Airway epithelial cells produce neurotrophins and promote the survival of eosinophils during allergic airway inflammation

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Background: Eosinophil-epithelial cell interactions make a major contribution to asthmatic airway inflammation. Nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), and other members of the neurotrophin family, originally defined as a class of neuronal growth factors, are now recognized to support the survival and activation of immune cells. Neurotrophin levels are increased in bronchoalveolar lavage fluid during allergic asthma.
Objective: We sought to investigate the role of neurotrophins as inflammatory mediators in eosinophil-epithelial cell interactions during the allergic immune response.
Methods: Neurotrophin expression in the lung was investigated by means of immunohistochemistry and ELISA in a mouse model of chronic experimental asthma. Coculture experiments were performed with airway epithelial cells and bronchoalveolar lavage fluid eosinophils.
Results: Neurotrophin levels increased continuously during chronic allergic airway inflammation, and airway epithelial cells were the major source of NGF and BDNF within the inflamed lung. Epithelial neurotrophin production was upregulated by IL-1β, TNF-α, and T_H2 cytokines. Lung eosinophils expressed the BDNF and NGF receptors tropomyosin-related kinase (Trk) A and TrkB, and coculture with airway epithelial cells resulted in enhanced epithelial neurotrophin production, as well as in prolonged survival of eosinophils. Eosinophil survival was completely abolished in the presence of the neurotrophin receptor Trk antagonist K252a.
Conclusion: During allergic inflammation, airway epithelial cells express increased amounts of NGF and BDNF that promote the survival of tissue eosinophils. Controlling epithelial neurotrophin production might be an important therapeutic target to prevent allergic airway eosinophilia.
Clinical implications: Attenuating the release of inflammatory mediators from the activated airway epithelium will become an important strategy to disrupt the pathogenesis of chronic allergic asthma. (J Allergy Clin Immunol 2006;117:787-94.)

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Supported by grants from the German National Science Foundation, Deutsche Forschungsgemeinschaft (Transregio 22, Projekt A9), and Stiftung für Pathobiochemie und Molekulare Diagnostik, DGKL.
Disclosure of potential conflict of interest: The authors have declared they have no conflict of interest.
Received for publication July 4, 2005; revised December 5, 2005; accepted for publication December 21, 2005.
Available online February 21, 2006.
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0091-6749/$32.00

doi:10.1016/j.jaci.2005.12.1339

Key words: *Allergy, asthma, neurotrophin, nerve growth factor, brain-derived neutrotrophic factor, eosinophils, survival, airway epithelium*

It is well known that eosinophils play an important role in the pathogenesis of allergic airway diseases because they contribute to the initiation and maintenance of the allergic response.[1] They are recruited at allergic inflammatory sites, and eosinophil accumulation into the inflamed lung is associated with both acute and chronic phases of allergic asthma.[2] The number of eosinophils within the allergic tissue depends primarily on the extent of cell infiltration during the acute inflammatory response. Mediators, such as IL-5 or eotaxin, control the infiltration process by regulating the maturation of eosinophils in the bone marrow, priming them for recruitment, and by regulating the eosinophil attraction into the tissues. However, on arrival at the site of inflammation, eosinophils depend on activation and survival signals; otherwise they undergo apoptosis and will be cleared by phagocytosis.[3] Therefore the tissue load of eosinophils is related to a balance between recruitment and apoptotic cell death, followed by phagocytosis. Cytokines such as IL-5 and GM-CSF are known to enhance eosinophil survival and thus contribute to the persistence of eosinophils at sites of allergic inflammation.[4,5] Recently, neurotrophins have been shown to exert an antiapoptotic effect on activated lung eosinophils in culture.[6] Neurotrophins, such as nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), and neurotrophin 3 (NT-3), were initially described as growth and survival factors for neuronal cells.[7] Neurotrophin's effects are mediated by cell-surface receptors, including the ligand-specific tropomyosin-related kinase (Trk) family and the pan-neurotrophin receptor $p75^{NTR}$.[8] NGF recognizes specifically TrkA, BDNF and neurotrophin 4/5 activate the TrkB receptor, and NT-3 recognizes primarily TrkC. Meanwhile, various cell types outside the nervous system, including immune cells and structural tissue cells, were identified as potential sources and targets of neurotrophins.[9] Although neurotrophins are able to maintain eosinophil survival in culture, we assume that a similar effect might also influence eosinophil homing within the inflamed airways. It is well known that NGF and BDNF levels are increased in bronchoalveolar fluids during allergic inflammation[10-13]; however, the cellular sources of neurotrophins in the allergic inflamed airways still remain unclear.

In the present study we therefore investigated neurotrophin expression in an animal model of chronic

Abbreviations used
7-AAD: 7-Amino-actinomycin D
BALF: Bronchoalveolar lavage fluid
BDNF: Brain-derived neurotrophic factor
MTEC: Murine tracheal epithelial cell
NGF: Nerve growth factor
NT-3: Neurotrophin 3
OVA: Ovalbumin
Trk: Tropomyosin-related kinase

experimental asthma and identified the epithelium as a major source of NGF and BDNF in the airways. The bronchial epithelium is a key position to translate and coordinate inflammatory signals between the luminal space and the lung tissue. Thereby eosinophil-epithelial cell interactions are thought to make a major contribution to asthmatic airway inflammation.[14] We hypothesized that neurotrophin synthesis by epithelial cells is tightly regulated during the allergic response and plays an important role in supporting eosinophil survival within the inflamed airway tissue. Here we show for the first time that eosinophil-epithelial interactions result in enhanced NGF/BDNF release by epithelial cells, as well as prolonged survival of eosinophils in culture.

METHODS

Animals

Female BALB/c mice between 8 and 12 weeks of age were purchased from Harlan Winkelmann (Borchen, Germany) and were kept under specific pathogen-free conditions. All animal experiments were performed according to ''The guidelines for the care and use of experimental animals'' prepared and published by the Society for Laboratory Animal Sciences (GV-SOLAS; Biberach a. d. Riss, 1988).

Allergic sensitization and airway allergen challenges

BALB/c mice were sensitized by means of 3 intraperitoneal injections of ovalbumin (OVA; 50 μg per injection; Sigma, Munich, Germany) and aluminium hydroxide (Pierce Biotechnology, Rockford, Ill) on days 0, 14, and 21. For isolation of bronchoalveolar lavage fluid (BALF) eosinophils, mice were challenged with 1% OVA aerosol on days 26, 27, and 28 (acute asthma). Immunohistologic analyses were performed from lungs of long-term allergen-exposed mice. Therefore mice were challenged with OVA aerosol twice a week for a 12-week period (chronic asthma).

Histologic analysis

Lung tissues and BALF eosinophil cytospin preparations were stained for NGF, BDNF, NT-3, TrkA, and TrkB by means of standard immunohistologic methods according to the manufacturer's instructions. Primary antibodies were as follows: rabbit anti-human NGF, BDNF, NT-3, TrkA, or TrkB (1:200; Santa Cruz Biotechnology, Santa Cruz, Calif). A biotinylated goat anti-rabbit IgG antibody (1:400, Vector Laboratories, Burlingame, Calif) was used as a secondary antibody. The biotinylated link antibody was labeled with an alkaline phosphatase–conjugated streptavidin (LSAB 2 system streptavidin AP; Dako Corporation, Glostrup, Denmark), followed by an incubation with the Vector Blue dye (lung tissue) or Vector Red (eosinophils, both Vector Laboratories), according to the manufacturer's instructions. Specificity of the primary antibody reactions was verified by incubating sections with irrelevant rabbit IgG antibodies (Vector Laboratories). Lung sections were stained with Congo red dye (Sigma) to identify eosinophils in the lung tissue. Congo red stains eosinophils and amyloid red-orange.

Isolation of primary tracheal epithelial cells

Primary murine tracheal epithelial cells (MTECs) were isolated as described elsewhere,[15] with slight modifications. Briefly, mice were killed, and the tracheas were transferred into dissociation media and incubated at 37°C for 60 minutes to release epithelial cells. The resultant cell suspension was incubated for 2 hours in a culture dish to remove contaminating nonepithelial cells by means of adherence. The nonattached cells were seeded into 24-well plates (4×10^5 cells/well) precoated with type VI collagen (Becton Dickinson, Mountain View, Calif) and were incubated for 4 days. Once the cells reached confluence, they were subcultured and used for further experiments. The purity of isolated epithelial cells was characterized by means of immunohistochemistry with antibodies against cytokeratin (pancytokeratin, Sigma) and was always greater than 99%.

Culture conditions of epithelial cells

LA-4 murine bronchial epithelial cells (American Type Culture Collection, Rockville, Md) were cultured in F12K Nutrient Mixture (Gibco, Carlsbad, Calif) supplemented with 15% FCS, 1% glutamine, 100 U/mL penicillin, and 100 μg/mL streptomycin (PAA Laboratories, Pasching, Austria). MTECs were cultured in airway epithelial cell growth medium supplemented with the supplement mixture, according to the manufacturer's instruction (Promocell, Heidelberg, Germany). Cells were seeded onto 24-well plates at concentrations of 2×10^5 cells/well. After 2 days of culture, cells had reached 80% confluence, and culture medium was changed. For analysis of basal neurotrophin production, cell-culture supernatants were collected 48 hours later and stored at −80°C for further analysis. Vectorial targeting of neurotrophin synthesis to the basolateral or apical (luminal) site was determined by culturing cells on the back of semipermeable culture inserts (PET membrane, 0.4-μm pore size; BD Labware, Franklin Lakes, NJ) until confluence. The culture medium was changed, and after another 2 days of culture, the supernatants of the cell-culture insert (basolateral compartment) and the cell-culture well (apical compartment) were removed.

The effect of the proinflammatory cytokines (IL-1β and TNF-α, all 10 ng/mL; PeproTech, Rocky Hill, NJ), T_H2-related cytokines (IL-4 and IL-13, PeproTech; IL-5, R&D Systems, Minneapolis, Minn; all 10 ng/mL), and the T_H1-related cytokine IFN-γ (PeproTech, 10 ng/mL) were analyzed by means of stimulation of LA-4 cells for 48 hours. In addition, LA-4 cells were cultivated with 0.1 μM dexamethasone (Fortecortin; Merck, Darmstadt, Germany), as described above. Cell-free supernatants were assayed for neurotrophins by using standard ELISA procedures, as described below.

Isolation of BALF eosinophils

After sensitization and airway allergen challenge, mice were killed 24 hours after the last challenge, and the tracheas were cannulated. Bronchoalveolar lavage was performed by flushing the lungs and airways 3 times with 800 μL of F12K Nutrient Mixture (Gibco) supplemented with 15% FCS, 1% glutamine, 100 U/mL penicillin, and 100 μg/mL streptomycin (all supplements were from PAA Laboratories). Cells were spun down onto glass slides with a cytospin (Thermo Electron Corp, Waltham, Mass), followed by staining with

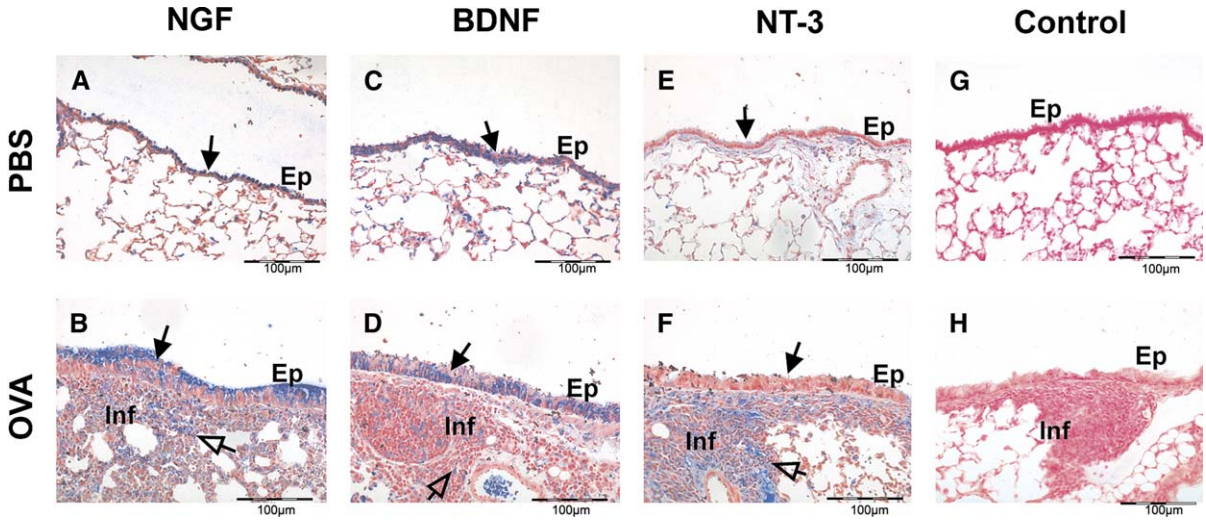

FIG 1. NGF, BDNF, and NT-3 expression in murine experimental asthma. Lung sections of PBS control mice *(PBS)* and OVA-sensitized mice *(OVA)* were stained for NGF (**A** and **B**), BDNF (**C** and **D**), and NT-3 (**E** and **F**). Rabbit IgG was used as a control antibody (**G** and **H**). *Ep*, Bronchial epithelium; *Inf*, inflammatory infiltrate. Original magnification 200×.

Diff-Quick (Dade, Marburg, Germany), to determine the purity of isolated eosinophils. The percentage of eosinophils was determined microscopically by using standard cytologic criteria, and they were greater than 95% of all BALF cells.

Coculture of eosinophils with airway epithelial cells

LA-4 cells were seeded onto 24-well plates at concentrations of 2×10^5 cells/well and were incubated for 48 hours until they reached 80% confluence. Cell-culture medium (F12K Nutrient Mixture, Gibco; supplemented with 15% FCS, 1% glutamine, 100 U/mL penicillin, and 100 μg/mL streptomycin, PAA Laboratories) was changed, and 2×10^5 isolated eosinophils were placed into a 24-well plate cell-culture insert (BD Labware). After further incubation for 48 hours, cell-free supernatants were collected and stored at −80°C until neurotrophin analysis. Eosinophils were cocultivated with LA-4 cells in the presence or absence of a selective inhibitor of the neurotrophin-specific tyrosine protein kinase (Trk) receptors (200 nM K252a; Alomone Labs, Jerusalem, Israel) to determine the survival rate of eosinophils.[16,17] After 1, 2, or 6 days, eosinophils were removed and subjected to a standard flow cytometric 7-amino-actinomycin D (7-AAD) dye exclusion survival assay, as described below.

Measurements of BDNF, NGF, and NT-3 concentrations by means of ELISA

BDNF, NGF, and NT-3 levels in supernatants were determined by using a commercially available ELISA, according to the manufacturer's instructions (Promega, Madison, Wis). Plates were read in a microplate autoreader (Tecan, Salzburg, Austria) at 405 nm. Cutoff levels were 8 pg/mL for NGF, BDNF, and NT-3.

PCR analysis

Neurotrophin and neurotrophin receptor mRNA expression was determined by means of RT-PCR, as described previously.[18] Quantitative neurotrophin mRNA expression in epithelial cells was determined by using real-time PCR (LightCycler System; Roche, Basel, Switzerland). PCR products were detected with SYBR Green (Qiagen, Hilden, Germany), according to the manufacturer's instructions. The primers were as follows: TrkA (forward: 5′-AGG TCT TTC TCG CTG AGT GC-3′, reverse: 5′-GGT GCA GAC TCC AAA GAA GC-3′), $TrkB_{gp145}$ (forward: 5′-CGA ACC TGC AGA TAC CCA AT-3′, reverse: 5′-TCA TGT GCT TGG AAA CCA AA-3′), NGF (forward: 5′-CCA AGC TCA CCT CAG TGT C-3′, reverse: 5′-TAC GGT TCT GCC TGT ACG-3′), BDNF (forward: 5′-GCT TTG TGT GGA CCC TGA GT-3′, reverse: 5′-TCG TCA GAC CTC TCG AAC CT-3′), and β-actin (forward: 5′-TTG GGT ATG GAA TCC TGT GG-3′, reverse: 5′-AGT ACT TGC GCT CAG GAG GA-3′).

Flow cytometric analysis of eosinophil viability

Survival of eosinophils was assessed by using a 7-AAD dye exclusion assay, according to the manufacturer's instructions (BD Labware). Analyses were performed with a FACSSort (BD Labware).

Statistics

Statistical significance for normally distributed samples was analyzed by using an unpaired *t* test. Nonnormal data or data with unequal variances were tested for significance with the Mann-Whitney rank sum test. *P* values of less than .05 were considered statistically significant.

RESULTS

Epithelium is a major source of NGF and BDNF during acute and chronic airway inflammation

It is known that acute allergic inflammation results in increased BALF levels of BDNF and NGF in OVA-challenged mice.[10,11] Here we further investigated the levels of neurotrophins in an animal model of chronic experimental asthma, which has been previously

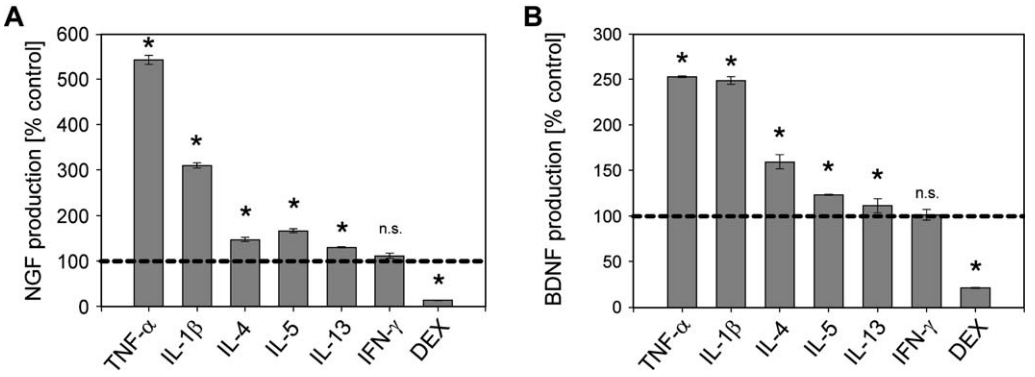

FIG 2. Stimulation of bronchial epithelial cells with various cytokines and dexamethasone *(DEX)*. NGF **(A)** and BDNF **(B)** levels were measured by means of ELISA after 48 hours. Data are given as means (n = 3) ± SD. **P* < .05, stimulation versus control. *n.s.,* Not significant.

described.[19] When compared with the acute inflammatory response, levels of NGF, BDNF, and NT-3 were further increased during long-term allergen exposures (NGF, 573.7 ± 302.3 pg/mL vs 983.9 ± 259.1 pg/mL; BDNF, 11.0 ± 1.9 pg/mL vs 15.4 ± 4.5 pg/mL; NT-3, 135.9 ± 46.3 pg/mL vs 675.6 ± 169.9 pg/mL; acute vs chronic, respectively). Immunohistochemical stainings for BDNF, NGF, and NT-3 were performed to identify the cellular sources of the neurotrophin production within the allergic inflamed lung. As demonstrated in Fig 1, lung sections of PBS control groups revealed a basal expression of NGF (Fig 1, *A*) and BDNF (Fig 1, *C*). Staining dominated in the bronchial epithelium (filled arrow). In contrast, immunoreactivity for NT-3 (Fig 1, *E*) was absent in the airway epithelium of nonsensitized mice. Also, a few cells in the alveolar tissue show immunoreactivity for NGF and BDNF, and neurotrophin expression by alveolar macrophages has been shown previously.[20]

During allergic airway inflammation, NGF and BDNF were strongly expressed by the bronchial epithelium (Fig 1, *B* and *D*, filled arrow); however, the inflammatory infiltrate showed also some weak immunoreactivity (open arrow). NT-3 was only produced by cells of the inflammatory infiltrate but not by bronchial epithelial cells (Fig 1, *F*).

Epithelial NGF and BDNF production is strongly upregulated by proinflammatory cytokines

Cell-culture experiments with LA-4 cells and primary epithelial cells were performed to investigate the regulation of neurotrophin production by epithelial cells. LA-4 cells produced continuously high amounts of NGF and BDNF but not NT-3: after 48 hours, NGF levels were 132 ± 16 pg/mL, and BDNF levels were 274 ± 26 pg/mL. Similar results were obtained with primary cultures of MTECs (NGF, 746 ± 33 pg/mL; BDNF, 118 ± 3 pg/mL), but again no NT-3 was detectable.

LA-4 cells are able to secrete neurotrophins toward the apical, as well as the basolateral, cell membrane. However, when cultured on semipermeable membranes, 70% of NGF (609 ± 4 pg) and 65% of BDNF (1279 ± 42 pg) was secreted into the basolateral compartment, whereas 30% of NGF (255 ± 15 pg) and 35% of BDNF (704 ± 3 pg) was secreted into the apical compartment.

Next we tested the influence of proinflammatory cytokines, such as IL-1β and TNF-α, as well as T_H2 cytokines, such as IL-4, IL-5, and IL-13, on neurotrophin production in airway epithelial cells. Stimulation with the proinflammatory cytokine TNF-α resulted in an about 5-fold increase in NGF synthesis, and with IL-1β there was an almost 3-fold increase in NGF synthesis (Fig 2, *A*). Stimulation with the T_H2 cytokines IL-4, IL-5, and IL-13 also augmented the NGF production, although to a lesser degree. In contrast, addition of the T_H1 cytokine IFN-γ had no significant effect on the production of NGF. Similar results were obtained for BDNF, with TNF-α and IL-1β inducing the strongest effects, followed by stimulation with IL-4, IL-5, and IL-13. The T_H1 cytokine IFN-γ did not affect production of BDNF (Fig 2, *B*).

The effect of glucocorticoids on epithelial neurotrophin expression was tested by means of incubation with 0.1 μM dexamethasone for 48 hours (Fig 2). Treatment with dexamethasone resulted in a marked suppression of NGF and, to a lesser extent, of BDNF. Thus NGF production is more suppressed by glucocorticoids than BDNF production in airway epithelia.

Lung eosinophils express the BDNF and NGF receptors TrkB and TrkA

Immunohistochemical stainings for the specific NGF and BDNF receptors TrkA and TrkB were performed to further investigate whether lung eosinophils are responsive to neurotrophin signaling in the inflamed lung (Fig 3). Although NGF and BDNF were mainly produced by the bronchial epithelium, the NGF receptor TrkA (Fig 3, *A*) and the BDNF receptor TrkB (Fig 3, *B*) were mainly expressed in cells within the inflammatory infiltrate. A large number of the infiltrated cells were identified as eosinophils by means of Congo red staining (data not shown).

We therefore analyzed the expression profile of neurotrophins and neurotrophin receptors on lung eosinophils isolated from the BALF of allergen-challenged mice.

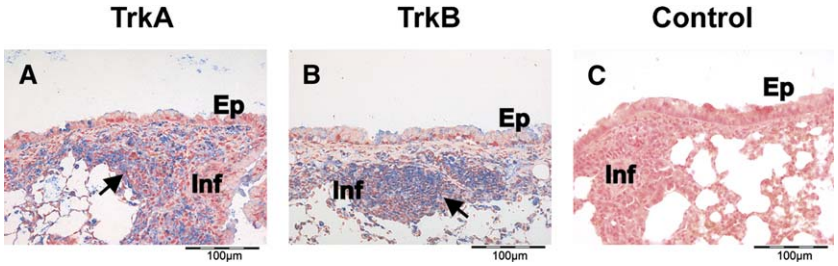

FIG 3. TrkA and TrkB expression in murine experimental asthma. Lung sections of OVA-sensitized mice were stained for TrkA **(A)** and TrkB **(B)**. Rabbit IgG was used as a control antibody **(C)**. *Ep*, Bronchial epithelium; *Inf*, inflammatory infiltrate. Original magnification 200×.

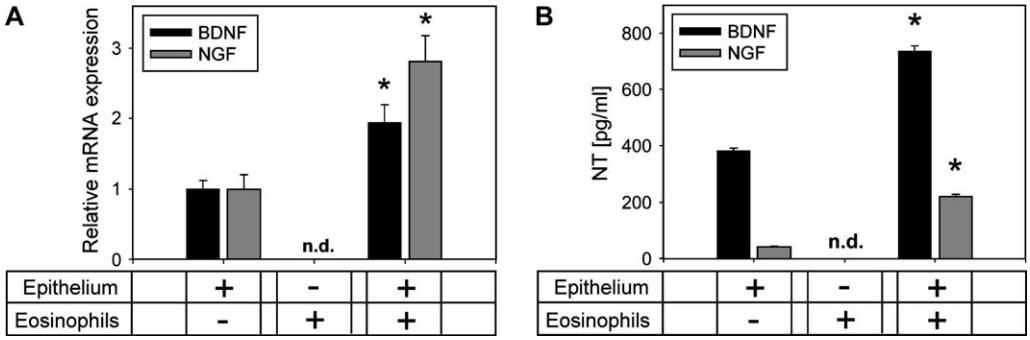

FIG 4. NGF and BDNF induction in bronchial epithelial cells during coculture with BALF eosinophils. **A**, Relative mRNA expression was analyzed with quantitative PCR. **B**, BDNF and NGF levels in the supernatant were measured by means of ELISA after 48 hours of coculture. Data are given as means (n = 3) ± SD. **P* < .05, epithelial cell monoculture versus epithelial-eosinophil coculture. *n.d.*, Not detectable.

BALF eosinophils cultured for 48 hours did not release NGF and BDNF (Fig 4); however, low amounts of NT-3 were detectable (data not shown). On the other hand, eosinophils were positively stained for the NGF receptor TrkA (Fig 5, *A*) and the BDNF receptor TrkB (Fig 5, *B*) and express the mRNA for TrkA and TrkB (Fig 5, *D*).

Eosinophil-epithelial interactions result in enhanced epithelial neurotrophin expression and prolonged survival of eosinophils

Eosinophil-epithelial cell interactions were studied in a coculture system *in vitro*. Although airway epithelial cells cultured alone produce NGF and BDNF, a significant upregulation of epithelial NGF and BDNF mRNA expression (Fig 4, *A*), as well as NGF and BDNF secretion (Fig 4, *B*), was found after 48 hours of coculture with BALF eosinophils.

Next we analyzed whether epithelial cells can prolong survival of lung eosinophils through the expression of neurotrophins. After 1, 2, and 6 days of culturing eosinophils alone (monoculture) or together with epithelial cells (coculture), eosinophils were analyzed for survival by means of flow cytometry with 7-AAD dye exclusion assay. As depicted in Fig 6, *A*, the viability of monocultured eosinophils continuously decreased to 78.0% ± 1.4% after 24 hours and to 39.4% ± 5.6% after 48 hours. After 6 days of monoculture, only about 25% of eosinophils

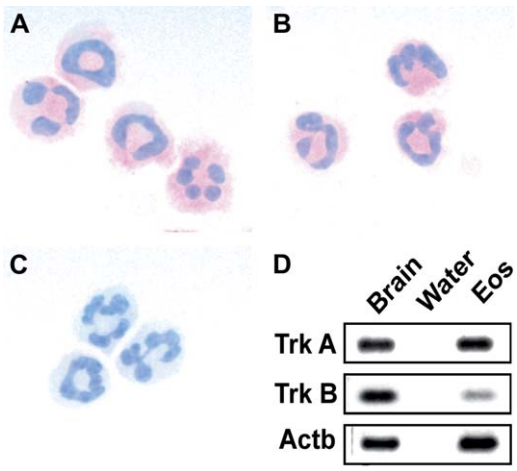

FIG 5. Analysis of TrkA and TrkB expression in BALF eosinophils. BALF eosinophils were spun on glass slides and were immunostained for TrkA **(A)** and TrkB **(B)** or rabbit IgG antibody control **(C)**. mRNA expression of isolated BALF eosinophils *(Eos)* was analyzed by using RT-PCR, with brain tissue as a positive control and water as a negative control **(D)**.

survived. However, when cocultured with airway epithelial cells, eosinophils showed a significantly improved survival. Cell-culture supernatants of airway epithelial cells were analyzed for IL-5 by means of ELISA and

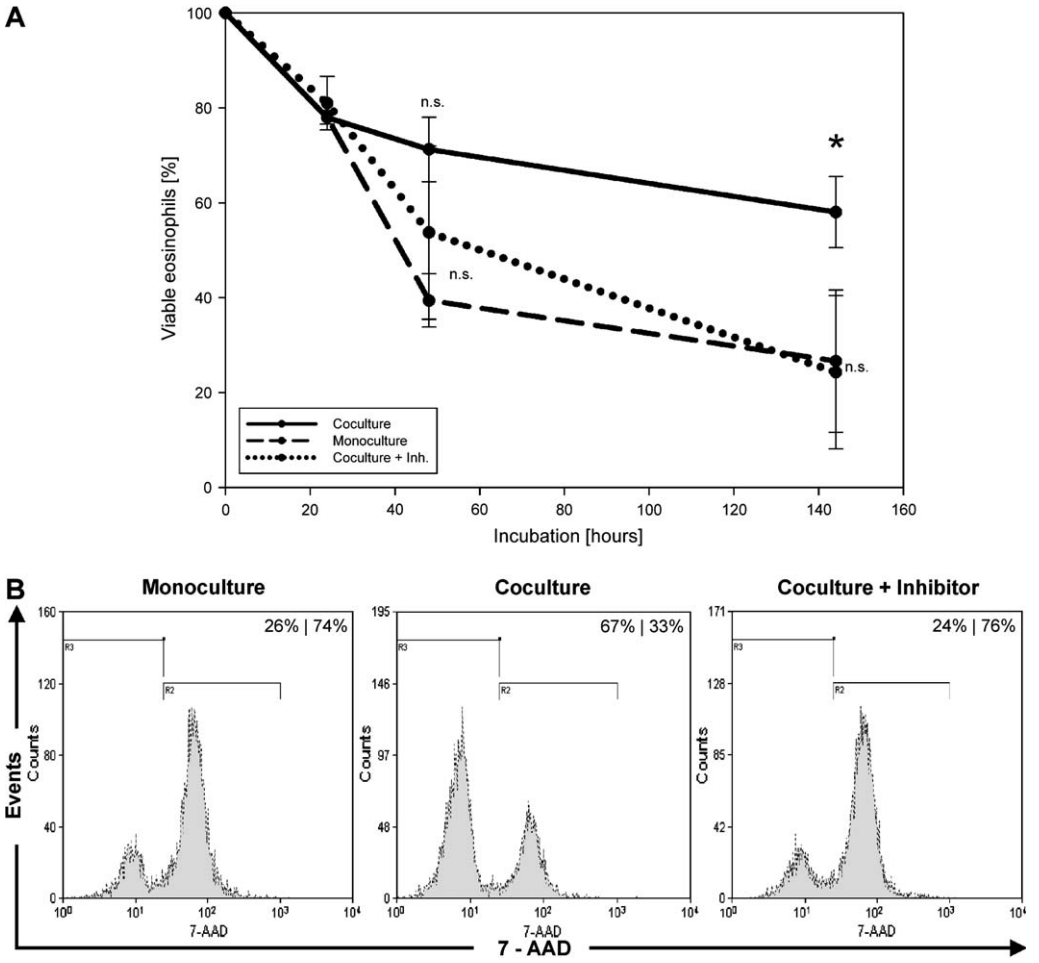

FIG 6. Analysis of epithelial cell–induced survival of BALF eosinophils. **A**, Survival of BALF eosinophils at indicated time points. Data are given as means (n = 5) ± SD. *$P < .05$. **B**, Representative FACS histogram after 6 days of culture. *n.s.,* Not significant; *Inh.,* inhibitor.

PCR and for GM-CSF by means of a cytometric bead assay to exclude the possibility that IL-5 or GM-CSF also contribute to lung eosinophil survival and were found to be negative for both cytokines (data not shown). Eosinophils were cultured with bronchial epithelial cells in the presence of a specific neurotrophin Trk receptor inhibitor, K252a, to confirm that prolonged survival depends on neurotrophin signaling. K252a completely inhibited the survival effect of epithelial cells, and eosinophil viability was significantly decreased comparable with the eosinophil monoculture level (Fig 6).

DISCUSSION

Allergic diseases, including bronchial asthma, are associated with increased neurotrophin levels detectable in the blood circulation, as well as in the local tissues.[21] In asthmatic patients neurotrophin levels in the BALF are increased after segmental allergen provocation,[12] and in various animal studies increased local concentrations of NGF and BDNF were observed during experimental allergic asthma.[9] The effect of neurotrophins on the development of airway hyperresponsiveness is well described, and neurotrophins directly trigger the activity of the bronchial nervous system.[22,23] In addition, there is growing evidence that neurotrophins might also exert various immunomodulatory functions,[24] but the exact mechanisms of how neurotrophins modulate the inflammatory response in the lung are still unclear.

During a repeated allergen challenge for at least 10 to 12 weeks, an increased inflammatory infiltration has been described by our group,[19] and in the present study we found that neurotrophin levels in BALF increase continuously during chronic allergic airway inflammation. However, we identified the bronchial epithelium as the major source of NGF and BDNF in the allergic lung, whereas the inflammatory infiltrate showed only some weak immunoreactivity for BDNF and NGF. Airway inflammation and clinical symptoms in allergic asthma

are clearly linked to increased production levels of proinflammatory cytokines, including IL-1β[25] and TNF-α,[26] as well as increased levels of T_H2 cytokines.[27] Here we show that IL-1β, TNF-α, and T_H2 cytokines strongly upregulate the NGF and BDNF production in airway epithelial cells. In contrast, IFN-γ, a T_H1 cytokine, did not alter BDNF or NGF expression in airway epithelial cells, indicating that the asthma-associated cytokine milieu is an important trigger for NGF and BDNF synthesis by airway epithelial cells.

In transgenic animal models we have previously shown that airway inflammation depends to a large extent on the production of NGF and on functional neurotrophin receptor expression. Mice with constitutive overexpression of NGF in the bronchial epithelium showed significantly higher numbers of eosinophils in the lung,[28] whereas eosinophil infiltration was markedly reduced in mice with homozygous disruption of the $p75^{NTR}$ gene.[29] In addition, application of neutralizing antibodies against NGF during allergen challenges also inhibited the influx of eosinophils into the airways.[11,28] Although these data clearly indicate a direct link between increased NGF signaling and eosinophil infiltration, the mechanism behind this phenomenon still remains poorly understood.

On the basis of our finding that the epithelial NGF/BDNF production is strongly upregulated by asthma-associated cytokines, we hypothesized that neurotrophins produced by the epithelial barrier might play an important role in regulating functional activities of eosinophils during the local inflammatory response. There are considerable data supporting the concept of a bidirectional communication between the epithelium and immune cells of the airways.[30] The bronchial epithelium actively participates in the inflammatory reaction through the synthesis of an arsenal of cytokines and other inflammatory mediators,[30] and it has also been shown that epithelial cells can directly interact with allergens, resulting in increased production of these cytokines.[31] Apart from the role of epithelial cells as sources of inflammatory cytokines, they might themselves be activated by inflammatory mediators released by immune cells or structural cells during the immune response.[30]

Because eosinophils represent an important cellular source of inflammatory cytokines, such as IL-4[32] and IL-13,[33] we hypothesized that eosinophil-epithelial cell interactions might trigger neurotrophin production by epithelial cells. It has been shown that eosinophils induce mucin production,[34] as well as release of chemokines, such as RANTES and monocyte chemoattractant protein 1,[35] in airway epithelial cells during coculture. In the present study we could show for the first time a significant upregulation of epithelial NGF, as well as BDNF, expression after cocultivation with lung eosinophils. Vice versa, induction of epithelial neurotrophins by eosinophils might modify biologic functions of eosinophils, such as activation, chemotaxis, and survival. The chemotactic activity of NGF,[36] as well as the induction of activation markers, such as CD69,[6] on eosinophils is well known. Moreover, several studies established that

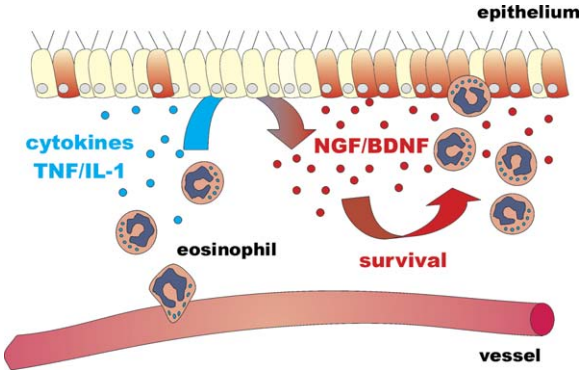

FIG 7. Neurotrophins mediate proinflammatory eosinophil-epithelial cell interactions during allergic airway inflammation.

neurotrophins are able to prolong the survival of eosinophils *in vitro*.[6,36] Quite recently, eosinophil apoptosis was inhibited by BDNF in patients with atopic dermatitis,[37] and the survival activity of neurotrophins is mediated by the neurotrophin-specific Trk receptors.[6] Neurotrophin receptor expression depends on the activation status because resting blood eosinophils in asthmatic subjects do not express Trk receptors compared with lung eosinophils from the same patients.[6] In atopic dermatitis peripheral blood eosinophils were shown to express the BDNF receptor TrkB,[37] potentially because of preactivation of circulating eosinophils, and it was speculated that enhanced BDNF concentrations might affect their survival in the blood through TrkB signaling.

In the present study we have demonstrated that eosinophil-epithelial interaction results in a prolonged survival of lung eosinophils, which express the neurotrophin receptors TrkA and TrkB. Because there was no direct cell-cell contact between eosinophils and epithelial cells, the survival effect must be mediated by soluble cytokines. Although in our airway epithelial cell culture neither IL-5 nor GM-CSF was detectable, other factors must promote the enhanced eosinophil survival. We conclude that the epithelial neurotrophin expression might be responsible for inducing eosinophil survival because the Trk receptor inhibitor K252a completely inhibited the survival effect of airway epithelial cells.

In summary, these findings provide the first evidence that airway epithelium regulates the survival of eosinophils in the lung through increased neurotrophin expression during chronic allergic airway inflammation (Fig 7). In this way the enhancement of epithelial neurotrophin synthesis by eosinophils, together with the maintenance of eosinophil survival, might amplify the allergic inflammation in a vicious cycle. Targeting the neurotrophin expression in airway epithelial cells might be an interesting tool for the treatment of airway inflammation in allergic asthma.

We thank Berit Sel for her expertise and help in performing the flow cytometric analysis and Stefanie Achenbach for excellent technical assistance.

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