

ORIGINAL ARTICLE

Brain-derived neurotrophic factor is increased in serum and skin levels of patients with chronic spontaneous urticaria

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Summary

Background Chronic spontaneous urticaria is triggered by many direct and indirect aggravating factors including autoreactive/autoimmune mechanisms, infections, non-allergic and pseudoallergic intolerance reactions. However, the role of neuroimmune mechanisms in chronic spontaneous urticaria so far is unclear.

Objective Thus, we wanted to address the regulation of the neurotrophin brain-derived neurotrophic factor (BDNF) in serum and inflammatory skin of patients with chronic spontaneous urticaria in comparison to subjects with healthy skin.

Methods Fifty adult patients with chronic spontaneous urticaria and 23 skin-healthy subjects were studied. Chronic spontaneous urticaria was defined as recurrent weals for more than 6 weeks. Autologous serum skin test was performed in all patients with chronic spontaneous urticaria and BDNF serum levels were analysed by enzyme immunoassay in all subjects. Furthermore, skin biopsies were taken from weals of eight patients with chronic spontaneous urticaria as well as from healthy skin of eight controls to evaluate the expression of BDNF and its receptors including tyrosine kinase (trk) B and pan-neurotrophin receptor p75^{NTR} by immunohistochemistry.

Results BDNF serum levels were detectable in all subjects studied. However, BDNF levels were significantly higher in patients with chronic spontaneous urticaria compared to non-atopic skin-healthy controls ($P < 0.001$). Furthermore, epidermal and dermal expression of BDNF and epidermal expression of p75^{NTR} was significantly higher in patients with chronic spontaneous urticaria compared with controls ($P < 0.05$ – 0.001). There was no difference with regard to the expression of trkB between chronic spontaneous urticaria and controls and no difference in BDNF serum levels between autologous serum skin test-positive ($n = 23$) and -negative ($n = 27$) patients with chronic spontaneous urticaria.

Conclusions and Clinical Relevance This study shows that BDNF is increased in serum and diseased skin of patients with chronic spontaneous urticaria, suggesting a role for neurotrophins in the pathophysiology of this chronic inflammatory skin disease. Further studies are needed to address the functional role of BDNF on key target effector cells in chronic spontaneous urticaria to establish new therapeutic implications.

Keywords brain-derived neurotrophic factor, chronic spontaneous urticaria, eosinophils, neurotrophins, skin inflammation

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Introduction

Brain-derived neurotrophic factor (BDNF) belongs to the family of neurotrophins, which have been well described for their primary activity, the growth of peripheral and

central nerves [1]. In the meantime, it became evident that neurotrophins exert a variety of immunomodulatory effects on non-neuronal cells including eosinophils, keratinocytes and mast cells which also produce neurotrophins [2–9].

Neurotrophins belong to a family of homologous proteins that exert their effects primarily as target-derived paracrine or autocrine neurotrophic factors. The biological effects of neurotrophins are mediated by binding either to the tyrosine kinase (trk) receptors to which mature neurotrophins bind with high affinity and specificity or the pan-neurotrophin receptor p75 (p75^{NTR}), a member of the tumour necrosis factor (TNF) super family, to which mature neurotrophins bind with low affinity and specificity [10]. TrkA represents the specific receptor for nerve growth factor (NGF), trkB for BDNF and neurotrophin (NT)-4 and trkC for NT-3 [11]. The expression of these neurotrophin receptors is widely distributed on neurons of the peripheral and the central nervous system. In addition, trkA-C and p75^{NTR} receptors are expressed on non-neuronal cells including smooth muscle cells, epithelial cells and immune cells including eosinophils, keratinocytes, melanocytes and mast cells [2, 5, 6, 8, 12–16].

The initial report about an increased NGF production in allergic rhinitis (AR), atopic dermatitis and urticaria was provided by Bonini and colleagues, indicating that patients with urticaria display significantly higher serum levels of NGF compared with controls [17]. However, the phenotype of urticaria (acute, chronic or physical) had not been specified in the latter study. Several studies confirmed these increased levels of NGF in atopic diseases including AR, allergic asthma and atopic dermatitis as shown by us and others [7, 17–22]. Interestingly, also BDNF levels are increased in AR, allergic asthma and atopic dermatitis [7, 18, 19, 22].

Chronic spontaneous urticaria is a common skin disorder defined by the rapid appearance of highly pruritic weals lasting <24 h for a time period of at least 6 weeks and often for decades [23]. Interestingly, chronic spontaneous urticaria is often accompanied by the appearance of angioedema which affects 50% of the patients. Because of pruritus and sleeplessness, life quality is significantly reduced [24]. In chronic spontaneous urticaria many direct and indirect triggering and aggravating factors including infections such as *Helicobacter pylori*, autoreactive/autoimmune mechanisms, non-allergic/pseudoallergic intolerance reactions have been described [25]. Further, an association between chronic spontaneous urticaria and autoimmune disease has been recognized in a subset of patients with functional IgG auto-antibodies (Ab) against the high-affinity receptor for IgE (FcεRIα) and less commonly against IgE. These patients have been described as having a more severe and difficult-to-control urticaria. In this regard, the autologous serum skin test was initially proposed as a surrogate marker for urticaria auto-Ab. However, the autologous serum skin test identifies presence of histamine releasing factors and displays only a moderate specificity as a marker for functional auto-Ab against IgE or FcεRI [26]. Thus, the autologous serum skin test is now used to define the autoreactivity of patients.

The inflammatory infiltrate in chronic spontaneous urticaria includes mast cells, basophils, T lymphocytes (CD4+ more than CD8+), monocytes, neutrophils and eosinophil granulocytes [27, 28]. Mast cell degranulation and histamine release are thought to play a major role in pruritus of chronic spontaneous urticaria. However, with regard to the inflammatory infiltrate and predominance of eosinophil granulocytes other mediators than histamine may be involved in the pathophysiology of chronic spontaneous urticaria.

The role of neuroimmune mechanisms in chronic spontaneous urticaria is not clear so far. Thus, we first investigated whether BDNF serum levels in chronic spontaneous urticaria are different from control subjects with healthy skin. Secondly we analysed whether BDNF levels differ between the autologous serum skin test-positive and -negative subtype of chronic spontaneous urticaria. Finally we investigated whether expression of BDNF and its receptors differs in inflammatory skin of patients with chronic spontaneous urticaria compared with healthy skin of control subjects.

Methods

Patients

Chronic spontaneous urticaria was defined as recurrent wealing occurring at least twice a week for more than 6 weeks. In patients with chronic spontaneous urticaria, activity was assessed using the activity score as recommended by the latest European guideline and previously described [23, 29]. The grading includes a score from 0 to 3 according to the number of weals and the severity of pruritus present at the time when blood samples were collected: 0 = no weals and no pruritus; 1 = mild <20 weals/24 h and mild pruritus; 2 = moderate 20–50 weals/24 h and moderate pruritus; 3 = intense >50 weals/24 h or large confluent areas of weals together with intense pruritus. After informed consent (approved by the local ethics committee) peripheral venous blood was obtained from 50 individuals with chronic spontaneous urticaria (mean age 49.0±21.0, *n* = 22 with additional recurrent angioedema) and 23 non-atopic subjects with healthy skin (mean age 43.8±16.5 years). At the time of blood draw, all patients were treated with second-generation antihistamines including ebastine, levocetirizine or desloratadine ranging from one to four tablets per day [30]. Patients did not receive systemic immunosuppressive treatment including corticosteroids or cyclosporine A or treatment with biologicals including anti-IgE therapy. Patients with physical urticaria were excluded from the study.

Autologous serum skin test

To perform the autologous serum skin test, we collected peripheral blood from each individual in a standardized

procedure at 9 o'clock in the morning. The serum was stored at room temperature for half an hour with subsequent centrifugation. Thereafter, 50 µL of serum were injected intradermally at the volar site of the forearm. Intradermal injection of saline solution (0.9% NaCl) was performed as negative control and skin prick test with 10 mg/mL histamine was used as positive control. Reactions showing a weal with a diameter of at least 3 mm greater than the control saline solution were considered as positive.

Analysis of serum brain-derived neurotrophic factor levels

We used a commercial enzyme-linked immunosorbent assay (BDNF: R&D Systems, Wiesbaden, Germany) to assess BDNF serum levels, according to the manufacturer's recommendations and as previously described [7, 18]. The specificity of the assay was confirmed by the manufacturers, the detection limit was 23.4 pg/mL.

Immunohistochemical studies

Skin biopsies of eight patients with chronic spontaneous urticaria were taken from weals that had appeared for <10 h. Skin biopsies taken from healthy skin of eight subjects with no history of atopic disease served as controls. The tissue samples were fixed in buffered formalin, dehydrated, embedded in paraffin wax and sectioned. After deparaffining and rehydrating, each tissue section was sliced into 6-µm-thick sections. Thereafter, H&E staining was performed of the sections, respectively. Further, immunohistochemistry was performed using the avidin-biotin-peroxidase kit (Vectastain ABC-kit, Vector Laboratories Inc., Burlingame, CA, USA) as previously described [7]. Primary antibody (Ab) or respective isotype control diluted in antibody diluent (DAKO Cytomation, Hamburg, Germany) were added and incubated for 24 h at 4 °C. For primary Ab, we used a concentration of 1 µg/mL p75^{NTR} [anti-human NGF receptor monoclonal Ab (mAb) directed against the extracellular domain, clone NGFR5; NeoMarkers, Fremont, CA, USA] and 1 µg/mL trkB (mAb directed against the extracellular domain; R&D Systems, Wiesbaden, Germany). BDNF (anti-human BDNF polyclonal Ab, Santa Cruz, CA, USA) was used in a dilution of 1 : 350. A

biotinylated goat anti rabbit or horse anti-mouse Ab was used as secondary Ab. Isotype-specific control staining was performed with monoclonal mouse IgG1 for p75^{NTR}, monoclonal mouse IgG2b for trkB and polyclonal rabbit IgG for BDNF (all R&D Systems).

Microscopic assessment of immunohistochemical staining

For quantification, epidermal and dermal serial sections excluding glands below the basement membrane were analysed by three independent 'blinded' observers. At least 10 high power fields were analysed in each sample. BDNF, p75^{NTR} and trkB immunoreactivity were assessed using a grade from 0 to 4 (absent, weak, moderate, strong, very strong) displayed as staining intensity [22]. All slides were microscopically examined and photo-documented (Olympus, Hamburg, Germany).

Statistical analysis

Statistical analysis was performed with the statistical software package SigmaStat (SigmaStat for Windows, Jandel Scientific Erkrath Wiesbaden, Germany). Data were analysed with either Student's *t*-test (normality test passed) or Mann-Whitney rank sum test (normality test failed). A *P*-value of <0.05 was considered to be statistically significant.

Results

Higher levels of serum brain-derived neurotrophic factor in chronic spontaneous urticaria

BDNF serum levels were detectable in both non-atopic controls with healthy skin and patients with chronic spontaneous urticaria (Table 1). BDNF serum levels were significantly higher in patients with chronic spontaneous urticaria compared with the non-atopic skin-healthy subjects (*P*<0.001) (Table 1). Twelve patients out of 50 patients with chronic spontaneous urticaria displayed weals and pruritus at the day of blood taking. In these 12 patients we, however, did not find a correlation of BDNF serum levels with the number of weals (data not shown) or with the urticaria activity score (Table 1).

Table 1. Increased BDNF serum samples of patients with chronic spontaneous urticaria compared with non-atopic controls

	Non-atopics, <i>n</i> = 23	CU patients, <i>n</i> = 50	ASST+CU, <i>n</i> = 23	ASST-CU, <i>n</i> = 27	UAS 0, <i>n</i> = 38	UAS 1, <i>n</i> = 6	UAS 2, <i>n</i> = 3	UAS 3, <i>n</i> = 3
BDNF (ng/mL)	6.57±0.17	13.19±0.32***	12.70±0.56	13.61±0.36	13.23±0.33	13.27±0.77	12.30±1.36	13.48±3.43

Increased levels of BDNF serum samples in patients with chronic spontaneous urticaria (*n* = 50) compared with non-atopic healthy subjects (non-atopics, *n* = 23,

****P*<0.001, Student's *t*-test). BDNF serum levels did not correlate with autologous serum skin test (ASST) or urticaria activity score (UAS). Displayed are mean BDNF levels±standard error of the mean.

BDNF, brain-derived neurotrophic factor; CU, chronic spontaneous urticaria.

No difference of brain-derived neurotrophic factor serum levels between autologous serum skin test-positive and negative patients

To analyse whether autoreactivity may play a role in BDNF regulation we performed the autologous serum skin test in all patients with chronic spontaneous urticaria.

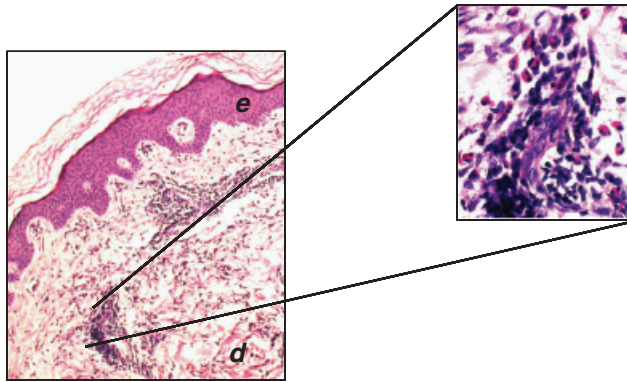


Fig. 1. Representative H&E staining of inflammatory skin derived from a patient with chronic spontaneous urticaria (CU, magnification $\times 200$). The inflammatory infiltrate in CU includes T lymphocytes and eosinophil granulocytes (magnification $\times 400$). e, epidermis; d, dermis.

Twenty-three patients with chronic spontaneous urticaria had a positive autologous serum skin test (mean age 51.6 ± 22.0 years, $n = 13$ with recurrent angioedema), whereas 27 patients with chronic spontaneous urticaria had a negative autologous serum skin test (mean age 42.6 ± 23.2 years, $n = 9$ with recurrent angioedema). Interestingly, there was no difference of BDNF serum levels between patients with positive autologous serum skin test or patients with negative autologous serum skin test with chronic spontaneous urticaria (Table 1).

Increased expression of brain-derived neurotrophic factor and $p75^{NTR}$ in the skin of patients with chronic spontaneous urticaria

Histology revealed the presence of an inflammatory infiltrate consisting mainly of eosinophil granulocytes and lymphocytes (Fig. 1). To further analyse the regulation of BDNF in the involved skin we performed an immunohistochemical analysis of skin biopsies in patients with chronic spontaneous urticaria (Fig. 2a) and non-atopic controls with healthy skin (Fig. 2b). BDNF expression was significantly higher in the epidermis ($P < 0.05$, Fig. 2c) and dermis ($P < 0.05$, Fig. 2d) of skin samples

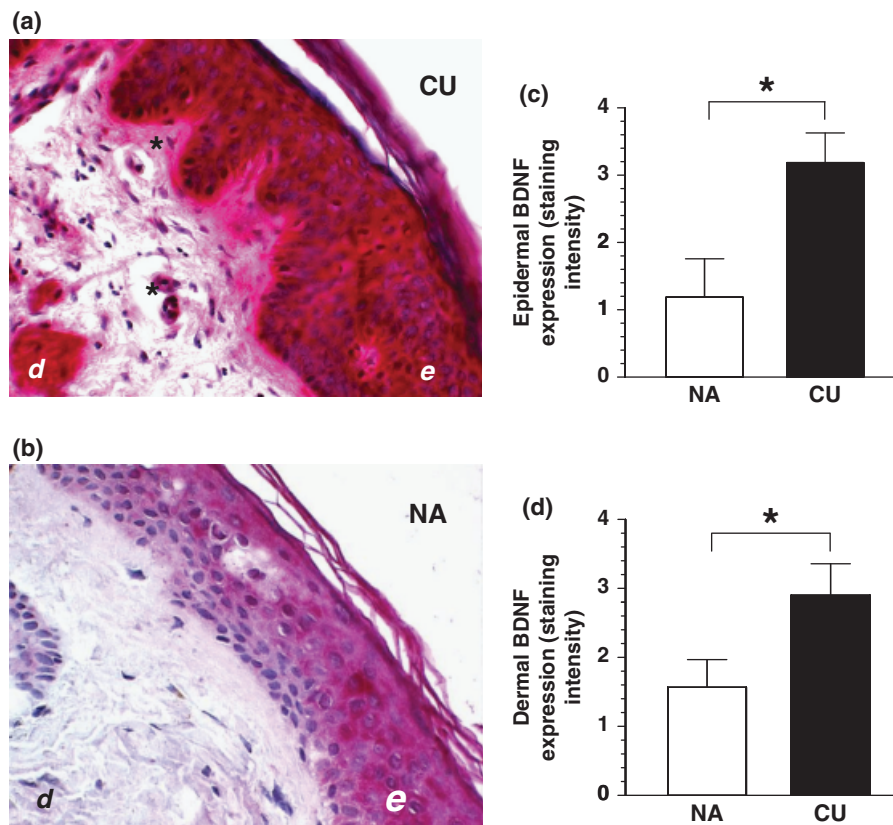


Fig. 2. Regulation of BDNF in inflammatory skin of chronic spontaneous urticaria [CU, (a)] and in non-atopic healthy skin [NA, (b)]. BDNF expression (displayed as staining intensity) was significantly higher in the epidermis [(c), $*P < 0.05$, Student's t -test] and dermis [(d), $*P < 0.05$, Student's t -test] of patients with CU ($n = 8$, black bars) compared with controls with healthy skin ($n = 8$, white bars). Asterisks point to eosinophil granulocytes in the skin of a patient with CU (a). e, epidermis; d, dermis; BDNF, brain-derived neurotrophic factor.

Table 2. Expression of BDNF and receptors in the skin of patients with chronic spontaneous urticaria compared with controls

	BDNF epidermal	BDNF dermal	p75 ^{NTR} epidermal	p75 ^{NTR} dermal	TrkB epidermal	TrkB dermal
Chronic spontaneous urticaria, <i>n</i> = 8	3.19±0.44*	2.9±0.45*	3.42±0.10***	3.00±0.28	0.01±0.00	0.61±0.10
Non-atopics, <i>n</i> = 8	1.19±0.57	1.57±0.39	0.79±0.33	2.05±0.40	0.29±0.022	0.99±0.31

Higher expression of epidermal BDNF and p75^{NTR} and dermal BDNF expression in the skin of patients with chronic spontaneous urticaria compared with non-atopic controls (**P* < 0.05; ****P* < 0.001, Student's *t*-test). Displayed is mean staining intensity ± standard error of the mean.

BDNF, brain-derived neurotrophic factor; trk, tyrosine kinase.

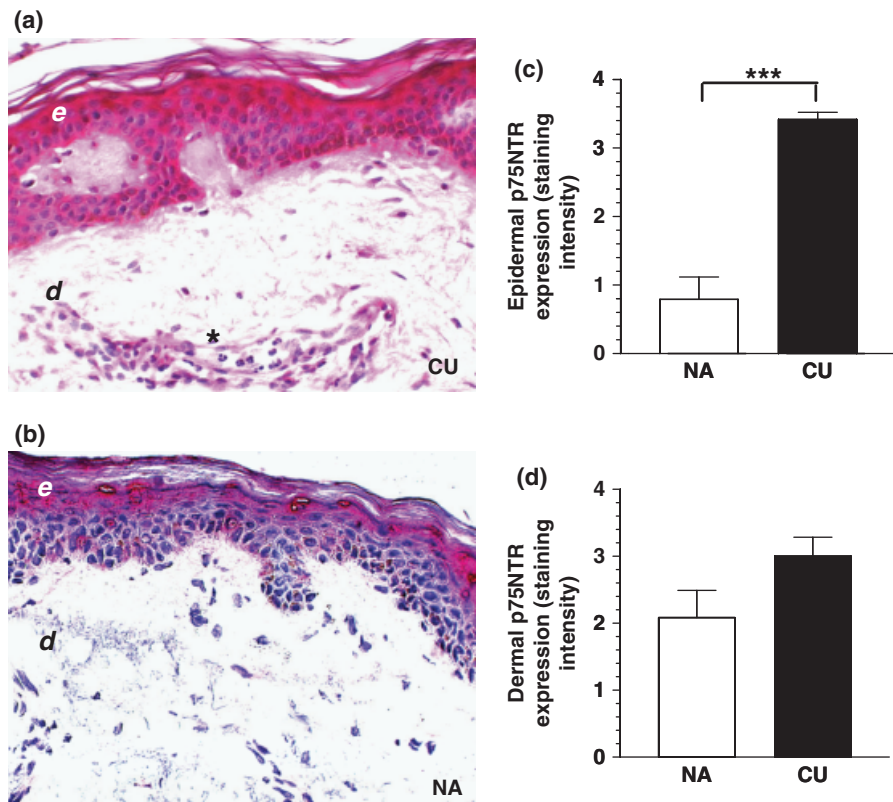


Fig. 3. Expression of p75^{NTR} in the skin of chronic spontaneous urticaria [CU, (a)] and healthy skin [NA, (b)]. Displayed is one representative histology out of eight different individuals. Asterisk marks eosinophilic infiltrate in CU. Increased expression of p75^{NTR} in the epidermis (e, epidermis) of patients with chronic spontaneous urticaria (CU, black bars, *n* = 8) compared with controls with healthy skin [NA, white bars, *n* = 8, (c), ****P* < 0.001, Student's *t*-test]. Dermal (d, dermis) expression of p75^{NTR} was similar in patients with CU (black bars) and controls with healthy skin [NA, white bars, (d), *n* = 8 different individuals for CU and NA, Student's *t*-test].

derived from patients with chronic spontaneous urticaria compared with control subjects with healthy skin (Figs 2a–d, Table 2). Further, epidermal expression of p75^{NTR} was significantly higher in chronic spontaneous urticaria patients compared to controls (*P* < 0.001, Figs 3a–c, Table 2). However, we did not find a significant difference in dermal p75^{NTR} (Fig. 3d, Table 2) and epidermal as well as dermal expression of trkB between controls and chronic spontaneous urticaria patients (Figs 4a–d, Table 2).

Discussion

This study provides evidence for higher serum and skin levels of the neurotrophin BDNF in patients with chronic

spontaneous urticaria compared with non-atopic control subjects with healthy skin. Interestingly, BDNF levels did not differ between autologous serum skin test-positive and -negative chronic spontaneous urticaria patients implying that BDNF regulation does not depend on auto-reactivity in chronic spontaneous urticaria. Another finding was that increased BDNF levels did not correlate with disease activity. This can possibly be explained by the fact that the majority of patients with chronic spontaneous urticaria included in the study already received a sufficient symptomatic therapy with H1 antihistamines, as 38 patients had no pruritus and no weals at the time of blood taking. Only 12 out of 50 patients with chronic urticaria displayed symptoms although treated with H1

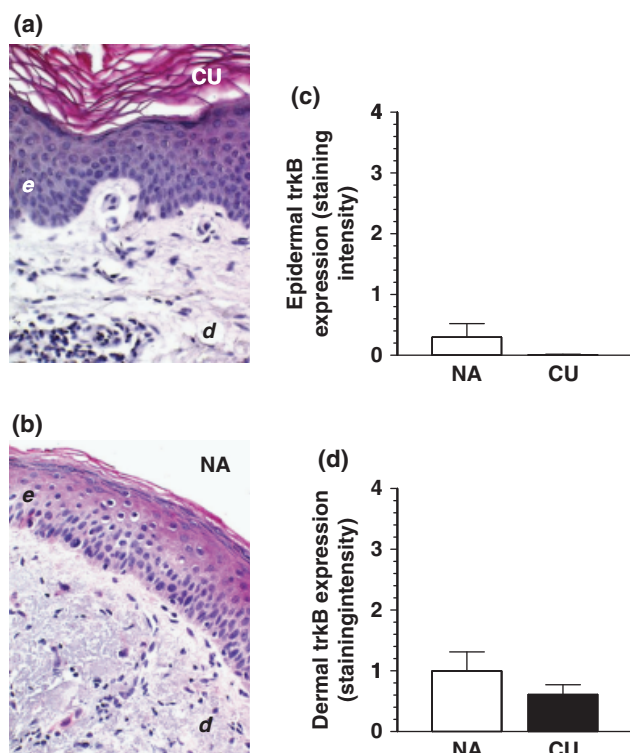


Fig. 4. Expression of trkB in the skin of chronic spontaneous urticaria [CU, (a)] compared with healthy non-atopic skin [NA, (b)]. No differences in the expression of trkB in the epidermis [(c), Mann-Whitney rank sum test] and dermis [(d), Mann-Whitney rank sum test] of patients with CU (black bars, $n = 8$) and controls with healthy skin (NA, white bars, $n = 8$) could be observed. e, epidermis; d, dermis; trk, tyrosine kinase.

antihistamines, ranging from mild ($n = 6$) to moderate ($n = 3$), and severe ($n = 3$). Furthermore, the analysis of the number of weals and the score of pruritus within one single day may not reflect the whole spectrum of disease activity as chronic spontaneous urticaria attacks including pruritus and weals occur unpredictably during the day or night.

Bonini and colleagues were the first to address a role of neurotrophins in patients with urticaria [17]. Analysing serum levels of NGF, they found increased levels in urticaria patients compared with healthy controls. In another study, it was found that NGF and BDNF levels were not increased in urticaria, which may be explained by methodological differences as these authors assessed extremely low levels of all neurotrophins and included patients with a very low severity score with no need for symptomatic treatment [31]. In the skin of patients with chronic, acute and delayed pressure urticaria it was shown that NGF expression was weak with no difference compared with skin-healthy controls [32]. With regard to the finding of Bonini and colleagues it is interesting to note that other chronic inflammatory skin diseases also including atopic dermatitis are characterized by increased levels of NGF and BDNF and a correlation to disease severity as shown by us and others [18–20]. Thus, neurotrophins

seem to be involved in the pathophysiology of several atopic and non-atopic inflammatory skin diseases.

It is well known that neurotrophins exert immunomodulatory functions not only on nerves but also on immune cells which has been best characterized for NGF [33]. Regarding BDNF we previously showed that it has a functional role on eosinophil granulocytes [7]. BDNF-induced chemotaxis and inhibited apoptosis of peripheral blood eosinophils of patients with the extrinsic type of atopic dermatitis, whereas BDNF had no functional effect on eosinophils derived from non-atopic skin-healthy subjects [6, 7]. In this regard, our data support our *in vitro* finding of BDNF-induced eosinophil chemotaxis with increased numbers of eosinophils in the skin of patients with chronic spontaneous urticaria possibly regulated by the increased expression of BDNF. Keratinocytes, which have been shown to synthesize and store BDNF [5] are likely candidates for the increased expression of BDNF in lesional skin of patients with chronic spontaneous urticaria. In this regard, keratinocytes may be activated by $\text{TNF-}\alpha$ [34], which is significantly increased in chronic spontaneous urticaria lesional skin [35]. Interestingly, we found a higher expression of p75^{NTR} in the epidermis of patients with chronic spontaneous urticaria compared with healthy skin of controls, but there was no difference in dermal p75^{NTR} expression. This finding is in contrast to the analysis of Hermes *et al.* [32], who assessed a lower expression of p75^{NTR} in the endothelium but only if they pooled all subtypes of urticaria including chronic, acute and delayed pressure urticaria compared with skin-healthy individuals. Epidermal and dermal trkB expression was almost not present in both chronic spontaneous urticaria and healthy skin of controls. In chronic spontaneous urticaria, this could be due to the increased expression of BDNF with subsequent trkB receptor binding in skin lesions. However, it would also be interesting to see whether healthy skin of patients with chronic spontaneous urticaria displays a different expression of BDNF in comparison to skin affected by weals, which we will address in a future study.

Our results further point to an important role of eosinophils in the inflammatory reaction of chronic spontaneous urticaria, as shown by the frequent eosinophil invasion in chronic spontaneous urticaria inflammatory skin. Indeed, the role of eosinophils in chronic spontaneous urticaria pathophysiology is also supported by the observation that anti-CD23 autoantibodies are present in about 65% of patients and that these antibodies can activate eosinophils which in turn release major basic protein and activate mast cells [36]. Further, eosinophils are the main cellular source of vascular endothelial growth factor in addition to tissue factor as assessed with double stainings in lesional skin of patients with chronic spontaneous urticaria, underlining their role in chronic spontaneous urticaria pathophysiology [37, 38]. In

addition, eosinophils have also been described to produce and release BDNF in patients with atopic dermatitis [7]. However, so far no functional data are available which would reflect the action of BDNF on peripheral blood eosinophils in patients with chronic spontaneous urticaria.

In conclusion, our data add novel aspects to the regulation and expression of BDNF in chronic spontaneous urticaria, suggesting a role of BDNF in the pathophysiology of this chronic inflammatory skin disease. Further investigations will need to address the functional role of BDNF on key target effector cells in chronic spontaneous urticaria to reveal novel treatment strategies.

Clinical relevance

BDNF displays a novel player in chronic urticaria with increased expression in serum and skin samples, indicating a role for neuroimmune interaction mechanisms in this ongoing chronic skin disease.

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The authors declare no conflicts of interest.

References

- Lewin GR, Barde YA. Physiology of the neurotrophins. *Annu Rev Neurosci* 1996; 19:289–317.
- Fischer TC, Lauenstein HD, Serowka F, Pilzner C, Groneberg DA, Welker P. Pan-neurotrophin receptor p75NTR expression is strongly induced in lesional atopic mast cells. *Clin Exp Allergy* 2008; 38:1168–73.
- Grewe M, Vogelsang K, Ruzicka T, Stege H, Krutmann J. Neurotrophin-4 production by human epidermal keratinocytes: increased expression in atopic dermatitis. *J Invest Dermatol* 2000; 114:1108–12.
- Peters EM, Raap U, Welker P *et al.* Neurotrophins act as neuroendocrine regulators of skin homeostasis in health and disease. *Horm Metab Res* 2007; 39:110–24.
- Marconi A, Terracina M, Fila C *et al.* Expression and function of neurotrophins and their receptors in cultured human keratinocytes. *J Invest Dermatol* 2003; 121:1515–21.
- Raap U, Deneka N, Bruder M, Kapp A, Wedi B. Differential up-regulation of neurotrophin receptors and functional activity of neurotrophins on peripheral blood eosinophils of patients with allergic rhinitis, atopic dermatitis and non-atopic subjects. *Clin Exp Allergy* 2008; 38:1493–8.
- Raap U, Goltz C, Deneka N *et al.* Brain-derived neurotrophic factor is increased in atopic dermatitis and modulates eosinophil functions compared with that seen in nonatopic subjects. *J Allergy Clin Immunol* 2005; 115:1268–75.
- Raap U, Kapp A. Neurotrophins in healthy and diseased skin. *G Ital Dermatol Venereol* 2010; 145:205–11.
- Noga O, Englmann C, Hanf G, Grutzkau A, Seybold J, Kunkel G. The production, storage and release of the neurotrophins nerve growth factor, brain-derived neurotrophic factor and neurotrophin-3 by human peripheral eosinophils in allergics and non-allergics. *Clin Exp Allergy* 2003; 33:649–54.
- Kaplan DR, Miller FD. Neurotrophin signal transduction in the nervous system. *Curr Opin Neurobiol* 2000; 10:381–91.
- Miller FD, Kaplan DR. Neurobiology. TRK makes the retrograde. *Science* 2002; 295:1471–3.
- Noga O, Englmann C, Hanf G, Grutzkau A, Guhl S, Kunkel G. Activation of the specific neurotrophin receptors TrkA, TrkB and TrkC influences the function of eosinophils. *Clin Exp Allergy* 2002; 32:1348–54.
- Hahn C, Islamian AP, Renz H, Nockher WA. Airway epithelial cells produce neurotrophins and promote the survival of eosinophils during allergic airway inflammation. *J Allergy Clin Immunol* 2006; 117:787–94.
- Nassenstein C, Kammertoens T, Veres TZ *et al.* Neuroimmune crosstalk in asthma: dual role of the neurotrophin receptor p75NTR. *J Allergy Clin Immunol* 2007; 120:1089–96.
- Schaper C, Glaser S, Groneberg DA, Kunkel G, Ewert R, Noga O. Nerve growth factor synthesis in human vascular smooth muscle cells and its regulation by dexamethasone. *Regul Pept* 2009; 157:3–7.
- Marconi A, Panza MC, Bonnet-Duquenois M *et al.* Expression and function of neurotrophins and their receptors in human melanocytes. *Int J Cosmet Sci* 2006; 28:255–61.
- Bonini S, Lambiase A, Bonini S *et al.* Circulating nerve growth factor levels are increased in humans with allergic diseases and asthma. *Proc Natl Acad Sci USA* 1996; 93:10955–60.
- Raap U, Werfel T, Goltz C *et al.* Circulating levels of brain-derived neurotrophic factor correlate with disease severity in the intrinsic type of atopic dermatitis. *Allergy* 2006; 61:1416–8.
- Namura K, Hasegawa G, Egawa M *et al.* Relationship of serum brain-derived neurotrophic factor level with other markers of disease severity in patients with atopic dermatitis. *Clin Immunol* 2007; 122:181–6.
- Toyoda M, Nakamura M, Makino T, Hino T, Kagoura M, Morohashi M. Nerve growth factor and substance P are useful plasma markers of disease activity in atopic dermatitis. *Br J Dermatol* 2002; 147:71–9.
- Virchow JC, Julius P, Lommatzsch M, Luttmann W, Renz H, Braun A. Neurotrophins are increased in bronchoalveolar lavage fluid after segmental allergen provocation. *Am J Respir Crit Care Med* 1998; 158:2002–5.
- Raap U, Fokkens W, Bruder M, Hoogsteden H, Kapp A, Braunstahl GJ. Modulation of neurotrophin and neurotrophin receptor expression in nasal mucosa after nasal allergen provocation in allergic rhinitis. *Allergy* 2008; 63:468–75.
- Zuberbier T, Asero R, Bindslev-Jensen C *et al.* EAACI/GA(2)LEN/EDF/WAO guideline: definition, classification and diagnosis of urticaria. *Allergy* 2009; 64:1417–26.
- Baiardini I, Giardini A, Pasquali M *et al.* Quality of life and patients' satisfaction in chronic urticaria and respiratory allergy. *Allergy* 2003; 58:621–3.
- Wedi B, Raap U, Kapp A. Chronic urticaria and infections. *Curr Opin Allergy Clin Immunol* 2004; 4:387–96.

- 26 Konstantinou GN, Asero R, Maurer M, Sabroe RA, Schmid-Grendelmeier P, Grattan CE. EAACI/GA(2)LEN task force consensus report: the autologous serum skin test in urticaria. *Allergy* 2009; **64**:1256–68.
- 27 Elias J, Boss E, Kaplan AP. Studies of the cellular infiltrate of chronic idiopathic urticaria: prominence of T-lymphocytes, monocytes, and mast cells. *J Allergy Clin Immunol* 1986; **78**:914–8.
- 28 Ying S, Kikuchi Y, Meng Q, Kay AB, Kaplan AP. TH1/TH2 cytokines and inflammatory cells in skin biopsy specimens from patients with chronic idiopathic urticaria: comparison with the allergen-induced late-phase cutaneous reaction. *J Allergy Clin Immunol* 2002; **109**:694–700.
- 29 Raap U, Wiczorek D, Gehring M *et al*. Increased levels of serum IL-31 in chronic spontaneous urticaria. *Exp Dermatol* 2010; **19**:464–6.
- 30 Zuberbier T, Asero R, Bindslev-Jensen C *et al*. EAACI/GA(2)LEN/EDF/WAO guideline: management of urticaria. *Allergy* 2009; **64**:1427–43.
- 31 Ozseker F, Buyukozturk S, Gelincik A *et al*. Neurotrophins: are they meaningful in chronic spontaneous urticaria? *Asian Pac J Allergy Immunol* 2008; **26**:83–8.
- 32 Hermes B, Zuberbier T, Haas N, Henz BM. Decreased cutaneous expression of stem cell factor and of the p75NGF receptor in urticaria. *Br J Dermatol* 2003; **148**:411–7.
- 33 Levi-Montalcini R, Dal Toso R, della VF, Skaper SD, Leon A. Update of the NGF saga. *J Neurol Sci* 1995; **130**:119–27.
- 34 Wittmann M, Zeitvogel J, Wang D, Werfel T. IL-27 is expressed in chronic human eczematous skin lesions and stimulates human keratinocytes. *J Allergy Clin Immunol* 2009; **124**:81–9.
- 35 Hermes B, Prochazka AK, Haas N, Jurgovsky K, Sticherling M, Henz BM. Up-regulation of TNF-alpha and IL-3 expression in lesional and uninvolved skin in different types of urticaria. *J Allergy Clin Immunol* 1999; **103**:307–14.
- 36 Puccetti A, Bason C, Simeoni S *et al*. In chronic idiopathic urticaria autoantibodies against Fc epsilonRII/CD23 induce histamine release via eosinophil activation. *Clin Exp Allergy* 2005; **35**:1599–607.
- 37 Tedeschi A, Asero R, Marzano AV *et al*. Plasma levels and skin-eosinophil-expression of vascular endothelial growth factor in patients with chronic urticaria. *Allergy* 2009; **64**:1616–22.
- 38 Cugno M, Marzano AV, Tedeschi A, Fannoni D, Venegoni L, Asero R. Expression of tissue factor by eosinophils in patients with chronic urticaria. *Int Arch Allergy Immunol* 2009; **148**:170–4.