

ORIGINAL PAPER

Differential up-regulation of neurotrophin receptors and functional activity of neurotrophins on peripheral blood eosinophils of patients with allergic rhinitis, atopic dermatitis and nonatopic subjects

U. Raap, N. Deneka, M. Bruder, A. Kapp and B. Wedi

Department of Dermatology and Allergology, Hannover Medical School, Hannover, Germany

Clinical and Experimental Allergy

Summary

Background Recent studies suggest that neurotrophins have a pivotal role in neuroimmune interactions. Indeed, in contrast to nonatopic subjects (NA), neurotrophins have been shown to be increased in atopic diseases such as allergic rhinitis (AR) and atopic dermatitis (AD).

Aim The aim of the study was to assess the functional role of nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin (NT)-3 and -4 and the expression of pan-neurotrophin receptor (p75^{NTR}) and tyrosine kinase (trk)A, -B and -C on peripheral blood eosinophils in AR, AD and NA.

Methods Peripheral blood eosinophils of patients with AR, AD and NA were purified by CD16 negative selection (purity > 98%). Neurotrophin receptor expression was analysed by FACS analysis. Apoptosis test (FACS analysis) and chemotactic index (modified Boyden chamber assay) were assessed after stimulation with BDNF, NT-3/-4 and NGF.

Results The expression of trkA-C and p75^{NTR} was significantly higher in AD > AR > NA ($P < 0.05$ – 0.001). Apoptosis was significantly inhibited by BDNF, NGF, NT-3 in AD ($P < 0.05$ – 0.001), by NT-3/-4 and NGF in AR ($P < 0.05$ – 0.01) and by NT-3 ($P < 0.05$ – 0.01) in NA eosinophils. Chemotaxis was significantly induced by BDNF and NT-3/4 ($P < 0.01$ – 0.001) in AD peripheral blood eosinophils.

Conclusion Neurotrophin receptor expression and neurotrophin functional activity was greatest in AD > AR > NA. AD eosinophils are pre-activated and may therefore better respond to neurotrophins. With this study, we provide new pathophysiologic insights into atopic diseases with a functional role of neurotrophins in peripheral blood eosinophils in AD and AR.

Keywords allergic rhinitis, apoptosis, atopic dermatitis, eosinophil granulocytes, neurotrophins

Submitted 21 September 2007; revised 25 March 2008; accepted 9 April 2008

Correspondence:

Ulrike Raap, Department of Dermatology and Allergology, Hannover Medical School, Ricklinger Str. 5, 30449 Hannover, Germany.
E-mail: raap.ulrike@mh-hannover.de

Introduction

Recent studies suggest that neurotrophins are important mediators for the bidirectional interaction between key target effector cells and peripheral nerves in the allergic inflammatory response [1]. Indeed, neurotrophins have long been regarded as neuronal mediators only. However, it has become evident that neurotrophins not only have a functional role in peripheral or central nerves but also in immune cells. Neurotrophins belong to a subgroup of structurally related proteins, including nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF),

neurotrophin (NT)-3 and -4. Their actions are mediated via the pan-neurotrophin receptor (p75^{NTR}) to which all mature neurotrophins bind with low affinity and specificity [2, 3]. In addition, neurotrophins bind with high affinity and specificity to receptors of the tyrosine kinase (trk) family. NGF binds to trkA, BDNF and NT-4 to trkB and NT-3 to trkC [4].

Neurotrophin receptors are expressed on several immune cells such as T and B cells [5, 6]. Also, peripheral blood eosinophils express neurotrophin receptors as shown by Noga et al. [7] in asthmatic subjects and by us [8] in atopic dermatitis (AD) and nonatopic healthy

subjects (NA). Interestingly, we revealed a higher expression of p75^{NTR} and trkB in AD peripheral blood eosinophils compared with NA, suggesting that the activation process of eosinophils might have an impact on neurotrophin receptor up-regulation [8].

In allergic asthma, several animal studies give evidence for a functional role of neurotrophins in bronchial hyper-reactivity and the enhancement of the inflammatory response [9, 10]. In AD, we have recently shown that neurotrophins are increased in the peripheral blood in comparison with other chronic inflammatory skin diseases such as psoriasis, underlining their possible impact in atopic diseases [11]. In addition, we have found evidence for an anti-apoptotic and chemotactic effect of BDNF on peripheral blood eosinophils of AD [8]. Peripheral blood eosinophils are also a source of neurotrophins, as shown by us for BDNF and by others for NGF, NT-3 and NT-4 [8, 12, 13]. However, the functional role of neurotrophins and neurotrophin receptor expression in peripheral blood eosinophils in atopic diseases such as allergic rhinitis (AR), AD and NA still remains unclear. Therefore, the aim of our study was to assess whether there is (i) a difference of neurotrophin receptor expression, (ii) a functional role of neurotrophins and (iii) a difference of neurotrophin functional activity in highly purified peripheral blood eosinophils of patients with AR and AD compared with NA.

Methods

Subject groups

Fifteen NA served as a control group (no history of AR, asthma or AD, no increased total IgE levels, no specific IgE Ab for at least seven frequent aeroallergens, negative skin prick test (SPT) reaction to a panel of 10 common aeroallergens); 15 patients with AR (history of intermittent AR, no AD, no asthma, increased total IgE levels, specific IgE Ab and positive SPT reactions to at least three out of 10 common aeroallergens); and 15 patients with AD of extrinsic type (positive history of AR, increased total IgE levels, specific IgE Ab and positive SPT reactions to at least three of ten frequent aeroallergens), defined according to the criteria of Hanifin and Rajka with a SCORAD score of at least 10%, were included in the study. All participants gave informed written consent to the study, which was approved by the medical ethics committee of the Hannover Medical School, Germany. None of the participants received any systemic or local immunosuppressive treatment for 2 weeks. All blood samples were taken out of the pollen season in winter time.

Blood samples and purification of eosinophils

Peripheral venous blood was obtained at nine o'clock in the morning to prevent daily variations. Eosinophils were

isolated by Ficoll density gradient centrifugation and subsequent CD16 negative selection with CD16 microbeads (Mitenyi Biotech[®], Bergisch Gladbach, Germany) as described previously [8, 14]. Eosinophil purity was >98% as assessed by FACS analysis (CD49d/CD16) and Kimura staining. Eosinophil viability was >99% as assessed by trypan blue dye exclusion.

Detection of neurotrophin receptors

2×10^5 eosinophils were incubated with primary Ab (1–5 µg/mL), all directed against the extracellular domain such as p75^{NTR} (mAb clone NGFR5; NeoMarkers, CA, USA), trkA-C (mAb; R&D Systems, Wiesbaden, Germany) or respective isotype controls (mAb IgG1 for p75^{NTR}, mAb IgG2a for trkA, mAb IgG2b for trkB/C, all R&D Systems) for 1 h at 4 °C. After washing steps, eosinophils were incubated with goat F(ab')₂ fragment anti-mouse IgG-FITC (Beckman Coulter, Marseille, France) for 30 min at 4 °C. Cells were analysed with a FACScan (Becton Dickinson, San Jose, CA, USA). Non-viable cells were gated out of the window and at least 10 000 events were analysed.

Eosinophil survival *in vitro*

1×10^6 eosinophils were resuspended in culture medium and added to a 24-well flat-bottomed culture plate in 1 mL aliquots. Eosinophils were stimulated with 10, 50 and 100 ng/mL BDNF, NT-3, NT-4 or NGF (50, 100, 500, 1000 ng/mL) (with endotoxin level <0.1 ng per 1 µg of cytokine, all R&D Systems), C5a (10^{-8} mol/L), GM-CSF (10 ng/mL) or dexamethasone (10^{-6} mol/L) (all Sigma-Aldrich Chemicals, Schnelldorf, Germany). Eosinophils were incubated at 37 °C, 5% CO₂ for 24 up to 120 h in RPMI 1640 with 10% heat-inactivated FCS including 2 mM L-glutamine, 10 000 U/mL penicillin and 10 mg/mL streptomycin (Seromed, Biochrome AG, Germany). Viability was assessed by trypan blue dye exclusion. Those eosinophils that excluded trypan blue after a 5-min incubation were considered to be viable cells.

Cytofluorometric analysis of eosinophil apoptosis

Apoptotic eosinophil nuclei were distinguished by their hypodiploid DNA content with the modified protocol of Nicoletti, as described previously [8, 15]. In addition, apoptosis was assessed with annexin-V propidium iodide labelling (Annexin V kit, Pharmingen, San Diego, CA, USA) as described previously [8]. The cells were analysed by flow cytometry within 1 h.

Chemotaxis assay

Chemotactic activity was assessed with Boyden chambers, which were filled with stimuli such as BDNF, NT-3/-4

(50 ng/mL, respectively), NGF (1000 ng/mL) or C5a (10^{-8} mol/L) as positive control and covered with polycarbonate filters (8 mm diameter). One hundred microlitres of an eosinophil suspension, concentration 5×10^5 per mL, was added to each chamber. After incubation for 3 h at 37 °C, the cells migrated to the lower part of the Boyden chambers and were lysed by adding 0.1% Triton X-100. β -glucuronidase activity in the lysates was determined photometrically using *p*-nitrophenyl β -glucuronide as a substrate. Values were calculated by a computer-assisted technique from a standard curve using known numbers of unchallenged eosinophils. Chemotactic activity was presented as a chemotactic index with the ratio of the number of migrating cells in the presence of stimulus/migrating cells in the presence of the medium.

Materials

If not otherwise stated, reagents were obtained from Sigma-Aldrich Chemicals.

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 the Mann-Whitney rank sum test (normality test failed). A *P*-value <0.05 was considered to be statistically significant.

Results

Increased expression of p75^{NTR}, tyrosine kinase A, B and C on eosinophils of patients with atopic dermatitis

Using FACS analysis, p75^{NTR} and trkA-C receptor expression was detected on highly purified peripheral blood eosinophils in all the groups studied. The p75^{NTR} expression was significantly higher in AD (mean $\pm$ SEM 24.9 ± 4.2) compared with AR (11.9 ± 5.4 ; $P < 0.05$) and NA eosinophils (8.4 ± 2.1 ; $P < 0.001$) (Fig. 1). The highest expression of trkA was obtained in AD eosinophils (6.7 ± 1.8) compared with AR (1.7 ± 1.2 ; $P < 0.05$) and NA (2.9 ± 0.8 ; $P < 0.05$). The number of trkB- and trkC-positive peripheral blood eosinophils was highest in AD (25.4 ± 4.6 for trkB; 12.7 ± 3.2 for trkC), with a significant difference compared with NA (9.7 ± 2.5 trkB; 4.6 ± 1.8 trkC; $P < 0.05$ – 0.01), however, with no significant difference as compared with eosinophils of AR (14.7 ± 6.7 for trkB; 9.1 ± 2.6 for trkC; Fig. 1).

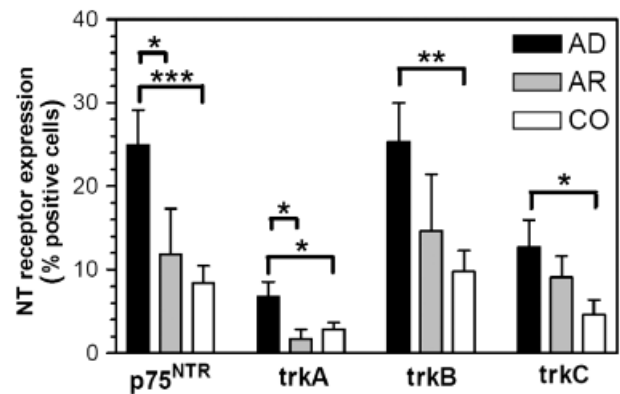


Fig. 1. Expression of neurotrophin receptor p75^{NTR}, tyrosine kinase (trk) A–C on highly purified peripheral blood eosinophils of patients with atopic dermatitis (AD) (black bar, $n = 15$), allergic rhinitis (AR) (grey bar, $n = 11$ – 15) and healthy control subjects (CO, unfilled bar, $n = 15$). Results are presented as mean $\pm$ SEM (* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$).

Neurotrophins inhibit apoptosis of peripheral blood eosinophils derived from patients with atopic dermatitis and allergic rhinitis

Peripheral blood eosinophils of AR and AD have a delayed programmed cell death in comparison with NA [15]. We confirmed these data in the present study. Stimulation of AD and AR eosinophils with NT-3 significantly inhibited apoptosis. In AR, a dosage of 100 ng/mL NT-3 was sufficient to inhibit eosinophil apoptosis ($P < 0.05$ – 0.01 , 24–120 h, data not shown), whereas in AD, eosinophils' lower concentrations (50 ng/mL) of NT-3 significantly inhibited eosinophil apoptosis ($P < 0.05$ – 0.001 , Fig. 2a). Also, in NA, NT-3 (100 ng/mL) significantly inhibited peripheral blood eosinophil apoptosis from 24 to 120 h in culture ($P < 0.05$ – 0.01 , data not shown). Stimulation of AD eosinophils with BDNF 10, 50 and 100 ng/mL, respectively, inhibited apoptosis in a dose-dependent manner. This was statistically significant for BDNF 10 ng/mL at 24, 72 and 120 h ($P < 0.05$, data not shown) and for BDNF 50 ng/mL at each time-point from 24 up to 120 h in culture ($P < 0.05$ – 0.001 , Fig. 2b). In contrast, BDNF stimulation did not modulate eosinophils apoptosis in NA and AR (data not shown). NT-4 (10 and 50 ng/mL) significantly inhibited peripheral blood eosinophil apoptosis in AR ($P < 0.05$ – 0.01 , data not shown), whereas there was no effect of NT-4 in AD and NA eosinophils. We could not find any functional effect of NGF using concentrations of 50, 100 and 500 ng/mL in all the subjects studied. However, stimulation of AD and AR eosinophils with NGF 1000 ng/mL significantly inhibited apoptosis at 24–120 h ($P < 0.05$ – 0.01 , for AD eosinophils, see Fig. 2c), whereas this effect was not found in NA eosinophils (data not shown). GM-CSF was used as an apoptosis-inhibiting control. The inhibition of peripheral blood eosinophil apoptosis by GM-CSF was twofold stronger compared to

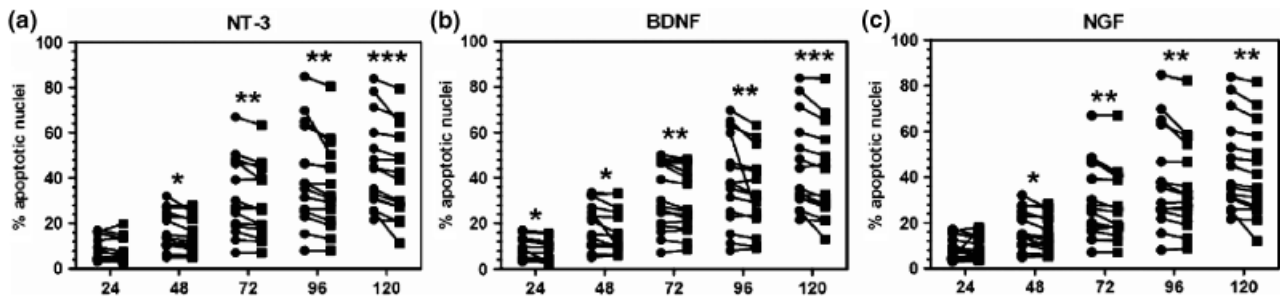


Fig. 2. Detection of eosinophil apoptosis displayed as percentage of apoptotic nuclei incubated with medium (dot) or with neurotrophins (square). Significant inhibition of peripheral blood eosinophil apoptosis of atopic dermatitis patients after stimulation with neurotrophin (NT)-3 50 ng/mL ($n = 15$) (a), brain-derived neurotrophic factor (BDNF) 50 ng/mL ($n = 15$) (b) and nerve growth factor (NGF) 1000 ng/mL ($n = 15$) (c) compared with the stimulation in medium alone after 24–120 h (x-axis) in culture (* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$).

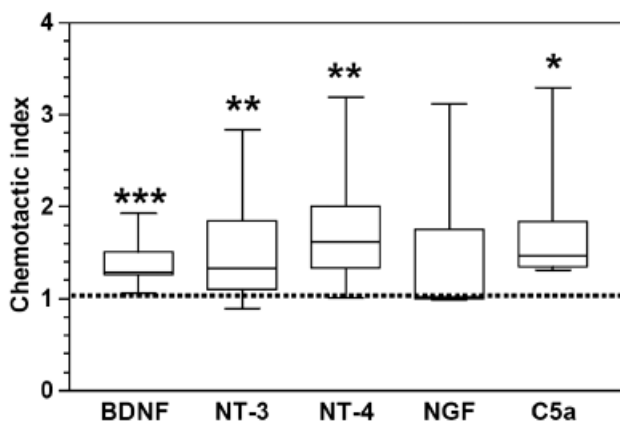


Fig. 3. Induction of chemotaxis displayed as a chemotactic index of atopic dermatitis peripheral blood eosinophils after stimulation with brain-derived neurotrophic factor (BDNF), neurotrophin (NT)-3, NT-4 and nerve growth factor (NGF) ($n = 10$ respectively; results are presented as box-blot graphs with median (line within the box) and interquartile range (* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$). C5a served as a positive control and induced chemotaxis in an expected manner.

the stimulation with BDNF, NT-3 and NGF (data not shown).

Brain-derived neurotrophic factor, neurotrophin-3 and neurotrophin-4 induce chemotaxis of eosinophils from patients with atopic dermatitis

BDNF (mean chemotactic index $\pm$ SEM; 1.38 ± 0.07), NT-3 (1.54 ± 0.15), NT-4 (1.79 ± 0.19) and C5a (1.71 ± 0.21) significantly induced eosinophil chemotaxis in AD eosinophils ($P < 0.05$ – 0.001) (Fig. 3), whereas this effect was not shown for NGF (1.45 ± 0.28). Stimulation of peripheral blood eosinophils of AR with BDNF (mean chemotactic index $\pm$ SEM, 0.96 ± 0.15), NT-3 (1.41 ± 0.29), NT-4 (1.08 ± 0.17) and NGF (0.99 ± 0.21) had no effect on the induction of chemotaxis, whereas C5a (2.30 ± 0.40) significantly induced

chemotaxis as compared with culture in medium ($P < 0.05$, data not shown). In NA, BDNF (1.15 ± 0.19), NT-3 (1.08 ± 0.047), NT-4 (1.12 ± 0.12) and NGF (1.00 ± 0.14) did not induce eosinophil chemotaxis, whereas C5a, which was used as a positive control, significantly induced eosinophil chemotaxis (1.76 ± 0.25 ; $P < 0.05$).

Discussion

Neurotrophins have been assessed in allergic diseases and their pivotal role has been described recently in several allergic inflammatory diseases including asthma, rhinitis and AD [8–11, 16–21]. 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 [22], the prototype of neurotrophins. However, investigations of neurotrophin-mediated effects on peripheral blood eosinophils – being potent effector cells in allergic inflammation of the skin, upper and lower airways – have not been compared thus far.

With our study, we provide evidence for the different expression profiles of neurotrophin receptors on peripheral blood eosinophils of atopic diseases such as AR and AD compared with nonatopic healthy controls. Interestingly, we found the highest expression of p75^{NTR} and trkA-C receptors in AD, followed by AR, whereas the receptor expression was the lowest in nonatopic control subjects. The expression of neurotrophin receptors in patients with airway allergy confirms the data of Noga et al. [7], who assessed the expression of trkA, -B and -C neurotrophin receptors on peripheral blood eosinophils in patients with AR and asthma. The high expression of neurotrophin receptors in AD could be explained by the fact that peripheral blood eosinophils of AD are pre-activated as shown by us previously [15, 23]. Indeed, the inflammatory response in AD affects a much larger organ in comparison with AR, giving another explanation as to why peripheral blood eosinophils may be more activated than in AR.

We also assessed the functional activity of neurotrophins by analysing chemotactic activity of BDNF, NT-3 and NT-4 on AD eosinophils. In our study, we could not find evidence for an induction of chemotaxis by NGF. Hamada et al. [24] assessed an induction of eosinophil chemotaxis by NGF with lower concentrations (50 ng/mL) and they also assessed an inhibition of peripheral blood eosinophil apoptosis with these NGF concentrations in NA. We could not find a functional effect of NGF with concentrations of 50, 100 and 500 ng/mL. However, using 1000 ng/mL NGF we assessed an inhibition of eosinophil apoptosis in AD and AR, which have *per se* a delayed programmed cell death in comparison with NA. We, therefore, used this high NGF dosage for investigating chemotactic activity.

In our study, NGF was able to inhibit peripheral blood eosinophil apoptosis, as well as BDNF, and NT-3 in AD. In AR, NGF, NT-4 and NT-3 inhibited eosinophil apoptosis whereas in NA, only NT-3 was able to inhibit eosinophil apoptosis. Peripheral blood eosinophils are a source of NGF and capable of releasing NGF on immunologic stimuli [13, 25]. Eosinophils also contain NT-3 and NT-4 as shown previously [12, 13]. In addition, peripheral blood eosinophils are a source of BDNF [8]. However, in the latter study, we only assessed a release of BDNF after stimulation of eosinophils with platelet-activating factor in AD and not in NA [8]. This underlines that eosinophils are not only a source of neurotrophins but are also capable of releasing neurotrophins such as BDNF at least in AD, which offers another explanation as to why these eosinophils may better respond to neurotrophins.

To summarize, our data provide evidence for the different up-regulation of neurotrophin receptors as well as their functional activities on peripheral blood eosinophils derived from patients with atopic diseases. AD seems to be the major target with regard to neurotrophin functional activity and receptor expression on peripheral blood eosinophils. As neurotrophins are capable of functioning bidirectionally between target effector cells and peripheral nerves, our findings reveal new pathophysiologic insights into AD pointing to neuroimmune interactions. Whether or not neurotrophins may also be expressed in allergic inflammatory skin such as AD needs to be addressed in future studies.

Acknowledgements

Ulrike Raap was funded by the Young investigators grant from the Hannover Medical School.

References

- 1 Nockher WA, Renz H. Neurotrophins in allergic diseases: from neuronal growth factors to intercellular signaling molecules. *J Allergy Clin Immunol* 2006; 117:583–9.
- 2 Lewin GR, Barde YA. Physiology of the neurotrophins. *Annu Rev Neurosci* 1996; 19:289–317.
- 3 Kaplan DR, Miller FD. Neurotrophin signal transduction in the nervous system. *Curr Opin Neurobiol* 2000; 10:381–91.
- 4 Miller FD, Kaplan DR. Neurobiology: TRK makes the retrograde. *Science* 2002; 295:1471–3.
- 5 Labouyrie E, Dubus P, Groppi A et al. Expression of neurotrophins and their receptors in human bone marrow. *Am J Pathol* 1999; 154:405–15.
- 6 Ehrhard PB, Erb P, Graumann U, Otten U. Expression of nerve growth factor and nerve growth factor receptor tyrosine kinase Trk in activated CD4-positive T-cell clones. *Proc Natl Acad Sci USA* 1993; 90:10984–8.
- 7 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.
- 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.
- 9 Kerzel S, Path G, Nockher WA et al. Pan-neurotrophin receptor p75 contributes to neuronal hyperreactivity and airway inflammation in a murine model of experimental asthma. *Am J Respir Cell Mol Biol* 2003; 28:170–8.
- 10 Path G, Braun A, Meents N et al. Augmentation of allergic early-phase reaction by nerve growth factor. *Am J Respir Crit Care Med* 2002; 166:818–26.
- 11 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.
- 12 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.
- 13 Kobayashi H, Gleich GJ, Butterfield JH, Kita H. Human eosinophils produce neurotrophins and secrete nerve growth factor on immunologic stimuli. *Blood* 2002; 99:2214–20.
- 14 Wedi B, Wiczorek D, Stunkel T, Breuer K, Kapp A. Staphylococcal exotoxins exert proinflammatory effects through inhibition of eosinophil apoptosis, increased surface antigen expression (CD11b, CD45, CD54, and CD69), and enhanced cytokine-activated oxidative burst, thereby triggering allergic inflammatory reactions. *J Allergy Clin Immunol* 2002; 109:477–84.
- 15 Wedi B, Raap U, Lewrick H, Kapp A. Delayed eosinophil programmed cell death in vitro: a common feature of inhalant allergy and extrinsic and intrinsic atopic dermatitis. *J Allergy Clin Immunol* 1997; 100:536–43.
- 16 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.
- 17 Sanico AM, Stanisiz AM, Gleeson TD et al. Nerve growth factor expression and release in allergic inflammatory disease of the upper airways. *Am J Respir Crit Care Med* 2000; 161:1631–5.
- 18 Aloe L, Bracci-Laudiero L, Alleva E, Lambiasi A, Micera A, Tirassa P. Emotional stress induced by parachute jumping enhances blood nerve growth factor levels and the distribution

- of nerve growth factor receptors in lymphocytes. *Proc Natl Acad Sci USA* 1994; **91**:10440–4.
- 19 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.
- 20 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.
- 21 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.
- 22 Levi-Montalcini R, Dal Toso R, della VF, Skaper SD, Leon A. Update of the NGF saga. *J Neurol Sci* 1995; **130**:119–27.
- 23 Wedi B, Raap U, Kapp A. Significant delay of apoptosis and Fas resistance in eosinophils of subjects with intrinsic and extrinsic type of atopic dermatitis. *Int Arch Allergy Immunol* 1999; **118**:234–5.
- 24 Hamada A, Watanabe N, Ohtomo H, Matsuda H. Nerve growth factor enhances survival and cytotoxic activity of human eosinophils. *Br J Haematol* 1996; **93**:299–302.
- 25 Solomon A, Aloe L, Pe'er J *et al*. Nerve growth factor is preformed in and activates human peripheral blood eosinophils. *J Allergy Clin Immunol* 1998; **102**:454–60.