

Brain-derived neurotrophic factor is increased in atopic dermatitis and modulates eosinophil functions compared with that seen in nonatopic subjects

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Background: Recently, the pivotal role of brain-derived neurotrophic factor (BDNF) has been described in allergic asthma. However, the role of this neurotrophin in atopic dermatitis (AD) still remains unknown.

Objective: The aim of this study was to investigate the functional role of BDNF on eosinophils and to assess BDNF levels in patients with AD and nonatopic control subjects.

Methods: p75 Neurotrophin receptor and tyrosine kinase B receptor expression was demonstrated by using FACS analysis and immunohistochemistry. BDNF levels were assessed with ELISA and FACS analysis. Chemotactic activity (modified Boyden chamber assay), eosinophil cationic protein release (fluoroenzyme immunoassay), respiratory burst (lucigenin-dependent chemiluminescence), and apoptosis (Nicoletti protocol and Annexin-V method) assays were used to assess BDNF functional activity.

Results: BDNF levels were increased in serum, plasma, eosinophils, and supernatants of stimulated eosinophils from patients with AD compared with levels seen in nonatopic control subjects ($P < .05-.001$). In addition, p75 neurotrophin receptor and tyrosine kinase B expression was higher on eosinophils from patients with AD compared with that seen on eosinophils from nonatopic control subjects ($P < .05-.001$). Eosinophil apoptosis was inhibited by BDNF ($P < .05-.01$) and chemotactic index was increased ($P < .001$) in BDNF-stimulated eosinophils from patients with AD, whereas this effect was not shown in eosinophils from nonatopic control subjects. However, no response of BDNF through the release of eosinophil cationic protein or reactive oxygen species was found.

Conclusion: This study provides the first evidence for a functional role of BDNF on eosinophils from patients with AD, probably mediated by an increased expression of BDNF receptors compared with that seen in nonatopic control subjects. In addition, higher intracellular, serum, and plasma BDNF levels, as well as the release of BDNF by eosinophils, underline the particular importance of BDNF in patients with

AD, pointing to new pathophysiologic aspects of this chronic inflammatory skin disease. (J Allergy Clin Immunol 2005;115:1268-75.)

Key words: Eosinophils, neurotrophins, atopic dermatitis, apoptosis, chemotaxis, brain-derived neurotrophic factor

Brain-derived neurotrophic factor (BDNF) was first cloned in 1989¹ as the second member of the neurotrophin family, which includes nerve growth factor (NGF) and neurotrophins 3 and 4/5. BDNF has been shown to regulate survival and differentiation of various neuronal populations, including sensory neurons.^{2,3} A wide range of immune cells, such as mast cells,⁴⁻⁶ T cells,^{7,8} B cells,^{9,10} endothelial cells,¹¹ natural killer cells,¹² macrophages,¹³ keratinocytes,¹⁴ and epithelial cells,¹⁵ have neurotrophin-producing capabilities. Neurons represent well-investigated sources of neurotrophin.¹⁶ Recent studies have shown that in addition to their neurotrophic activity, neurotrophins also appear to be important mediators in the interaction between both immune and nerve cells in inflammatory conditions. In this regard increased plasma levels of NGF have been found in asthmatic subjects and also in patients with atopic dermatitis (AD), contributing to disease activity.^{17,18}

The neurotrophin effects are mediated by binding to 2 different receptor types. On the one hand, there is the tyrosine kinase (trk) family of receptors to which neurotrophins bind with high affinity and specificity, such as trkA for NGF, trkB for BDNF and neurotrophin 4, and trkC for neurotrophin 3.¹⁹ On the other hand, there is the pan-neurotrophin receptor (NTR) p75, a member of the TNF superfamily, to which all neurotrophins bind with low affinity.²⁰ Neurotrophin receptors were found to be expressed on several immune cells, such as T and B cells.^{21,22} Recently, functional neurotrophin receptor expression was also found on peripheral blood eosinophils in inhalant allergy.²³ However, neurotrophin receptor expression and potential functional roles of neurotrophins on eosinophils in allergic inflammatory skin diseases still remain unclear.

Human eosinophils play an important role in the pathogenesis of several inflammatory diseases. Locally generated cytokines and chemokines regulate their accumulation and activation in tissue. At sites of inflammation,

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Abbreviations used

AD:	Atopic dermatitis
BDNF:	Brain-derived neurotrophic factor
ECP:	Eosinophil cationic protein
NGF:	Nerve growth factor
NTR:	Neurotrophin receptor
trk:	Tyrosine kinase

eosinophils are believed to be responsible for tissue damage through the release of oxygen species, cytotoxic granule proteins, and several proinflammatory cytokines.²⁴ Although the storage of NGF, neurotrophin 3, and BDNF in eosinophils has been recently described in patients with allergic asthma²⁵ and for NGF also in patients with AD,²⁶ it is still questionable whether eosinophils might be a source of BDNF also in AD.

On the basis of the pleiotropic effects of NGF on different inflammatory effector cells in atopic diseases, we hypothesized that the neurotrophin BDNF might exert immunomodulatory functions in patients with AD. Neurotrophin receptor expression and functional effects of BDNF were investigated on highly purified peripheral blood eosinophils *in vitro* to address this question. In addition, BDNF levels were investigated in serum, plasma, eosinophils, and eosinophil supernatants of patients with AD and nonatopic control subjects.

METHODS

Subjects

After informed consent (approved by the ethics committee), peripheral venous blood was obtained from nonatopic control subjects (no history of intermittent or persistent allergic rhinitis, asthma, or AD; no increased total IgE levels; no specific IgE antibodies for at least 7 frequent aeroallergens; and negative skin prick test reactions to a panel of 10 common aeroallergens). Patients with AD of the extrinsic type (positive history of intermittent allergic rhinitis, increased total IgE levels, specific IgE antibodies, and positive skin prick test reactions to at least 3 of 10 frequent aeroallergens) defined according to the criteria of Hanifin and Rajka with a SCORAD score of at least 10% were included in the study. Blood samples were taken at 9 AM to prevent daily variations. Subjects did not receive systemic or local immunosuppressive treatment.

Purification of eosinophils

Eosinophils were isolated by means of Ficoll density gradient centrifugation and subsequent CD16 negative selection with CD16 microbeads (Miltenyi Biotech, Bergisch-Gladbach, Germany), as previously described.²⁷ All readouts were performed with highly purified eosinophils (purity >98%), as assessed by means of FACS analysis (CD49d/CD16) and Kimura staining, and viability was greater than 99%, as assessed by means of trypan blue dye exclusion.

Immunocytochemistry

To examine neurotrophin receptor expression, immunocytochemistry was performed on alcohol-fixed cytospin preparations of eosinophils with an avidin-biotin-peroxidase kit according to the manufacturer's instructions (Vectastain ABC-kit; Alexis, Germany), as described elsewhere.²⁸ Primary antibody or respective isotype

control diluted in antibody diluent (DAKO Cytomation, Hamburg, Germany) were added in optimized concentrations (1–10 µg/mL) and incubated for 1 hour at room temperature. Paraffin samples (6 µm) of human neuroinoma were stained with p75NTR (monoclonal mouse anti-human NGF receptor antibody directed against the extracellular domain; clone NGFR5, NeoMarkers, Calif) and trkB (polyclonal rabbit anti-human trkB antibody; Chemicon International, Calif), respectively, to ascertain antibody specificity and revealed positively stained sensory nerves (data not shown). All slides were microscopically examined and photodocumented (Olympus).

Eosinophil survival *in vitro*

Eosinophils (1×10^6) were stimulated with 10, 50, and 100 ng/mL BDNF or the respective isotype control (mouse IgG1κ), C5a (10^{-8} mol/L), platelet-activating factor (PAF) (10^{-5} to 10^{-7} mol/L), GM-CSF (10 ng/mL), and dexamethasone (all R&D Systems, Minneapolis, Minn) at 37°C and 5% CO₂ for 24 up to 120 hours in RPMI 1640 with 10% heat-inactivated FCS, 2 mM L-glutamine, 10,000 U/mL penicillin, and 10 mg/mL streptomycin (Seromed, Biochrome AG, Germany). Viability was assessed by means of trypan blue dye exclusion.

Cytofluorometric analysis of eosinophil apoptosis

Apoptotic eosinophil nuclei were distinguished by their hypodiploid DNA content from the diploid DNA content of normal eosinophil nuclei with a modification of the protocol of Nicoletti, as described previously.^{27,29} Moreover, apoptosis was assessed with annexin-V propidium iodide labeling (Annexin V kit; Pharmingen, San Diego, Calif), as described in detail elsewhere.³⁰

Chemotaxis assay

Chemotactic activity was assessed with Boyden chambers, which were filled with medium and stimuli, such as BDNF or C5a, as positive controls and covered with polycarbonate filters. One hundred microliters of an eosinophil suspension (concentration of 5×10^5 /mL) was added to each chamber. After incubation for 1 hour 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, with *p*-nitrophenyl β-glucuronide as a substrate. Values were calculated by means of a computer-assisted technique from a standard curve with known numbers of unchallenged eosinophils. Chemotactic activity was presented as chemotactic index, with the ratio of the number of migrating cells in the presence of stimulus/migrating cells in the presence of medium.

Detection of reactive oxygen species

The release of reactive oxygen species was quantitated by using a single-photon imaging system (MTP reader; Hamamatsu Photonics, Herrsching, Germany), as described previously.³¹ Eosinophils were stimulated with 10, 50, and 100 ng/mL BDNF for 1 hour. Integral counts were obtained from a 30-minute incubation interval immediately after the addition of C5a (10^{-8} mol/L) and were indicated as intensity counts. Measurements were done in triplicate at 37°C.

Eosinophil cationic protein release

Eosinophils (5×10^6) were suspended in 1 mL of PBS containing 1 mM Ca²⁺ and 0.5 mM Mg²⁺. Assays were performed in 96-well flat-bottom culture plates in the presence of 5 µg/mL cytochalasin B with 5×10^5 cells/well. Cells incubated in medium alone or stimulated with BDNF (10, 50, and 100 ng/mL) were incubated for 60 minutes at 37°C. Eosinophil cationic protein (ECP) release was determined after addition of cetyltrimethyl-ammonium bromide to

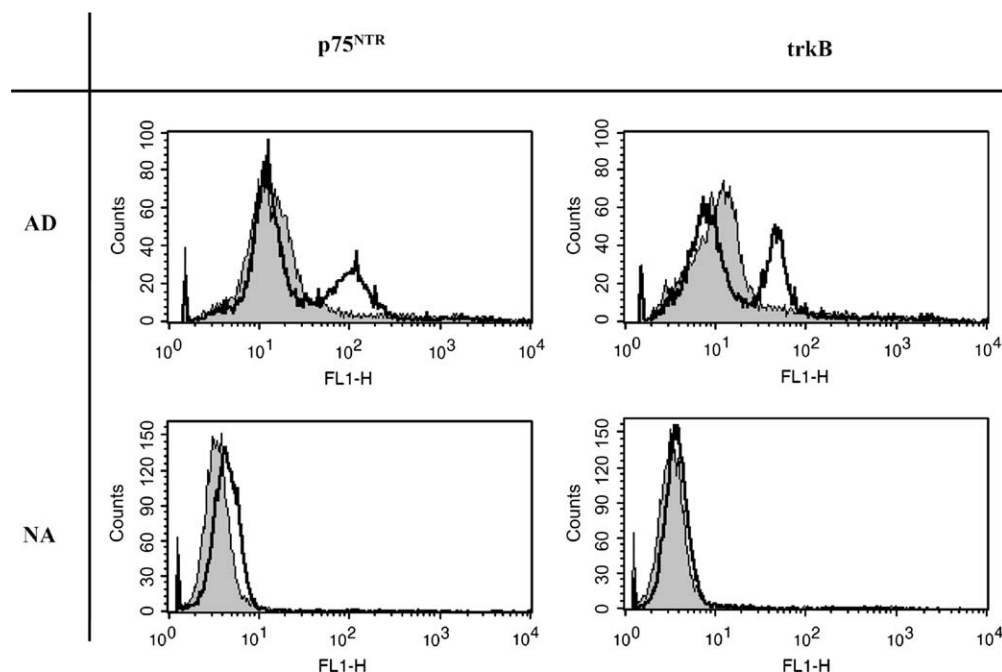


FIG 1. p75NTR and trkB surface expression on eosinophils from patients with AD and nonatopic control subjects. The filled histogram represents respective isotype control (mouse IgG1 or rabbit IgG), and the overlaid histogram (not filled) represents p75NTR or trkB expression. Presented is one experiment of 7.

the cells at a final concentration of 0.3%. ECP was detected in the supernatants by using a commercial fluoroenzyme immunoassay (UniCap; Pharmacia Diagnostics, Uppsala, Sweden).

Measurement of BDNF protein

A commercial ELISA (R&D Systems, Wiesbaden, Germany) was used to measure BDNF protein in eosinophil cell lysates, serum, plasma, and supernatant, according to the manufacturer's recommendations. The specificity of the assay was confirmed by the manufacturer, and the detection limit was 39 pg/mL.

p75NTR, trkB, and CD69 surface detection

Eosinophils (2×10^5) were incubated with mouse monoclonal anti-p75NTR (10 μ g/mL) or mouse IgG1 isotype control (10 μ g/mL, R&D Systems) and polyclonal rabbit anti-trkB antibody (diluted 1:2500) or rabbit IgG isotype control (diluted 1:2500, R&D Systems) for 1 hour at 4°C. Afterward, cells were incubated with FITC-labeled goat anti-mouse or goat anti-rabbit antibody (Beckmann Coulter, Marseille, France) for 30 minutes at 4°C.

Eosinophils were stimulated with 100 ng/mL BDNF for 24 to 120 hours and incubated with phycoerythrin-labeled monoclonal mouse-anti human CD69 (Immunotech, Marseille, France) or mouse IgG1 isotype control for 1 hour at 4°C to assess CD69 upregulation. Cells were analyzed with a FACScan (Becton Dickinson, San Jose, Calif).

BDNF intracellular staining

For intracellular staining, 2×10^5 eosinophils were prepared according to the manufacturer's instructions (BD Biosciences, San Diego, Calif). Eosinophils were incubated with 10 μ L of a monoclonal anti-human BDNF antibody or mouse IgG1 isotype control (both R&D Systems) for 50 minutes at 4°C. After washing steps, cells were incubated with goat anti-mouse FITC-labeled antibody for 20 to 30 minutes at 4°C. Intracellular BDNF levels were determined over time in

culture in unstimulated and GM-CSF-stimulated (10 ng/mL) eosinophils. Analysis was performed with FACScan (Becton Dickinson).

Materials

Propidium iodide was purchased from MoBiTec (Göttingen, Germany). If not otherwise stated, reagents were obtained from Sigma-Aldrich Chemicals (Schnellendorf, Germany).

Statistical analysis

Results are presented as means $\pm$ SEM or SD. The statistical significance between study groups was analyzed by using the unpaired Student *t* test and the paired Student *t* test for comparison in the group itself. A *P* value of less than .05 was accepted as statistically significant.

RESULTS

Increased expression of p75NTR and trkB on eosinophils from patients with AD

By using immunohistochemistry, p75NTR and trkB receptor expression was detected on highly purified eosinophils from patients with AD (data not shown). In addition, p75NTR and trkB expression profile was also assessed with FACS analysis (Fig 1). Interestingly, p75NTR expression was significantly higher in eosinophils from patients with AD compared with that seen in eosinophils from nonatopic control subjects ($P < .05$; Fig 2, A). Similar results were obtained for trkB ($P < .001$; Fig 2, B). Comparing the neurotrophin receptor expression profile in eosinophils from patients with AD itself, expression of trkB was significantly higher than that of p75NTR ($P < .01$; Fig 2, C).

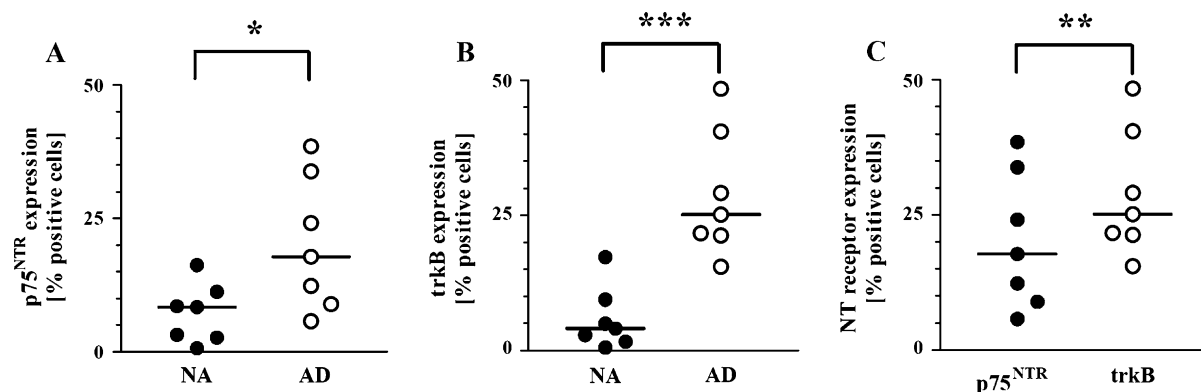


FIG 2. A, Significantly increased expression of p75^{NTR} presented as the percentage of positive cells of eosinophils from patients with AD compared with those from nonatopic control subjects (* $P < .05$). B, Increased expression of trkB on eosinophils from patients with AD compared with those from nonatopic control subjects (*** $P < .001$). C, Increased expression of trkB compared with p75^{NTR} on eosinophils from patients with AD (* $P < .05$, $n = 7$).

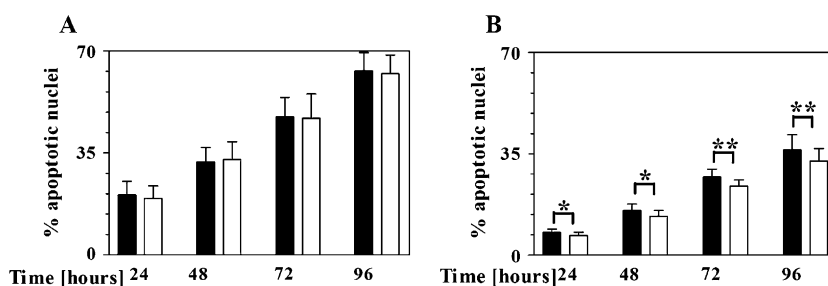


FIG 3. Preincubation with BDNF (50 ng/mL) significantly inhibited apoptosis of eosinophils from patients with AD (B) but not that of eosinophils from nonatopic control subjects (A; * $P < .05$, ** $P < .01$). Depicted are eosinophils incubated for 24 to 96 hours in culture with medium (filled bars) or with BDNF (open bars). Statistical analysis was performed with paired t test, and data are presented as means $\pm$ SEM ($n = 10$).

BDNF inhibited apoptosis and induced chemotaxis of eosinophils from patients with AD

With increasing time in culture, we found an increased number of apoptotic eosinophils in both patients with AD and nonatopic control subjects. As already published by us,³² apoptosis of eosinophils from patients with AD was significantly inhibited compared with that seen in eosinophils from nonatopic control subjects. Moreover, stimulation of eosinophils from patients with AD with 10, 50, and 100 ng/mL BDNF, respectively, inhibited apoptosis in a dose-dependent manner (Fig 3, B). This was statistically significant for 10 ng/mL BDNF at 24, 72, and 120 hours ($P < .05$) and for 50 ng/mL BDNF at each time point from 24 up to 120 hours in culture ($P < .05-.01$). In contrast, BDNF stimulation of eosinophils from nonatopic control subjects had no effect (Fig 3, A). Stimulation with GM-CSF as an apoptosis-inhibiting control resulted in a significant inhibition of eosinophil apoptosis in both groups, whereas dexamethasone significantly induced apoptosis (data not shown).

BDNF significantly induced eosinophil chemotaxis in eosinophils from patients with AD ($P < .001$; Fig 4, A) but not in eosinophils from nonatopic control subjects

(Fig 4, B). C5a was used as a positive control and induced eosinophil chemotaxis as expected.

No effect of BDNF on CD69 receptor upregulation, release of ECP, and reactive oxygen species

Eosinophils from patients with AD were stimulated with 50 ng/mL BDNF for 24 to 120 hours to assess CD69 upregulation. Compared with unstimulated eosinophils, CD69 expression was not increased after stimulation with BDNF (data not shown).

BDNF at concentrations of 10, 50, and 100 ng/mL, respectively, had no influence on the release of ECP and reactive oxygen species either in patients with AD or nonatopic control subjects (data not shown).

BDNF levels were increased in serum, plasma, eosinophils, and eosinophil supernatants in patients with AD

BDNF levels were detected in serum and plasma of both groups studied. However, BDNF serum levels were significantly higher in patients with AD compared with levels in nonatopic control subjects ($P < .001$; Fig 5, B).

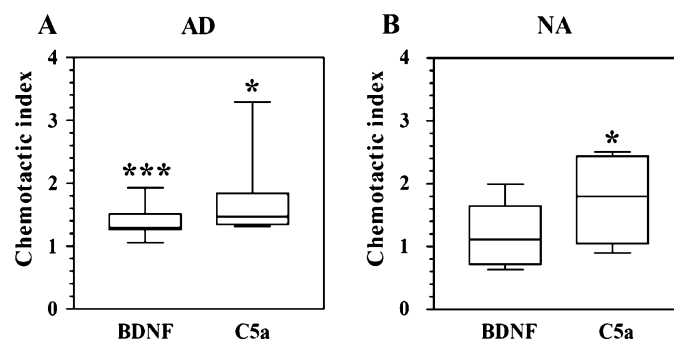


FIG 4. BDNF induced chemotaxis of eosinophils from patients with AD ($***P < .001$, **A**), whereas stimulation of eosinophils from nonatopic control subjects showed no chemotactic activity (**B**). Cells were incubated with BDNF, and incubation with C5a served as a positive control and induced chemotaxis in an expected pattern in both groups studied ($*P < .05$). The results are presented as means $\pm$ SEM of 10 different experiments.

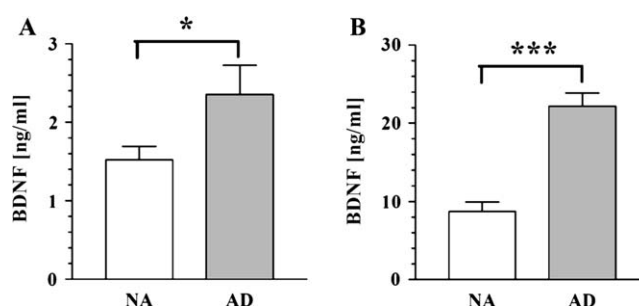


FIG 5. BDNF levels of plasma (**A**) and serum (**B**) of patients with AD and nonatopic control subjects. Columns represent means $\pm$ SEM. Open bars represent nonatopic control subjects, whereas filled bars represent patients with AD. Serum and plasma BDNF levels were significantly increased in patients with AD compared with nonatopic control subjects ($*P < .05$, $***P < .001$; $n = 11$).

Similarly, BDNF plasma levels were significantly higher in patients with AD ($P < .05$; Fig 5, A).

In freshly isolated and disrupted eosinophils, BDNF levels were detected by means of ELISA (Fig 6, A). Again, eosinophils from patients with AD showed significantly higher levels of BDNF compared with those from nonatopic control subjects ($P < .001$; Fig 6, A). In addition, stimulation of eosinophils with platelet-activating factor significantly increased BDNF levels in supernatants of patients with AD ($P < .05$; Fig 6, B), whereas BDNF was not detectable in supernatants of nonatopic control subjects (data not shown). To further underline the differing amount of BDNF in patients with AD and nonatopic control subjects, intracellular staining of BDNF was performed. Again, intracellular BDNF levels were higher in eosinophils from patients with AD ($P < .01$; Fig 7, A). In addition, stimulation of eosinophils from patients with AD with GM-CSF (10 ng/mL) resulted in a significant increase of intracellular BDNF levels after 48 to 96 hours in culture ($P < .01$; Fig 7, B), whereas this effect was not found in eosinophils from nonatopic control subjects (not shown).

DISCUSSION

Neurotrophins have been assessed in allergic diseases, and their pivotal role has recently been described in

several inflammatory conditions.^{17,33-37} It is well known that neurotrophins exert immunomodulatory functions not only on nerves but also on immune cells, which has been characterized best for NGF,³⁸ the prototype of neurotrophins. However, direct effects of neurotrophins on eosinophils, being potent effector cells in inflammatory conditions of the skin, have not been investigated thus far.

The results presented in this study demonstrate for the first time that BDNF levels are increased in serum, plasma, and eosinophils of patients with AD. In addition, eosinophils from patients with AD are capable of releasing BDNF, as assessed in supernatants of stimulated cells. Moreover, there is first evidence for p75NTR and trkB expression on peripheral blood eosinophils, which is greater in patients with AD compared with in nonatopic control subjects. Furthermore, it has been shown that BDNF induces chemotaxis and inhibits apoptosis of eosinophils in patients with AD but not in nonatopic control subjects, underlining the pivotal role of BDNF in inflammatory conditions.

As shown previously, NGF enhances survival of peripheral blood eosinophils of healthy human subjects.³⁹ However, the survival-enhancing activity of neurotrophins still remains heterogeneous because another group found no effect on viability by BDNF and NGF on peripheral blood eosinophils of asthmatic subjects.²³ However, bronchoalveolar lavage eosinophils were

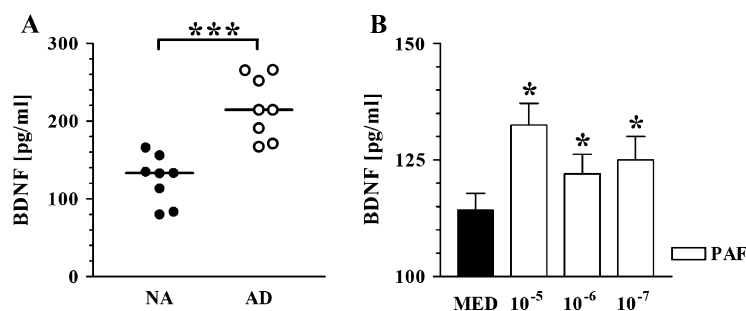


FIG 6. A, BDNF levels of freshly isolated and disrupted eosinophils. Scattergram represents single eosinophils and medians of data points from experimental groups. *Filled dots* represent nonatopic control subjects, and *open dots* represent patients with AD (** $P < .001$, $n = 8$). **B**, Increased BDNF levels in supernatants of eosinophils from patients with AD after stimulation with platelet-activating factor for 24 hours (*open bars*) compared with medium (*MED*) incubation (*filled bar*; * $P < .05$; $n = 4$).

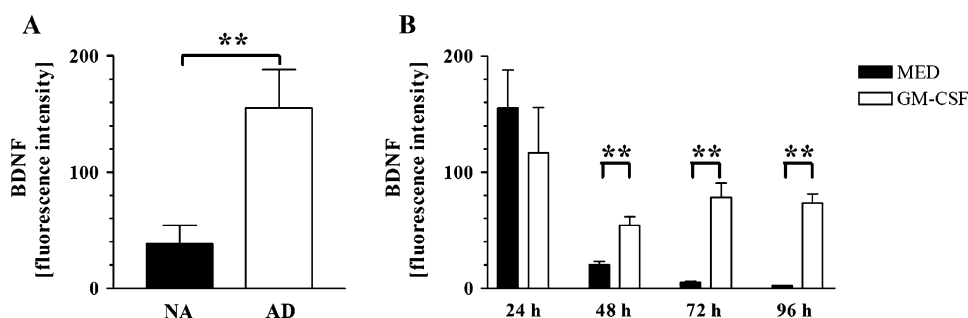


FIG 7. A, Increased intracellular BDNF levels depicted as geometric mean fluorescence intensity $\pm$ SEM of eosinophils from patients with AD (*open column*) compared with levels of eosinophils from nonatopic control subjects (*filled column*) (** $P < .01$; $n = 8$). **B**, Increased intracellular BDNF levels of eosinophils from patients with AD after GM-CSF stimulation (*open column*) compared with incubation with medium (*MED*) alone presented as geometric mean fluorescence intensity $\pm$ SEM (*filled bars*; ** $P < .01$; $n = 4$).

found to have an increased viability after allergen provocation and stimulation with NGF and BDNF.⁴⁰ In the present study eosinophil apoptosis was significantly inhibited in patients with AD compared with that seen in nonatopic control subjects, as previously shown by us.^{31,41} In addition, stimulation with BDNF significantly inhibited eosinophil apoptosis in patients with AD but not in nonatopic control subjects. As in asthmatic subjects, eosinophils in patients with AD are preactivated and might therefore respond to BDNF stimulation with inhibition of programmed cell death. Furthermore, neurotrophin receptors, such as p75NTR and trkB, were found to be particularly expressed on eosinophils from patients with AD. This could very likely explain why BDNF exerts immunomodulatory functions especially in eosinophils from patients with AD but not in eosinophils from nonatopic control subjects.

Several lines of evidence have shown that neurotrophins, such as NGF and BDNF, regulate Bcl2 levels in the nervous system.⁴² This effect was also shown for neurotrophin 3 recently, with the upregulation of the anti-apoptotic Bcl2 in oligodendrocyte progenitor cells preventing apoptosis.⁴³ Antiapoptotic effects might also be displayed through a mechanism involving the recruitment of the PI-3 kinase/Akt signaling pathway.⁴⁴ However, the underlying mechanisms of neurotrophin-

induced inhibition of apoptosis in eosinophils still has to be elucidated.

It has been shown that NGF activates eosinophil functions through the release of eosinophil cytotoxic granule proteins and induction of eosinophil chemotaxis.^{39,45} However, after stimulation with BDNF, we found no response by release of ECP or increased production of superoxide anions. In addition, we found no further upregulation of the eosinophil activation marker CD69 on eosinophils from patients with AD. Because eosinophils are a source of BDNF itself, it is very likely that additional BDNF stimulation of the preactivated eosinophil from patients with AD does not further upregulate CD69. Also, in asthmatic subjects CD69 was upregulated on peripheral blood eosinophils,³² and stimulation with NGF, BDNF, neurotrophin 3, and neurotrophin 4 did not further increase its expression.⁴⁰

It is well known that NGF induces migration of lung and skin fibroblasts and chemotaxis of macrophages.⁴⁶ In contrast, thus far, BDNF has not been described to exert chemotactic activity on effector cells of the inflammatory cascade.⁴⁷ Our data provide the first evidence for a chemotactic role of BDNF in patients with AD, indicating a role in proinflammatory actions.

NGF levels are significantly increased in asthma, rhinitis, and AD.^{17,37} Also, mental stress increases NGF

levels,³⁶ whereas kissing downregulates NGF and BDNF plasma levels.⁴⁸ In our study BDNF levels were significantly higher in serum and plasma from patients with AD compared with that from nonatopic control subjects. In addition, eosinophils from patients with AD were capable of BDNF release, as assessed in supernatants. Similar to NGF, endogenously released BDNF in patients with AD might have an effect on tissue remodeling after acute or chronic inflammation. The mechanism of increases in BDNF levels could possibly be explained by an autocrine production of BDNF by eosinophils, which are a source of BDNF itself and are increased in peripheral blood and skin lesions of patients with AD. However, other immunomodulatory cells, such as keratinocytes and endothelial cells, are possible releasers of BDNF as well.^{14,49}

BDNF also promotes hematopoietic colony growth and might participate in wound healing and tissue repair, contributing to skin remodeling in inflammatory conditions.⁵⁰ Therefore our observations might also have important implications for the pathophysiologic role of eosinophils in mucosal immunity and inflammation, especially in patients with AD. Thus eosinophil production of BDNF might represent a well-integrated mechanism for maintaining and/or restoring homeostatic function of tissues during or after inflammation. In addition, eosinophils express p75NTR and trkB neurotrophin receptors, suggesting that the synthesis and release of BDNF could be an autocrine mechanism of this leukocyte.

In conclusion, this study provided the first evidence for a functional role of BDNF on peripheral blood eosinophils from patients with AD. In addition, increased BDNF levels suggest the systemic involvement of neurotrophic factors in patients with AD in closing the loop of neuroimmune interactions in this chronic inflammatory skin disease.

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