

Nerve growth factor or IL-3 induces more IL-13 production from basophils of allergic subjects than from basophils of nonallergic subjects

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Background: Studies show that nerve growth factor (NGF) exhibits immunomodulatory activity. This neurotrophin is found at high levels in the serum of asthmatic individuals, is released during allergic reactions, and is reported to augment *in vitro* histamine and leukotriene C4 release by human basophils.

Objective: Because basophils represent a substantial source of IL-4 and IL-13, we tested the effects of NGF on the secretion of these cytokines by cells prepared from allergic subjects and cells prepared from nonallergic subjects.

Methods: Cytokine and histamine were measured in culture supernatants by ELISA and fluorimetry, respectively. Both real-time RT-PCR and conventional RT-PCR were used to measure IL-13 mRNA expression. NGF receptor expression was determined by 2-color flow cytometry.

Results: Basophil suspensions from allergic subjects secreted some 2.5-fold greater levels of IL-13 when cultured with NGF than did cells prepared from normal control subjects. Flow cytometry revealed no significant differences in TrkA receptors on basophils to explain these findings. The levels of IL-13 secreted by the 2 groups of donors also differed when cells were activated with IL-3 but not when they were activated with anti-IgE antibody. Both NGF and IL-3 failed to induce IL-13 in cell cultures depleted of basophils, suggesting that the measurable IL-13 was indeed basophil-derived. Real-time RT-PCR showed an average induction of IL-13 message above medium control that was 4.3 (± 1.7)-fold with NGF and 8.9 (± 3.7)-fold with IL-3. Finally, NGF priming resulted in a remarkable enhancement of IL-13 induced by anti-IgE. This was significantly greater than the priming observed for either the IL-4 or histamine when this stimulus was used.

Conclusion: NGF (like IL-3) can both directly stimulate IL-13 secretion and modulate IgE-mediated responses in basophils. Its enhanced effect on cells from allergic individuals raises the importance of this cytokine in the pathogenesis of allergic disease. (*J Allergy Clin Immunol* 2001;108:387-93.)

Key words: *Neurotrophin, cytokine, allergy, basophil, histamine, IgE*

Abbreviations used

BEC:	Basophil-enriched cell
CT:	Cycle threshold
IMDM:	Isocove's modified Dulbecco's medium
MFI:	Mean fluorescence intensity
NGF:	Nerve growth factor
PAG:	PIPES/albumin/glucose
PIPES:	Piperazine-N,N'-bis-2-ethanesulfonic acid

Nerve growth factor (NGF), a protein derived from many different cell types, belongs to a family of molecules that are designated *neurotrophins* and best known for their biological effects on the nervous system.¹ Recent studies demonstrate additional effects of neurotrophins, particularly NGF, in both hematopoietic and immunomodulatory processes.² In fact, a variety of immune cells, including T lymphocytes,³ B lymphocytes,³ mast cells,^{4,5} and eosinophils,⁶ are reported to secrete NGF.

There is mounting evidence for the involvement of NGF in allergic disease. Increased levels of this neurotrophin have been demonstrated in the plasma of individuals presenting with allergic disease in comparison with that of normal controls.⁷ Markedly increased levels have also been detected in bronchoalveolar lavage fluids after experimental segmental allergen challenge.⁸ In addition, it has recently been shown that baseline levels of NGF are elevated in nasal lavage fluids obtained from patients with allergic rhinitis in comparison with those obtained from normal controls and that these levels rapidly increase after allergen challenge.⁹

Among NGF's biological effects is its ability to support the development and differentiation of myeloid progenitor cells,¹⁰ thus acting as a colony-stimulating factor for both eosinophils and basophils.^{11,12} Most significantly, NGF has been shown to modulate the function of these cells by directly activating eosinophils for release of eosinophil cationic protein⁶ and priming basophils for increased histamine and leukotriene C4 (LTC4) release in response to both IgE-dependent and IgE-independent stimuli.¹³ Studies have shown that these effects of NGF on basophils are mediated through the tyrosine kinase receptor TrkA, which is expressed at high levels on these cells.¹⁴

As one of the immune cells recruited to allergic lesions, basophils have become recognized as a major source of IL-

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4 and IL-13, two of the so-called T_H2 cytokines that play a commanding role in the pathogenesis of allergic reactions (reviewed by Schroeder et al¹⁵). Whereas activation through the high-affinity IgE receptor Fc ϵ RI induces the generation of both cytokines in basophils, there is a growing body of evidence that IL-4 and IL-13 might be differentially regulated in response to IgE-independent stimuli; several groups have shown that IL-3, though a poor inducer of IL-4, is a potent stimulus for IL-13 secretion by basophils.^{16,17}

There is little information regarding whether cytokines other than IL-3 directly induce IL-4 and/or IL-13 secretion by basophils or whether such cytokines are more active on cells obtained from allergic individuals than on cells from nonallergic individuals. In this study, we present evidence that NGF, like IL-3, enhances both IL-4 and IL-13 secretion mediated through Fc ϵ RI activation. Furthermore, this neurotrophin directly induces IL-13 from basophils with parameters similar but not identical to those seen with IL-3 activation. Most importantly, we found the induction of IL-13 message and its protein by either NGF or IL-3 to be significantly greater in cells obtained from allergic individuals than in cells from normal controls. These findings further support the importance of NGF and IL-3 in the pathogenesis of allergic disease.

MATERIALS AND METHODS

Special reagents

The following reagents were purchased: crystallized human serum albumin (Calbiochem-Behring Corp, La Jolla, Calif); piperazine-N,N'-bis-2-ethanesulfonic acid (PIPES), FBS, crystallized BSA, and human IgG (Sigma Chemical Co, St Louis, Mo); gentamicin, Dulbecco's PBS, Iscove's modified Dulbecco's medium (IMDM) and RPMI-1640 (both containing L-glutamine and 25 mmol/L HEPES), and nonessential amino acids (100X stock) (Life Technologies, Inc, Grand Island, NY); Percoll (Pharmacia Biotech, Inc, Piscataway, NJ); and recombinant human IL-3 and NGF β (Biosource, Camarillo, Calif). The polyclonal antihuman IgE antibody used in these experiments was made in a goat.¹⁸ Antibodies used for flow cytometry were obtained commercially (goat anti-IgE FITC antibody from Kirkegaard & Perry Laboratories, Inc, Gaithersburg, Md; anti-TrkA from Oncogene, Cambridge, Mass).

Special buffers and media

10x PIPES buffer (250 mmol/L PIPES, 1.10 mol/L NaCl, 50 mmol/L KCl, pH 7.4) was stored at 4°C as a stock solution. PIPES/albumin/glucose (PAG) was made by diluting 1 part 10x PIPES with 9 parts deionized water; it contained 0.003% human serum albumin and 0.1% D-glucose. PAG-EDTA also contained 4 mmol/L EDTA. Isotonic Percoll (referred to in this article as 100% Percoll) was prepared by mixing 1 part 10x PIPES and 9 parts Percoll. Percoll solutions of 55% (density, 1.072 g/mL) and 61% (density, 1.081) were made by mixing the appropriate amounts of 100% Percoll with 1x PIPES. Conditioned medium (C-IMDM) consisted of IMDM supplemented with 5% heat-inactivated (56°C for 30 minutes) FBS, 1x nonessential amino acids, and 5 μ g/mL gentamicin.

Cell preparation

Peripheral blood basophils were prepared from well-characterized subjects who had been grouped as allergic (n = 18) or nonallergic (n = 12) according to skin test reactivity to a panel of common

allergens. None, however, had been taking antiallergic medication at the time blood was drawn, nor were any symptomatic. Venous blood was anticoagulated in 10 mmol/L EDTA. Basophil-enriched cell (BEC) suspensions (purity, 2% to 21%) were prepared on double Percoll gradients (55% layered onto 61%), as previously described.¹⁹ After density centrifugation, basophils were concentrated at the 61% Percoll interface, harvested, and washed twice in PAG-EDTA at room temperature and then once in PAG at 4°C. For many experiments, the cell concentrate at the 55% Percoll interface, which consisted of lymphocytes and monocytes but was less than 0.01% basophils, was washed free of platelets and used as basophil-depleted cells. Additional experiments used ultrapure basophil suspensions (purity, >98%) prepared by negative selection. Briefly, BEC suspensions were incubated with a cocktail of IgG₁ monoclonal antibodies (CD2, CD3, CD14, CD16, CD24, CD34, CD36, CD45RA, CD56, glycophorin A); this was followed by incubation with antimouse IgG-coated magnetic colloid beads, according to the manufacturer's protocol (StemCell Technologies, Inc, Vancouver, British Columbia, Canada). Cells were then applied to an LS cell separation column mounted to a Midi MACS magnet (Miltenyi Corp, Auburn, Calif). Basophils were collected as they passed through the column, washed, and then counted through use of Alcian-blue.²⁰

Culture conditions

For cytokine secretion, cells were cultured in 96-well flat-bottomed microtiter plates (Costar, Cambridge, Mass) through use of C-IMDM.¹⁹ Cells were added to wells in 100 μ L of medium and incubated for 15 minutes to allow equilibration to 37°C (5% CO₂) before the addition of 100 μ L of stimulus (NGF, IL-3, or anti-IgE) at twice the final concentration. When pure basophil suspensions (purity, >98%) were used, culture volumes were performed in a total volume of 100 μ L. For effects of priming on IgE-mediated secretion, cells were pretreated with NGF for 15 minutes before stimulation with anti-IgE for 4 hours or for 18 hours to measure IL-4 and IL-13, respectively. Cytokine measurements were determined in cell-free supernatants from duplicate cultures through use of ELISA kits for IL-4 (Biosource, Irvine, Calif) and IL-13 (Immunotech, Westbrook, Me).

FACS analysis for TrkA expression

Immunostaining for TrkA expression on basophils was performed through use of 2-color flow cytometry. BEC suspensions isolated on Percoll were incubated for 60 minutes on ice in PBS plus 5% nonfat dry milk containing 1 mg/mL human IgG to block Fc receptors. Without being washed, the cells were then divided into 25- μ L aliquots to each of which an equal volume of buffer containing 1:25 dilution of anti-IgE/FITC and 20 μ g/mL of either anti-TrkA or IgG1 isotype control was added. After 30 minutes, the cells were washed once in PBS plus 0.5% BSA before the addition of goat antimouse IgG phycoerythrin-conjugated antibodies (Biosource, Irvine, Calif) for an additional 30 minutes. Cells were then washed 3 more times before being analyzed through use of a FacsCalibur flow cytometer (Becton Dickinson Immunocytometry Systems, San Jose, Calif) set up to detect green and red fluorescence. Mean fluorescence intensities (MFIs) for the isotype control were subtracted from those obtained with anti-TrkA to determine specific MFI (sMFI).

RT-PCR

Basophils were purified to >98%, as described above. Approximately 0.5 to 1.0 $\times 10^6$ cells in 0.125 mL of C-IMDM were allowed to equilibrate to incubator conditions (37°C; 5% CO₂) for 15 minutes in autoclaved (RNAase-free) 1.5-mL polypropylene microcentrifuge tubes. Cells were then challenged with an equal volume of medium alone, NGF (10 ng/mL), or IL-3 (100 ng/mL). After 4 hours' incubation, the cell cultures were centrifuged (12,000g) for 7

to 10 seconds and the supernatants removed. Total RNA was extracted through use of the RNeasy protocol (Tel-test, Inc, Friendswood, Tex). After isopropanol precipitation for 1 hour on ice, the RNA was washed with 70% ethanol, semidried under vacuum, resuspended in 25 μ L of diethyl pyrocarbonate-treated water, and stored at -80°C , as previously described.²¹ Real-time RT-PCR was performed in special optical tubes of a 96-well microtiter plate (PE Applied Biosystems, Foster City, Calif) and formatted for the ABI PRISM 7700 Sequence Detector (PE Applied Biosystems). The following primers/probe combinations were designed through use of the Primer Express 1.7 software package (PE Applied Biosystems) and used for IL-13 mRNA detection: IL-13 forward, 5'-AGGATGCTGAGCGGATTCTG-3'; IL-13 reverse, 5'-AAACTGGGCCACCTCGATT-3'; IL-13 specific probe, 5'-TTCCAGCTGTCATGTCGAGACACC-3'. The reporter dye was 6-carboxy-fluorescein, and the quencher was 6-carboxy-tetramethyl-rhodamine. One-step RT-PCR was performed with a TaqMan Gold RT-PCR kit (PE Applied Biosystems) used according to the manufacturer's protocol, with the exception that a total volume of 25 μ L (20 μ L master mix plus 5 μ L RNA) was used in each reaction. Thermal cycler conditions for RT were 48°C and 30 minutes. For amplification, a 10-minute incubation at 95°C was done to activate the AmpliTaq Gold; this was followed by 40 cycles with 15 seconds at 95°C and 60 seconds at 60°C for each cycle. Changes in cycle thresholds (CTs) were calculated from comparison of the CT values obtained with cells cultured in medium alone and the values obtained with cells activated with either NGF or IL-3. Relative quantitation (equal to $2^{\Delta\text{CT}}$) was normalized to total RNA levels. Conventional RT-PCR to amplify a larger IL-13 cDNA product (429 base pairs) was also performed through use of primer sets and conditions described elsewhere.^{17,24}

RESULTS

NGF induces IL-13 secretion by human basophils

To address the ability of NGF to modulate cytokine secretion by human basophils, we evaluated its potential to directly induce IL-4 and/or IL-13 from highly purified basophils (purity, >99%) cultured during a time course of 1 to 48 hours. In an experiment shown in Fig 1, which is representative of 2 others, IL-13 was not detected after a 2-hour incubation with NGF (20 ng/mL), but it was seen after 6 hours; these levels were only slightly increased after 18 hours' incubation. In contrast, NGF induced only marginally detectable levels of IL-4 after 20 hours in the same culture supernatants. On the basis of these data, an incubation time of 18 to 20 hours was used in the experiments described below to investigate NGF-mediated IL-13 secretion.

NGF dose-response curve for induction of IL-13

A wide range of NGF concentrations were then tested to determine the dose-response curve for NGF-mediated secretion of IL-13. Fig 2 shows that the lowest concentration of NGF tested, 0.1 ng/mL, induced IL-13 secretion averaging ~ 10 ng/mL ($n = 6$). These levels of IL-13 more than doubled when the NGF concentration was increased 10-fold to 1 ng/mL; only marginal increases in IL-13 were seen after the NGF was increased to 10 and 100 ng/mL. In all subsequent experiments, a concentra-

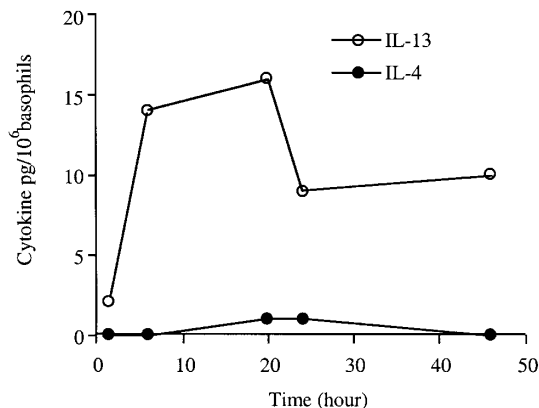


FIG 1. Time course for the induction of IL-13 protein in human basophils treated with NGF. Basophils (purity, >99%) were cultured in NGF (20 ng/mL) for the indicated times. Cell-free supernatants were then harvested and tested for both IL-4 and IL-13 protein by ELISA. Values shown are from a representative experiment. Level of detection values for IL-13 and IL-4 were 2.0 and 0.4 pg/10⁶ basophils, respectively.

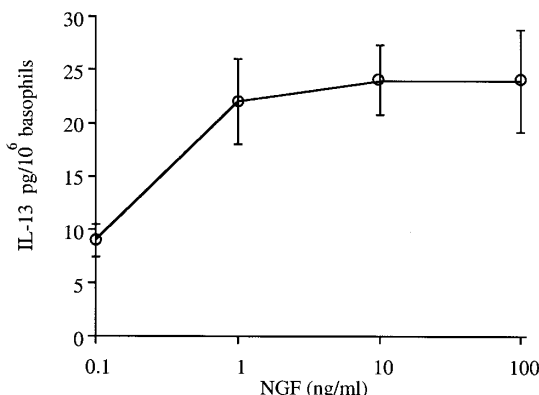


FIG 2. Dose-response curve for the induction of IL-13 by NGF. BEC suspensions were prepared from whole blood through use of double Percoll density centrifugation and incubated with different concentrations of NGF for 18 hours. IL-13 levels were determined by ELISA. Values represent the means $\pm$ SEMs of 6 experiments using cells from different donors.

tion of 10 ng/mL was used to assess the effect of NGF on IL-13 secretion.

IL-13 secretion by basophils from allergic subjects versus basophils from nonallergic subjects

We next tested whether the induction of IL-13 by NGF, IL-3, or anti-IgE antibody varied among cells prepared from allergic subjects versus cells prepared from nonallergic subjects. For these experiments, we used BEC suspensions (purity, 2% to 21%) rapidly prepared on Percoll to assess cytokine secretion; we used relatively fresh cells that had not been subjected to purification protocols which might have affected their response. To control for IL-13 produced by other cell types, we also investigated whether NGF induced IL-13 secretion in leukocyte cultures depleted of basophils.

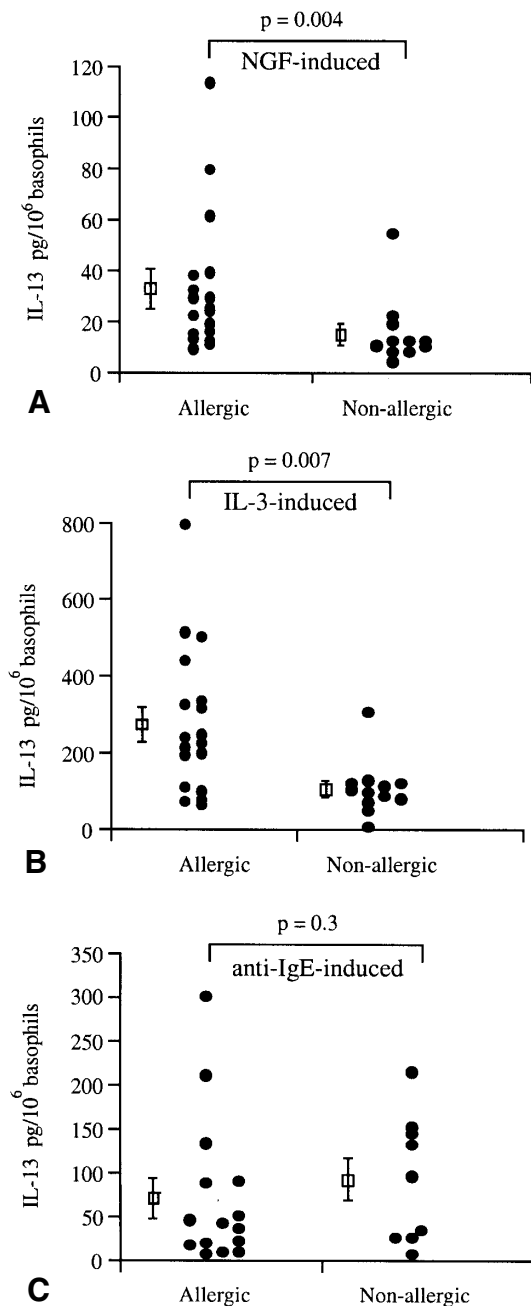


FIG 3. Comparison of IL-13 secretion by BEC suspensions prepared from allergic subjects ($n = 18$) and normal control subjects ($n = 12$). Basophil suspensions (purity, 2% to 21%) were stimulated for 18 hours with 10 ng/mL NGF (**A**), 100 ng/mL IL-3 (**B**), or 10 ng/mL anti-IgE antibody (**C**). Levels of IL-13 were determined by ELISA. Individual values are represented by solid circles; the mean $\pm$ SEM value is shown to the left for each condition. P values were calculated through use of nonparametric analysis (Mann-Whitney).

As shown in Fig 3, A, NGF induced IL-13 levels averaging 33 ± 8 pg/10⁶ basophils (range, 8 to 115 pg/10⁶ basophils) from cells of allergic subjects. This amount was nearly 2.5-fold greater than the amount of IL-13 secreted by cells from nonallergic donors. NGF did induce low levels of IL-4 protein in 3 of the super-

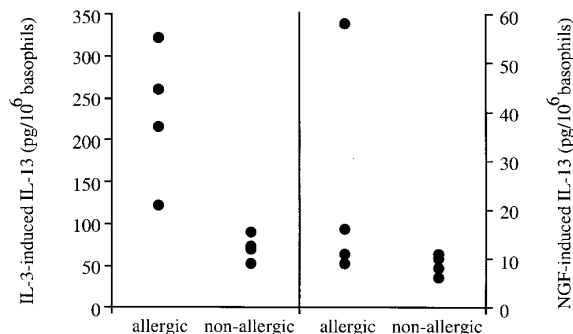


FIG 4. Comparison of the IL-13 levels secreted in response to NGF (**A**) and IL-3 (**B**) stimulation from basophils purified (purity, >98%) from allergic subjects ($n = 4$) and normal control subjects ($n = 4$). Basophils were prepared through use of the combination of double Percoll density centrifugation and negative selection. Cell-free supernatants were harvested after 18 hours' incubation for determination of IL-13 protein by ELISA.

natants taken from cultures for which cells from allergic subjects had been used; however, this small number did not allow for statistical analysis (data not shown). As expected, high levels of IL-13 were produced with IL-3 treatment (Fig 3, B). Once again, however, the cells prepared from allergic subjects secreted levels of this cytokine that were some 2.5-fold greater than the levels secreted by cells from nonallergic subjects (274 ± 45 pg/10⁶ and 106 ± 21 pg/10⁶ basophils, respectively). Interestingly, there was no significant difference in the levels of IL-13 secreted by cells of allergic subjects versus cells of nonallergic subjects when they were challenged with anti-IgE (Fig 3, C). It is important to note that IL-13 was not detected in the supernatants of basophil-depleted cultures (data not shown), suggesting that basophils were the source for most, if not all, of the IL-13 generated in response to NGF and IL-3. Furthermore, the IL-13 produced in the basophil-enriched cultures, when plotted in picograms per 10⁶ total cells versus percent basophils, showed a significant correlation for both NGF ($r = 0.95$, $P = .0001$) and IL-3 ($r = 0.6$, $P = .01$) stimulation (data not shown). Finally, differences were seen in the levels of IL-13 secreted by ultrapure basophil suspensions (purity, >98%) prepared from allergic versus nonallergic subjects (Fig 4), though purification generally reduced the relative amounts of this cytokine induced by both NGF and IL-3.

NGF induced IL-13 mRNA

To assess whether the IL-13 induced by NGF was evident at the level of gene expression, we next investigated the accumulation of IL-13 mRNA in basophils (purity, >98%) using quantitative real-time RT-PCR. With this assay, PCR amplification products for IL-13 were measured after each cycle during a 40-cycle run. Differences between samples in the amount of message were determined by the number of cycles required to reach a given threshold (ie, the CT value). Fig 5, A shows the relative fold induction of IL-13 mRNA in cells from 5 different donors, which averaged $4.3 (\pm 1.7)$ -fold and $8.9 (\pm$

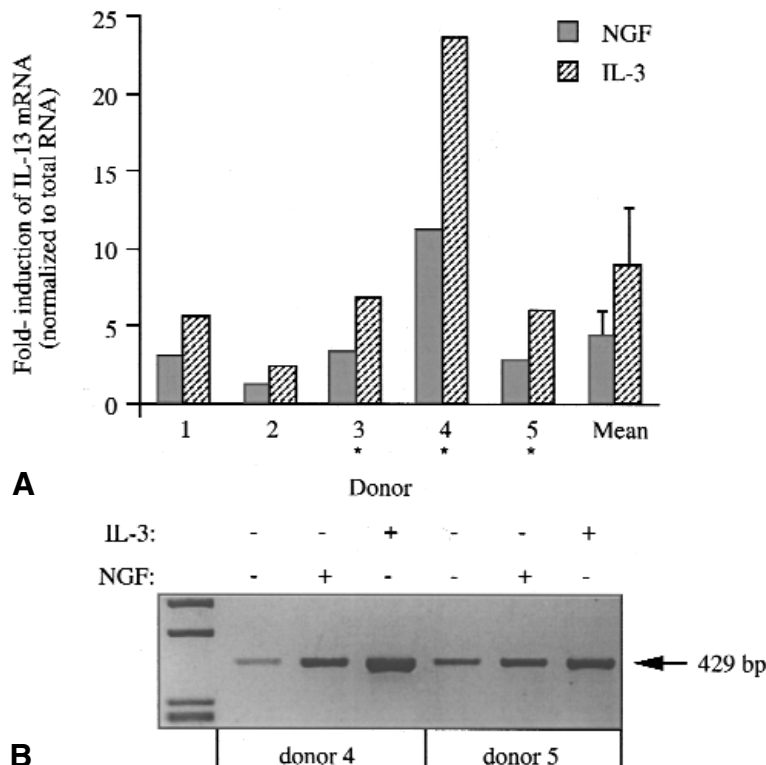


FIG 5. Induction of IL-13 mRNA in basophils stimulated with NGF versus basophils stimulated with IL-3. **A**, Total RNA was isolated from equal numbers of basophils (purity, >98%) and analyzed for IL-13 mRNA through use of real-time RT-PCR. Both individual and mean $\pm$ SEM results are shown; cells used were from 5 different donors (*asterisk* denotes allergic subject). Values represent the fold induction above the IL-13 message expressed in cells culture in medium alone and after normalizing to total RNA content. **B**, Gel image from conventional RT-PCR shows an increase in a 429-base pair IL-13 product with either NGF or IL-3 stimulation; the same RNA from donors 4 and 5 was used.

3.7)-fold greater with NGF and IL-3 stimulation, respectively, in comparison with cells cultured in medium alone. Two specimens (from donors 4 and 5) were also examined through use of conventional RT-PCR. As shown in Fig 5, *B*, there were increases in a single product of 429 base pairs (approximately one third of the full-length message) with either NGF or IL-3 stimulation; these results were qualitatively similar to those seen with the real-time RT-PCR.

TrkA on basophils of allergic subjects versus basophils of nonallergic subjects

The enhancing effects of NGF on basophil histamine release are mediated through high-affinity receptors (TrkA) expressed on these cells.¹⁴ We therefore investigated, through use of flow cytometry, whether the greater amounts of IL-13 secreted by basophils from allergic subjects in comparison with basophils from nonallergic subjects were reflective of the levels of TrkA found on these cells. Fig 6, *A* shows a representative histogram demonstrating that TrkA is readily detected on basophils. However, as shown in Fig 6, *B*, there were no significant differences between the sMFIs for this receptor between the 2 groups of donors. In addition, no correlations were found between the amounts of IL-13 secreted and the levels of TrkA expressed (data not shown).

NGF modulation of IgE-mediated responses

Priming with NGF has been shown to enhance the levels of histamine and LTC₄ secreted by basophils in response to IgE-dependent stimulation.¹³ Therefore, in a final set of experiments, we investigated the relative priming activity mediated by NGF for the secretion of IL-13 and IL-4 to compare it with that for histamine after activation with 1, 10, and 100 ng/mL of anti-IgE antibody. As shown in Fig 7, NGF enhanced histamine equally well for each concentration of antibody tested. Its greatest effect on cytokine secretion, however, occurred at the lower amounts of anti-IgE used for basophil activation (ie, 1 and 10 ng/mL). Remarkably, NGF caused an enhancement of IL-13 that was significantly greater than that seen for either IL-4 or histamine when cells were challenged with 1 ng/mL of anti-IgE.

DISCUSSION

We have identified NGF as a modulator of cytokine secretion in human basophils, providing additional evidence that this neuropeptide plays a role in the pathogenesis of allergic disease. NGF preferentially stimulated IL-13 generation in these cells by direct induction as well as by enhanced secretion after IgE-mediated activa-

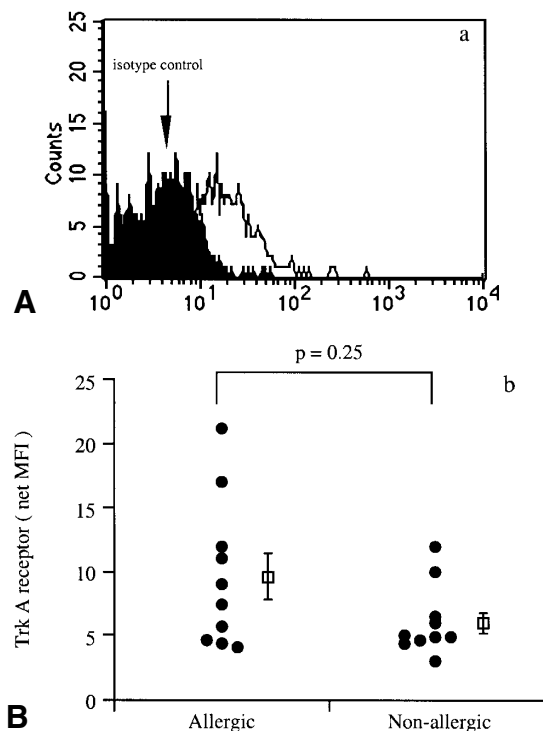


FIG 6. TrkA receptor expression on basophils from allergic subjects versus basophils from nonallergic subjects. Cells isolated by density centrifugation were prepared for 2-color flow cytometry. **A**, Representative histogram shows TrkA receptor expression compared with staining with an isotype control. **B**, sMFI for TrkA ($n = 10$ for each group shown). P value was determined by Mann-Whitney U test.

tion. The ability of NGF to stimulate IL-13 generation was greatest in allergic subjects. Furthermore, this disparity in IL-13 production between the 2 groups was displayed in cultures containing basophils and not in suspensions depleted of these cells, thus adding to the significance of the role that basophils have in modulating allergic responses.

Previous studies suggest that serum NGF levels are increased in disease states manifesting T_H2 -like profiles.² Interestingly, the concentrations of NGF that induced in vitro IL-13 secretion (ie, 0.1 to 10.0 ng/mL) in our study were remarkably similar to the pathologic levels of this neurotrophin measured in the serum of asthmatic subjects and individuals presenting with multiple allergies.⁷ This finding implies that the circulating in vivo concentrations of NGF not only might affect neural hyperresponsiveness but also might in fact be sufficient to induce IL-13 production in basophils, even in the absence of IgE/antigen interactions. Indeed, total serum IgE levels are reported to correlate with serum NGF in individuals with asthma,⁷ which might result from the ability of IL-13, like IL-4, to promote the synthesis of this immunoglobulin.

Studies show that increased levels of NGF are also associated with conditions involving a high degree of tissue remodeling. This is true not only in allergic diseases

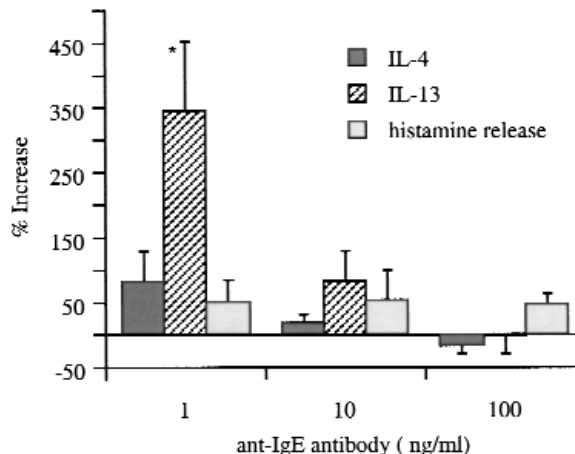


FIG 7. Priming effect of NGF for IL-13, IL-4, and histamine released by anti-IgE. Basophil-enriched suspensions from nonallergic subjects were pretreated for 15 minutes with NGF (10 ng/mL). Cells were then activated with 1, 10, and 100 ng/mL anti-IgE. Cell-free supernatants were harvested after 4 hours' incubation for IL-4 and histamine and after 18 hours' incubation for IL-13. Values are means $\pm$ SEMs ($n = 5$) for the percent increase after subtraction of any values obtained with NGF and anti-IgE alone. Control release values with 1, 10, and 100 ng/mL anti-IgE, respectively, were as follows: $14\% \pm 5\%$, $45\% \pm 9\%$, and $55\% \pm 11\%$ total histamine; 98 ± 42 , 322 ± 128 , and 99 ± 54 pg/ 10^6 basophils for IL-4; and 52 ± 37 , 99 ± 29 , and 43 ± 22 pg/ 10^6 basophils for IL-13. Asterisk denotes a significant difference ($P < .05$) in the percent increase versus that seen for IL-4 or histamine.

such as asthma and vernal conjunctivitis but also in nonallergic conditions, including multiple sclerosis, systemic lupus, and rheumatoid arthritis. It is intriguing to consider that such associations might result from the ability of NGF to directly induce IL-13, because there is evidence that IL-13 has a role in modulating collagen homeostasis in fibroblasts.²³ Thus the link between NGF in both allergic diseases and autoimmune diseases might be its ability to induce IL-13.

Increased levels of NGF have also been measured in lavage fluids taken from sites of allergic lesions, particularly in the upper and lower airways.^{8,9} Baseline levels of NGF in the noses of subjects with allergic rhinitis are significantly higher than those detected in normal controls, and allergen challenge causes an acute release of this neurotrophin. Because basophils, like eosinophils and lymphocytes, selectively infiltrate allergic lesions in the skin, nose, and lung, it also seems possible that the NGF secreted locally in these reactions could affect basophil cytokine generation, either acting directly to induce IL-13 or acting to enhance allergen-mediated release of this cytokine as well as IL-4, histamine, and LTC₄. It is important to note that the basophils infiltrating late-phase responses in the lung have been shown to secrete IL-4²³ and probably contribute to the IL-13 that is also seen in these reactions.²⁴ Accordingly, though the NGF secreted in allergic reactions of the airways might have a role in increasing neuronal hyperresponsiveness to various substances or irritants, it also likely functions in amplifying allergic inflammation by promoting IL-13 and IL-4 production.

Because reports have shown that TrkA is the only identifiable neurotrophin receptor found on basophils,¹⁴ we investigated whether differences in its expression might correlate with the levels of IL-13 induced by NGF. However, we could not detect any significant differences between the levels of TrkA expressed on cells from allergic subjects and the levels expressed on cells from normal controls. It seems rather that other properties account for the greater potential for IL-13 generation in basophils of allergic individuals after stimulation with NGF. One theoretical explanation for these differences is the in vivo "priming" of basophils that results from exposure to various cytokines, including IL-5, GM-CSF, and histamine releasing factor, but also from exposure to IL-3 and/or NGF as well. Thus the increased production of IL-13 occurring in cells of allergic individuals ex vivo might result from their priming to specific cytokines in vivo. It remains to be determined, however, what the particular cytokines or agents responsible for this priming are.

Finally, it has been known for several years that NGF, much like IL-3, is capable of priming for increased histamine and LTC₄ release from basophils activated by a variety of secretagogues.¹³ However, NGF does not directly induce the release of these mediators, just as IL-3 is reported to have limited potential in causing their release from cells of selected allergic subjects.²⁵ It is therefore not surprising that NGF failed to significantly induce IL-4 in this study. Our experience would support the belief that IL-4 generation in basophils is closely coupled to signals resulting from FcεRI-mediated activation, whereas multiple pathways appear to exist for the generation of IL-13.²⁶ If so, then IL-13 might be induced by a variety of stimuli, both IgE-dependent and IgE-independent, and NGF and IL-3 might fall into the latter category. Presently, it is not known whether other cytokines or factors preferentially activate IL-13 in basophils. Ochensberger et al,¹⁶ who tested several different growth factors, including GM-CSF and IL-5 as well as various chemokines, reported that none of them—other than IL-3—directly induced IL-13 and that only a few marginally enhanced these cytokines when combined with C5a or N-formyl-methionyl-leucyl-phenylalanine.¹⁶ Thus though IL-13 generation in basophils seems less restricted to activation through FcεRI than is IL-4, it is still somewhat selectively regulated. The fact that both NGF and IL-3 are stimuli for IL-13 should spark further interest in the role played by these cytokines in the pathogenesis of allergic disease.

This paper is dedicated to the memory of E.M.R.

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