *in vitro* basophil and mast cell histamine release assays.

To better clarify the possible pathogenic mechanisms by which hive formation might occur, recent literature has often tried to characterize the dermal cellular infiltrate found in CIU.

Mast cells are considered the primary effector cells in urticaria and have often been reported to be significantly higher (up to a 9–10-fold increased number) in chronic idiopathic urticarial wheals compared with normal controls (11). Dermal mast cells appear to be maximally increased in recent fully developed lesions (12,13) (while after 6 h, wheals contain fewer degranulated mast cells) (14) and they even seem to show an increased releasability to TNF-α and IL-3 (15,16).

Histologically, however, it has been shown that the inflammatory infiltrate in CIU also includes mononuclear cells, represented by monocytes (approximately 22%), T lymphocytes (approximately 47%) and almost no B lymphocytes (17). Although some authors have found T-cell subpopulations to be highly variable from patient to patient with no overall difference from the ratio observed in plasma (17), newer studies have pointed out a preponderance of CD4+ over CD8+ T cells (16,18).

Therefore, as in other inflammatory dermatoses, it has been suggested that CD4+ T cells might play a central role in the pathogenesis of the disease, generating histamine releasing factors, partly as yet unknown, that trigger mast cell degranulation (19,20).

Furthermore, when CIU perivascular infiltrate is predominantly leukocytic, the term 'neutrophilic urticaria' (NU) is often used (21,22) to indicate an acute-phase pattern of reaction also observed in cholinergic and cold urticaria. Even though no data have yet been published regarding the possible pathomechanism involved in NU, a recent study (23) demonstrated the deposition of immuno-components in a large percentage of patients with CIU and suggested that the activation of complement fragments might influence the infiltration pattern of leukocytes.

The role of eosinophils in the pathogenesis of CIU is still unclear but as an increase of eosinophilic cationic protein (ECP), peroxidase (EPO) and major basic protein (MBP) has been found

in lesions of CIU (24–26), these mediators are judged to possibly contribute to mast cell degranulation and skin inflammation (27).

In this novel study, we aimed to further define the immunophenotypic features of the inflammatory response of CIU in the subgroup of patients with positive ASST. The cellular composition and markers of activation of the dermal infiltrate were investigated in our selected patients, comparing biopsies from lesional and unaffected skin with healthy skin of normal donors and with psoriatic skin. In order to clarify possible pathomechanisms of the dermatosis, we also focused our attention on the detection of adhesion molecules, hypothesizing their preferential expression to be a potential signal for the cellular recruitment.

Methods

Patients

Upon informed consent seven patients (four males and three females; age range 22–57 years) with chronic urticaria and a positive response to intradermal autologous serum were studied.

The ASST was performed according to Grattan *et al.* (2). Antihistamines were discontinued one week before skin testing and none of the patients was taking any other drug. The clinical features and the duration of the skin disease are shown in Table 1.

Baseline investigations in all patients included the detection of circulating immuno-complexes (CIC), autoantibodies (antinuclear, antigastric parietal cells, antipancreatic islet cells, antimitochondria, antithyroglobulin, antiperoxidase) and anti-Helicobacter Pylori antibodies (Table 2).

A negative response to ASST, predominant physical urticaria, allergic urticaria (IgE-mediated), urticaria vasculitis, C1-esterase inhibitor deficiency, drug or alcohol abuse, pregnancy and lactation were considered as criteria for exclusion.

Table 1. Clinical features and skin disease duration in patients with chronic idiopathic urticaria and positive autologous serum skin test

Sex	4 M/3F
Age	22–57 years
Duration of urticaria	5–120 months
Exacerbating factors	Non-steroid anti-inflammatory drugs (NSAID), acetilsalicylic acid

Table 2. Baseline investigations performed in selected patients

Histamine-release assay	Positive in all patients
Circulating immunocomplexes (CIC)	–
Antinuclear Abs	Present in two patients
Anti-gastric parietal cells Abs	Present in one patient
Anti-pancreatic islet cells Abs	–
Anti-mitochondria Abs	–
Anti-thyroglobulin Abs	Present in one patient
Anti-peroxidase Abs	Present in one patient
Anti-Helicobacter Pylori Abs	–
Others	Antismooth muscle Abs in one patient Anti-TSH receptor Abs in 1 patient

Biopsies

Four-mm punch biopsies were taken in all seven patients from a spontaneous wheal that was present at the time of the ASST. All biopsies were performed 2 h to 6 h after the wheals appeared.

Five of the same patients were also biopsied on unaffected skin, five additional biopsies were taken from healthy donors and five from patients affected by psoriasis vulgaris (PASI ranging from 12 to 38).

Biopsies were performed under local ring anesthesia (without adrenaline) and covered in cryoprotectant (Tissue-Tek, Miles Inc, Philadelphia, PA) before storage in a refrigerator at -80°C .

All biopsies showed an interstitial dermal oedema, dilated venules with endothelial swelling and a perivascular and interstitial mixed-cell infiltrate according to the hypothesized diagnosis of urticaria. We did not observe any sign of vasculitis-like endothelial degeneration, vessel necrosis, leucocytoclasia, fibrin deposition or erythrocyte extravasation.

Immunohistochemical procedure

Tissue specimens were cut into 5- μm sections, which were stained immunohistochemically for mast cells using monoclonal antibodies against tryptase at 1:50 (Dako Ltd, High Wycombe, UK). Monoclonal antibodies for human T lymphocytes included the following: CD3 at 1:20 pan-specific for T cells, CD4 at 1:50 specific for T helper, and CD8 at 1:20 specific for cytotoxic/suppressor T cells (Dako). Neutrophils were stained with monoclonal antibodies against myeloperoxidase (MPO) at 1:100 (Dako); eosinophils were labelled by anti-EG1 at 1:150 and anti-EG2 at 1:300 monoclonal antibodies (Pharmacia & Upjohn Diagnostics Ab, Uppsala, Sweden) that bind the epitope of eosinophil cationic protein (ECP) in the non-secreted and secreted form.

We studied the related cytokines in tissue specimens using monoclonal antibodies anti-IL-4 at 1:20, anti-IL-5 at 1:50, anti-IL-8 at 1:100, anti-IFN- γ at 1:100, anti-TGF β_1 at 1:30 (R & D Systems, Europe Ltd, Abingdon, UK). Monoclonal antibodies for integrins included the following: ICAM-1 at 1:50 (Immunotech, Marseille, France), V-CAM at 1:50 (R & D Systems), and ELAM at 1:100 (Novocastra Laboratories Ltd, Newcastle upon Tyne, UK). We also studied two chemokines using antibodies against CCR3 at 1:50 and anti-CXCR3 at 1:50 (R & D Systems). Before staining, frozen sections were air-dried and fixed in acetone (5 min). Immunolabelling was performed using rabbit antimouse bridging antibodies conjugated with alkaline phosphatase (1:10 dilution, 30 min; Dako Ltd) followed by incubation with murine alkaline phosphatase antialkaline phosphatase (APAAP) complexes (1:30 dilution, 30 min; Dako Ltd).

Two independent observers made a 'blind' assessment of the histological sections. For quantitative analysis, stained cells were counted and added in three adjacent microscopic ($\times 250$) fields of each section, and expressed as the mean $\pm$ SD.

Statistical analysis

Results were statistically evaluated by Wilcoxon's test for paired data to compare the lesional and unaffected skin of the urticaria patients, with a statistical significance $P \leq 0.05$. Wilcoxon's test for unpaired data was used to determine statistical differences between spontaneous wheals and healthy normal skin and psoriasis.

Results

Cellular infiltrate

Tryptase-positive (+) mast cells were detected in the upper and lower dermis with a perivascular and periadnexal distribution. Their mean was

Table 3. Composition of cellular infiltrate in spontaneous wheal and unaffected skin of patients with chronic idiopathic urticaria and positive autologous serum skin test compared with healthy controls

	Spontaneous wheal	Uninvolved skin	<i>P</i>	Healthy controls	<i>P</i>
CD3	35.3 $\pm$ 18.2	52.7 $\pm$ 8.3	$P < 0.05$	4.6 $\pm$ 2.2	$P < 0.05$
CD4	29.1 $\pm$ 11.3	45.6 $\pm$ 12.8	$P < 0.05$	7.5 $\pm$ 1.8	$P < 0.05$
CD8	11.2 $\pm$ 6.7	19.6 $\pm$ 8.5	n.s.	3.9 $\pm$ 1	$P < 0.05$
Tryptase	21.9 $\pm$ 9.5	19 $\pm$ 5.8	n.s.	23.1 $\pm$ 0.7	n.s.
EG1	21.5 $\pm$ 8.6	3.9 $\pm$ 4.1	$P < 0.05$	3.6 $\pm$ 2.5	$P < 0.05$
EG2	26.3 $\pm$ 6.5	4.5 $\pm$ 3.2	$P < 0.05$	2.6 $\pm$ 0.2	$P < 0.05$
MPO	86.1 $\pm$ 41.1	15.2 $\pm$ 7.6	$P < 0.05$	13.6 $\pm$ 3.7	$P < 0.05$

n.s.: not significant.

similar in both spontaneous wheal and unaffected skin (Table 3).

T-cell counts for CD3 supplied statistically significant difference between spontaneous wheal and unaffected areas ($P \leq 0.05$). In lesional skin, CD4 and CD8+ T cells showed a prevalent perivascular distribution, with the CD4+ T cells outnumbering the CD8+ 0. The T ratio (2:1) was also maintained in unaffected skin (Table 3; Fig. 1).

Lesional skin showed a superficial perivascular MPO expression together with an epidermal staining in two cases, possibly an expression of focal neutrophilic exocytosis; in unaffected skin biopsies only a few MPO+ cells were scattered in the dermis. Neutrophils represented the main infiltrating population in spontaneous wheals actually outnumbering the mean of unaffected skin (Table 3; Fig. 2).

An intense staining for EG1+ eosinophils was found in all lesional biopsies of the perivascular areas of the superficial and middle dermis, whereas in only one biopsy from unaffected skin was a focal expression with a similar distribution pattern detected (Table 3).



Figure 1. Alkaline phosphatase antialkaline phosphatase, $\times 200$. CD4+ T cells mainly distributed in the perivascular dermal areas of spontaneous wheals.

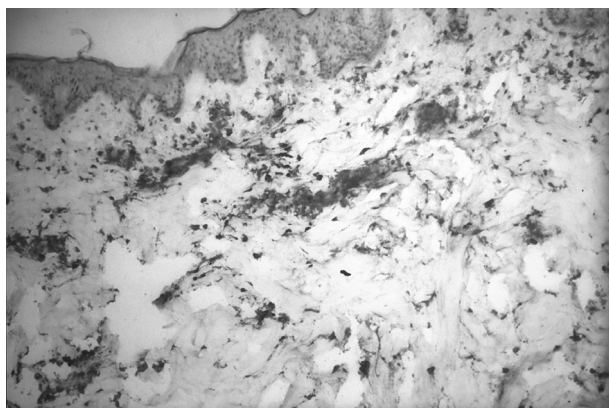


Figure 2. Alkaline phosphatase antialkaline phosphatase, $\times 200$. MPO-positive neutrophil granulocytes were the most numerous cell population present in the lesional skin of chronic autoimmune urticaria patients.

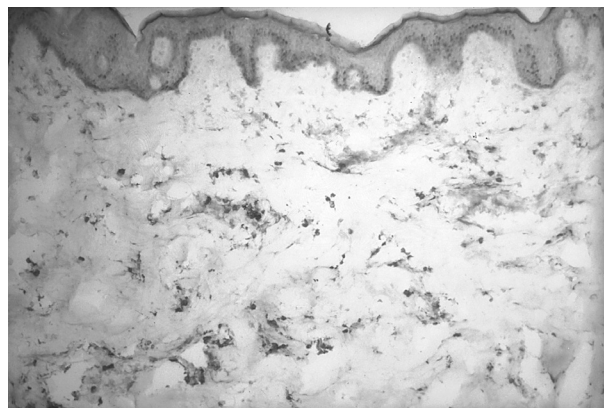


Figure 4. Alkaline phosphatase antialkaline phosphatase, $\times 200$. IL-4-positive cells with a prevalent intracellular staining were preferentially localized in the perivascular dermal sites of spontaneous wheals.

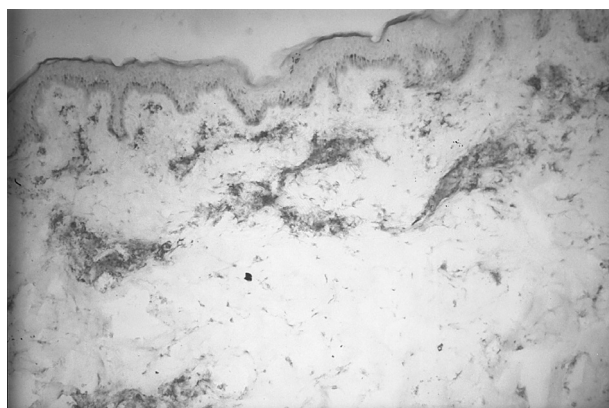


Figure 3. Alkaline phosphatase antialkaline phosphatase, $\times 200$. EG2-positive cells in spontaneous wheals.

Activated eosinophils are identified by the anti-EG2 monoclonal antibody, which binds to the epitope of human ECP present in the secreted form. Such staining was found in the superficial perivascular dermis of spontaneous wheals with an intense expression, while the intensity of expression in unaffected skin was significantly lower (Table 3; Fig. 3).

In healthy subjects used as control, T-cell subpopulations were poorly represented in both the epidermis and the dermis. Tryptase+ mast cells were demonstrated in the perivascular areas of the superficial dermis, together with MPO+ cells. Cell counts for EG1+ eosinophils gave mean values overlapping those of unaffected skin. EG2 staining was always negative (Table 3).

Cytokines, chemokines and chemokine receptors

In urticarial lesions, as well as in unaffected skin of the same patients, a focal expression for IFN- γ

was detected on infiltrating cells of the perivascular sites of the dermis.

IL-4-positive (+) cells were demonstrated in the perivascular sites of the upper dermis and also around appendages, with a predominant intracellular staining (Fig. 4).

An intense extracellular staining for IL-5 was demonstrated in spontaneous wheals in the dermal perivascular areas. Moreover, IL-5 showed focal staining throughout the epidermis. In unaffected skin, IL-5+ cells were scattered in the dermis and focally grouped at the dermo-epidermal junction in all biopsies (Fig. 5).

IL-8 expression was observed in the perivascular dermis of lesional skin with a mean value of 16.5 ± 13.8 . When analyzing healthy skin of the same urticarial patients, moderate staining for IL-8 was also found in the basal layer of the epidermis and in the perivascular areas of the dermis (Table 4).

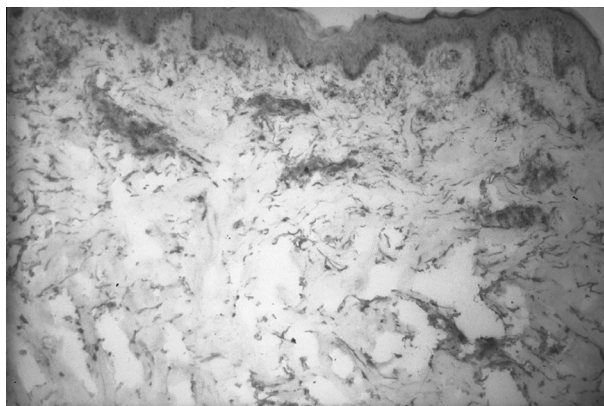


Figure 5. Alkaline phosphatase antialkaline phosphatase, $\times 200$. Intense, mainly extracellular, IL-5 staining was documented in lesional skin of chronic autoimmune urticaria patients.

Table 4. Cytokines, chemokines and chemokine receptors in spontaneous wheal and unaffected skin of patients with chronic idiopathic urticaria and positive autologous serum skin test compared with healthy controls and psoriatic skin

	Spontaneous wheal	Uninvolved skin	P	Healthy controls	P	Psoriasis	P
IFN- γ	3.0 $\pm$ 2.1	1.1 $\pm$ 0.9	$P < 0.05$	neg.	n.d.	24.6 $\pm$ 9.8	$P < 0.05$
IL-4	18.3 $\pm$ 13.5	7.0 $\pm$ 1.9	n.s.	neg.	n.d.	3.1 $\pm$ 1.8	$P < 0.05$
IL-5	35.5 $\pm$ 18.8	4.1 $\pm$ 2.8	$P < 0.05$	neg.	n.d.	3.0 $\pm$ 1.5	$P < 0.05$
IL-8	16.5 $\pm$ 13.8	17.3 $\pm$ 6.0	n.s.	13.2 $\pm$ 5.8	n.s.	19.7 $\pm$ 8.4	n.s.
CCR3	11.6 $\pm$ 3.4	14.2 $\pm$ 4.5	n.s.	5.2 $\pm$ 2.8	n.s.	9.9 $\pm$ 6.1	n.s.
CXCR3	46.6 $\pm$ 13.2	44.5 $\pm$ 11.7	n.s.	22.7 $\pm$ 15.4	$P < 0.05$	52.3 $\pm$ 3.3	n.s.

n.s.: not significant; n.d.: not determinable but significant; neg.: negative.

As to the lesional biopsies, focal staining for the eotaxin-specific receptor CCR3 was identified in the superficial perivascular areas, while CXCR3 (IP-10, Mig receptor) expression was detected in the epidermis (with a more marked intensity in the basal and suprabasal layers) and in both perivascular and vascular dermal areas (Fig. 6). In unaffected skin, CCR3 and CXCR3 did not show a significantly different expression than in lesional sites.

Healthy controls did not show positive staining either for IFN- γ , or IL-4 or IL-5. IL-8 was focally expressed on basal keratinocytes and endothelial cells (Table 4). CCR3+ cells could be observed both in perivascular/vascular sites and focally in the dermis, while the chemokine CXCR3 was found in the dermis and in the basal epidermis with focal staining (Table 5).

In psoriatic patients IFN- γ staining was moderately observed in papillar and perivascular dermal sites; IL-4 and IL-5 were only focally seen, and IL-8 was especially appreciated in perivascular sites. The chemokine CCR3 was poorly expressed, while CXCR3 was intensely found in the basal/suprabasal epidermal keratinocytes and in the dermal vascular and perivascular areas (Table 4).



Figure 6. Alkaline phosphatase antialkaline phosphatase, $\times 200$. Basal and suprabasal epidermal staining and moderate perivascular expression for CXCR3 in spontaneous wheals.

Cellular adhesion molecules

Spontaneous wheals showed an intense and diffuse staining for ICAM-1 mainly localized in the vascular and perivascular dermal sites. Vascular cell adhesion molecule (VCAM) expression was mainly found in the dermal perivascular superficial sites in both spontaneous wheal and unaffected skin. A focal significant staining for ELAM was detected particularly on endothelial cells of the upper dermis in lesional sites (Table 5).

In unaffected skin no differences could be detected for CAMs concerning distribution. However, ELAM showed a significantly reduced expression if compared to lesional skin.

On apparently normal skin of healthy patients, CAMs were significantly lower expressed on endothelial cells of the upper dermis.

Discussion

In the present study we have tried to characterize the cellular infiltrate immunophenotype and molecular products found in spontaneous wheals from patients with CIU and positive ASST.

Our data describe a defined stage of the urticarial skin infiltrating process, and can be usefully compared to the inflammatory response evolution of the autologous serum-induced wheals supplied by Grattan (27) in 1990, finally helping to better understand pathogenesis and effector mechanisms of CIU.

Table 5. Cellular adhesion molecule expression in spontaneous wheal and unaffected skin of patients with chronic idiopathic urticaria and positive autologous serum skin test compared with healthy controls

	Spontaneous wheal	Uninvolved skin	P	Healthy controls	P
ICAM-1	43.2 $\pm$ 7.2	31.1 $\pm$ 12.1	n.s.	18.5 $\pm$ 6.0	$P < 0.05$
ELAM	18.5 $\pm$ 7.4	6.7 $\pm$ 4.6	$P < 0.05$	3.3 $\pm$ 1.8	$P < 0.05$
VCAM	16.9 $\pm$ 6.0	14 $\pm$ 7.7	n.s.	6.0 $\pm$ 4.1	$P < 0.05$

n.s.: not significant.

Actually, the author performed his study (27) on serial biopsies from autologous serum-induced wheals, noting that perivascular neutrophils and eosinophils showed a tendency to increase from 30 min to 2 h; lymphocytes were also increased by 2 h, CD4+ cells becoming prevalent on CD8+ cells at 24 h. At 48 h, neutrophils were clearing but eosinophils and lymphocytes persisted. Grattan also proved (27,28) mast cell degranulation by electron microscopy and a subsequent reduction of stainable mast cells in these specimens when compared with those in the saline skin test. Taken together, all these findings make the pathology of the ASST strictly resemble IgE-mediated late-phase responses and support the hypothesis that a circulating factor causes mast cell degranulation in CIU.

Unlike what is reported in the literature (11,13), where a considerable increment of mast cells in spontaneous wheal is highlighted, the number of mast cells in our patients was not significantly different from that present in unaffected skin of the same patients and from specimens from healthy controls.

Such an observation, however, does not disagree with the predominant role of tryptase+ mast cells and related mediators in hive formation, but merely suggests we should consider their activation to be very early, as has been already proposed by Barlow (14). It is likely that, in most of our patients, we seized the moment in which degranulation had happened and the immunohistochemical staining was therefore no longer able to detect cell morphology and localization.

As a result of our data, CD4+ T cells were more numerous than CD8+ T cells (T ratio 2:1) and their number was lower than in unaffected skin. This behaviour is, indeed, similar to that observed for mast cells and suggests a wider role for T cells not only confined to the lesional skin; we may even assume a systemic activation for T cells able to trigger the onset of the wheals at any moment over the entire skin, as already suggested by Henz (12). In fact, by analogy with other inflammatory dermatoses (19,20), CD4+ T cells might generate histamine-releasing factors, being able to induce mast cell degranulation and granulocyte chemotaxis.

Our analysis has also demonstrated the presence, exclusively in lesional skin, of a T-cell population with a prevalent Th2-like pattern, a result of its preferential production of IL-4 and IL-5 rather than IFN- γ .

This last finding is worth noting, especially considering the proven role of Th2 cytokines in keratinocyte activation and inflammatory cell recruitment through the stimulation of adhesion molecule expression on endothelium (29,30). As is

known, IL-4 up-regulates adhesion molecules involved in eosinophil and mast cells tropism such as ICAM-1 and VCAM; it also increases the adhesiveness of endothelial cells to eosinophils and basophils (31). Moreover, IL-4 acts on tissue eosinophil recruitment by promoting eosinophil transmigration from vascular endothelium and on neutrophils by enhancing their respiratory burst as well as their phagocytic properties. Finally, IL-4 and IL-5 provide optimal help for humoral responses and for mast cell and eosinophil growth and differentiation (31).

Neutrophils and eosinophils can be seen to have significantly increased in lesional skin compared with unaffected skin, as previously suggested by Sabroe (32). Their preponderance, together with their evident state of activation (most of the cells appeared degranulated on staining for their specific protein) only seen in the spontaneous wheal areas, provides evidence for a fundamental role of granulocytes in the development of tissue lesions in CIU. Referring to neutrophils in particular their homing in CIU seems to be poorly promoted by IL-8; this chemokine was in fact preferentially expressed in lesional skin rather than in controls, but without significant difference. In this respect, analogous data were already found by Hermes et al. (16).

Trafficking of activated T cells and other leukocytes into inflammatory sites is a tightly controlled process directed by multiple molecules, adhesion molecules and chemokines in particular. Recent *in vitro* studies have demonstrated that Th1 and Th2 cells express distinct sets of chemokine receptors that might regulate the recruitment of these cells. In particular CXCR3 and CCR5 have been associated with the Th1 cytokine profile, whereas CCR3, CCR4 and CCR8 have been associated with the Th2 phenotype (28). We would have expected to find a marked staining for CCR3 in the perivascular areas of spontaneous wheals, but this was not significantly different from the staining of unaffected skin in the same patients. As regards the expression of CXCR3 in lesional skin, we found an intense staining in the basal and suprabasal layers of the epidermis and moderate in both perivascular and vascular dermal areas. A similar expression for CCR3 and CXCR3 was also seen in biopsies from autologous serum-induced wheals at 30 min, with an even slighter positivity (data not shown). We might therefore speculate that the involvement of the chemokine receptor CCR3 could occur early in spontaneous wheals, being, however, not a necessary pathway for the development of the disease. On the other hand, CXCR3 might play a major pathogenetic role in

CAIU, supposedly because of its poor expression in healthy controls.

As regards adhesion molecules, in spontaneous wheals our data demonstrated an increased expression of all studied CAMs (ICAM-1, VCAM and ELAM) if compared with healthy controls, while previous data by Zuberbier (33), concerning immunohistochemical and serum detection of CAMs, failed to obtain significant differences. When the comparison was made with unaffected skin, no significant differences could be detected for VCAM and ICAM, even if a slight increase was observed in lesional skin. These last data are in agreement with those of Haas (34), who could not detect any increase of CAMs in early whealing reactions, and seem to confirm for VCAM and ICAM-1 the systemic activation suggested earlier for mast cells and lymphocytes.

A different trend was instead observed for ELAM, which showed in unaffected skin a significantly reduced expression when compared with lesional skin. Among adhesion molecules E-selectin provides rolling adhesion of circulating leukocytes and a transient and reversible interaction that initiates leukocyte extravasation. Etter et al. (35) have demonstrated the importance of E-selectin expression for neutrophil adherence and the modified kinetics of the selectin in the presence of IL-4 and IL-13, which seem to control the period of induced E-selectin expression and appear to be confined to the acute phase of leukocyte emigration. The marked staining we found in spontaneous wheals for ELAM-1 together with the intense expression for Th2-like cytokines acted in accordance with the previous observation by Etter (35).

In conclusion, it is our aim to pay special attention to some of our results, particularly to the lack of a significant difference in the expression of tryptase+ mast cells, CD8 T lymphocytes, IL-8, CXCR3, CCR3, and a few CAMs between lesional and unaffected skin of CIU patients. Taken together such data seem to point to a wide immunological activation that does not involve only lesional tissues, but possibly extends to the immune system of all skin. All cutaneous surfaces would in this way be ready to respond at any moment to triggering stimuli, as represented by a serum factor, not yet identified, able promptly to evoke mast cell degranulation.

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