

Nerve growth factor influences IgE-mediated human basophil activation: functional properties and intracellular mechanisms compared with IL-3

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Received 22 October 2004; received in revised form 6 December 2004; accepted 9 December 2004

Abstract

NGF and IL-3 play a unique role in supporting human basophil differentiation and mediator secretion. Their importance in allergic disease is underlined further by studies showing elevated levels of these factors in asthmatics. Here, we compared the abilities of IL-3 and NGF to stimulate basophil histamine, IL-4 or IL-13 release, either directly or in conjunction with IgE-dependent stimulation and assessed the intracellular signals responsible. Our results show that the ability of IL-3 and NGF to enhance IgE-dependent histamine release are similar. Both factors also potentiated IgE-dependent IL-13 secretion to a greater degree than the release of histamine or IL-4. At high concentrations (100 ng/ml), IL-3 and NGF alone were capable of releasing cytokines but little histamine. These abilities of IL-3 and NGF to modulate basophil activation were sensitive to blockade by specific inhibitors of PI 3-kinase, p38 MAPK and PLC, but not PKC, suggesting that their effects are mediated considerably by pathways comparable to IgE-dependent signalling.

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Keywords: Basophils; Signal transduction; Inflammatory mediators; IgE; NGF; IL-3

1. Introduction

During the course of an allergic reaction, mast cells and basophils, which are recruited at sites of allergic inflammation, release histamine and other mediators which significantly contribute to the symptomology of

allergic disease. In recent years, however, it has become apparent that a plethora of cytokines (e.g. IL-3, IL-5, GM-CSF, NGF) [1] and chemokines (RANTES, MIP-1 α , eotaxins) [2,3] are found in allergic inflammation and can modulate the functions of the above effector cells. In humans, basophils are particularly sensitive to priming by a number of cytokines and chemokines which potentiate their ability to degranulate by IgE-dependent triggers (i.e. allergens) (reviewed in Ref. [4]). Conversely, human mast cells are less responsive to priming except for the

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action of stem cell factor [5]. This suggests that basophil, but not mast cell, responsiveness to allergen challenge is crucially controlled by the cytokine milieu in affected tissues.

The ability of basophils to invade various organs during allergic reactions has been demonstrated in the lung, nose and skin in patients with allergic asthma [6–8], rhinitis [9] and atopic dermatitis [10], respectively. The likelihood of their modulation by priming factors is underlined by studies showing increased levels of NGF in both allergic rhinitis and asthma [11,12] and elevated expression of IL-3, IL-5 and GM-CSF during allergen-induced cutaneous late phase responses in atopic individuals [1]. It is interesting to note that the most potent enhancing factor for basophils, namely IL-3, is also important for their differentiation and development, a function which has not been reported for chemokines (reviewed in Ref. [4]). IL-5 and GM-CSF, which share a common receptor β -subunit with IL-3, may also contribute to basophil differentiation but they are less effective at priming these cells for enhanced mediator release [13–15]. Also, in vivo studies using rhesus monkeys treated with IL-3 have shown an increase in basophil numbers and symptoms of immediate hypersensitivity reactions which point to the unique importance of this factor in basophil biology [16,17].

Although IL-3 and other priming cytokines are usually poor stimuli for in vitro basophil mediator release in the absence of co-stimulation with allergens, Sin et al. showed that basophils from allergic subjects are capable of releasing IL-13 upon direct stimulation with either IL-3 or NGF [18]. IL-13 is an important immunomodulator and, together with IL-4 (also rapidly released from basophils), it can enhance B cell IgE synthesis as well as support the recruitment of inflammatory cells. This direct stimulatory action of IL-3 may partially explain the observations described above in primates treated by the cytokine.

Similar functions have also been ascribed to the neurotrophin NGF which, like IL-3 and related cytokines, also appears to play a role in basophil differentiation [19]. There is particular interest in characterising the effects of NGF on basophils since this action is unique among the neurotrophins [20]. Furthermore, NGF binds to an entirely different receptor than for other cytokines but shares similar

functional capabilities with IL-3 [20–23]. The intracellular signalling events triggered by NGF and IL-3 in basophils are also poorly understood. While it has been shown that IL-3 and NGF enhance basophil ERK1&2 phosphorylation, regulating PLA₂ and subsequent LTC₄ synthesis [23], targets responsible for enhanced histamine or cytokine secretion have not been characterised. Given the enhanced production of NGF and IL-3 from a variety of immune cells during an allergic reaction and their positive influence on the immunomodulatory effects of basophils, our aims were to compare the effects of NGF with that of IL-3 on basophil mediator release in order to delineate possible differences in their actions. In addition, we addressed whether IL-3 and NGF can modulate p38 MAPK activation, which is a universally important enzyme involved in IgE-mediated signalling in basophils.

2. Materials and methods

2.1. Reagents and materials

The following reagents were purchased: anti-mouse Ig horseradish peroxidase, ECL-plus (Amersham, Buckinghamshire, UK); LY294002, PD098059 (Alexis, Grünberg, Germany); IL-4 ELISA (BioSource, Camarillo, CA, USA); SB203580, U73122 (Calbiochem, Bad Soden, Germany); IL-13 ELISA (Boehringer Ingelheim Bioproducts, Heidelberg, Germany); MACS basophil isolation kit (Miltenyi Biotec, Bergisch Gladbach, Germany); anti-rabbit Ig horseradish peroxidase, rabbit anti-ERK1&2, rabbit anti-p38 MAPK, rabbit anti-phospho p38 MAPK (New England Biolabs, Frankfurt am Main, Germany); Ficoll-Paque (Pharmacia Biotec, Uppsala, Sweden); anti-human IgE, mouse anti-diphosphorylated ERK1&2, Bisindolylmaleimide I, Bisindolylmaleimide II, 2× concentrated Laemmli sample buffer, rhIL-3, rhNGF- β (Sigma, St. Louis, USA). All other standard reagents were purchased at the highest grade obtainable.

2.2. Basophil purification

Human basophils were obtained from freshly prepared Buffy coats (leukocyte concentrate of 450

ml venous blood) from healthy blood donors, with consent, undergoing routine blood donation. Basophils were enriched by Ficoll density centrifugation followed by elutriation and purified to over 90% purity by negative selection using magnetic cell sorting. We previously showed that this technique for basophil enrichment does not lead to functional differences between purified and unpurified basophils [24]. Basophil purity was assessed by alcian blue staining and averaged $95.2 \pm 1.0\%$ and the viability of the cells, which exceeded 95%, was ascertained by Trypan blue exclusion.

2.3. Kinase inhibitors

LY294002, SB203580 and PD098059 were dissolved in DMSO, all at 0.1 M, and subsequently diluted in HEPES-buffered-Tyrodé's solution shortly before use. U73122 was dissolved in ethanol at 1 M and diluted in HEPES-buffered-Tyrodé's solution accordingly. At working dilutions of the compounds (5 μM), no effects were observed due to DMSO or ethanol in the absence of the drugs.

2.4. Cell stimulation and mediator assay

Purified basophils ($1\text{--}2 \times 10^5$ nucleated cells per ml) were resuspended in HEPES-buffered-Tyrodé's solution (400 μl per tube), containing 1 mM CaCl_2 and, following a short warming period (5 min), cells were preincubated with various concentrations of either the priming cytokines and/or kinase antagonists for 15 min (unless stated otherwise) before stimulation with anti-IgE (1/25 000 dilution unless stated otherwise). Controls consisted of cells incubated with the stimulus in the absence of the priming factors and/or kinase inhibitors and cells incubated in buffer alone. In mediator release studies, basophils were stimulated for either 30 min, for histamine release, or 5 hours for the secretion of IL-4 and IL-13. Cells stimulated for 30 min were then centrifuged and histamine contents in the supernatants together with the cell pellets, which were lysed with perchloric acid (4%), were measured using a spectrofluorometric autoanalyser. Percentage histamine releases were determined from the total histamine content in the sum of pellet and supernatant tubes. Basophils incubated for 5 h were centrifuged and the respective release of IL-4 and IL-

13 in the supernatants was assayed by ELISA (according to the manufacturer's instructions). The minimum sensitivity of the ELISAs were 3 pg/ml for IL-4 and 1 pg/ml for IL-13. Statistical significances were assessed using a paired Student's *t*-test.

2.5. Western blot analysis

Purified basophils ($>98\%$ pure, 2×10^5 /sample) were treated as indicated and incubated at 37°C for periods of up to 15 min. After these times, reactions were terminated rapidly by placing tubes in ice. All tubes were then immediately centrifuged, supernatants removed for histamine analysis (in order to gauge the response) and cell pellets lysed by vigorous mixing in 15 μl lysis buffer (50 mM Tris-HCl pH 7.5, 5 mM EDTA, 10 mM EGTA, 5 mM DTT, 1% Nonidet P-40, 1 mM PMSF, 100 $\mu\text{g/ml}$ aprotinin, 20 $\mu\text{g/ml}$ leupeptin and 10 mM benzamide). The samples were stored at -80°C prior to Western blotting. An equal volume of $2\times$ concentrated Laemmli sample buffer was added to the lysed cell pellets which were then boiled for 3 min. Proteins were separated by 12% SDS-PAGE and blotted onto nitro-cellulose membranes. The m.w. of the resolved proteins was visualized using prestained m.w. rainbow markers which were also included for each SDS-PAGE run. Membranes were blocked for 4 h in 5% skimmed milk in TBST buffer (20 mM Tris-HCl, pH 7.5, 137 mM NaCl, 0.1% Tween 20) with gentle agitation. Following 3×5 min washes in TBST, membranes were incubated overnight (at 4°C) with specific mouse or rabbit antibodies to either phosphorylated or total ERK1&2 or p38 MAPK. Following incubation with the various primary antibodies, membranes were successively washed (4×5 min, TBST) and incubated with either anti-mouse or anti-rabbit Ig horseradish peroxidase for 2 h with gentle agitation. After washing unbound secondary antibody, proteins were visualized by autoradiography according to the manufacturers instructions (ECL plus, Amersham, Buckinghamshire, UK). Comparisons of phosphorylated (activated) signalling proteins were made not only on the basis of equal cell numbers but by observing the even signal intensity of total expression. After detection, membranes were stripped for 15–25 min using Re-blot plus reagent (Chemicon international), washed in TBST (4×5 min) and reprobed.

3. Results

3.1. Effects of IL-3 and NGF on basophil histamine, IL-4 and IL-13 secretion

The ability of various concentrations of NGF or IL-3 to modulate basophil mediator secretion either alone

or in conjunction with anti-IgE is shown in Fig. 1. In the absence of anti-IgE, low doses of IL-3 and NGF were poor inducers of histamine release (Fig. 1A). However, at concentrations of 10 ng/ml or greater a small but significant ($p<0.04$) increase of the release was observed with both priming factors, with IL-3 having significantly more effect than NGF ($p<0.05$).

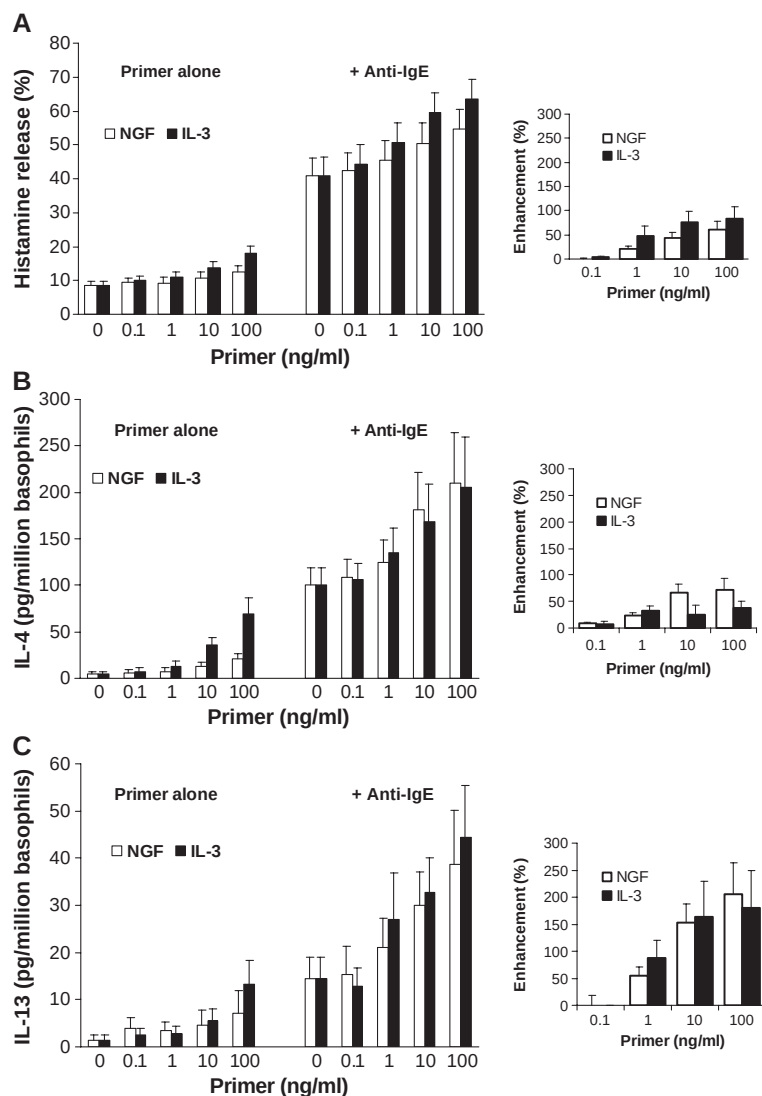


Fig. 1. Dose dependent effects of NGF and IL-3, either alone or in conjunction with anti-IgE, on histamine (A), IL-4 (B) and IL-13 (C) secretion from purified human basophils ($n=9-12$). Basophils were stimulated with various concentrations of the priming factors for 15 min before the addition of anti-IgE (1/25000 dilution), where indicated, and incubated for a further 30 min for the release of histamine and 5 h for cytokine synthesis. The panels shown to the right of each figure depict the percentage enhancement of respective mediators calculated from the same data and corrected for secretions caused by the priming factors themselves. Data are shown as means $\pm$ SEM from either 12 (for histamine) or 9 (IL-4 and IL-13) separate experiments.

Both cytokines were able to dose-dependently and significantly ($p<0.001$) potentiate anti-IgE induced histamine release, with IL-3 being significantly more efficacious than NGF in comparison ($p<0.01$).

At the level of cytokine release from basophils, both IL-3 and NGF caused pronounced enhancement of both IL-4 (Fig. 1B) and IL-13 (Fig. 1C) secretion in combination with anti-IgE. These increases reached statistical significance ($p=0.05$) using 1 ng/ml of either IL-3 or NGF regarding both IL-4 and IL-13 release. At concentrations of 10–100 ng/ml, the priming factors enhanced anti-IgE induced IL-13 secretion more significantly ($p<0.01$) than IL-4 ($p<0.04$). However, in contrast to histamine release, where IL-3 consistently had more priming effect than NGF, the absolute amounts of cytokines released were similar in the presence of either priming factor. In terms of IL-4 production, the picture was different when expressing the data as a percentage enhancement and taking into account the IL-4 release caused by the priming factors alone (Fig. 1B right hand panel). Here, NGF was actually a ‘better’ primer, since it released little IL-4 alone but enhanced IgE-dependent IL-4 release to a greater degree than for histamine. Conversely, at high concentrations (>10 ng/ml) IL-3 caused considerable IL-4 release by itself in 6 out of 9 donors tested which exceeded the half maximal levels achieved by anti-IgE stimulation of basophils. Therefore, the overall potentiation of IgE-dependent IL-4 release was more pronounced for NGF than IL-3 once the data were corrected for the release of the cytokine caused by the priming factors alone.

In terms of IL-13 secretion, both IL-3 and NGF were capable of releasing this cytokine by themselves at 100 ng/ml, which for IL-3 was almost at the level of that caused by anti-IgE stimulation. However, this ability of IL-3, in particular, to release IL-13 alone was highly variable, i.e. donor dependent, three of which from the nine failed to appreciably release the cytokine even at maximum IL-3 concentrations. We have previously made similar observations (which were not included here since not all IL-3 concentrations were employed) where even less donors showed basophil IL-13 release to IL-3, despite IgE-dependent enhancement. This is in contrast to earlier reports showing more consistent direct ability of IL-3 to cause release of IL-13 from basophils [18,25].

At 100 ng/ml, NGF alone released less IL-13, when compared to the effects of IL-3. However, in terms of priming IgE-dependent IL-13 release both NGF and IL-3 were generally equally capable of enhancing IL-13 release caused by anti-IgE. Interestingly, this potentiatory effect, even when taking the direct actions of both factors into account (i.e. in the absence of anti-IgE), was strikingly greater than their ability to enhance either histamine or IL-4 release from basophils (Fig. 1 right hand panels). This clearly suggests that potentiatory mechanisms controlling IgE-dependent IL-13 production by IL-3 or NGF differ from IL-4 or histamine release.

3.2. The potentiatory actions of NGF and IL-3 are dependent on preincubation before IgE-receptor activation

Fig. 2 shows that preincubating basophils with NGF or IL-3 for short periods before IgE-dependent stimulation is necessary for maximal enhancement of basophil histamine release. Although NGF, as already shown in Fig. 1, produced less enhancement than IL-3, their potentiatory abilities followed similar kinetics. Both factors required at least 15

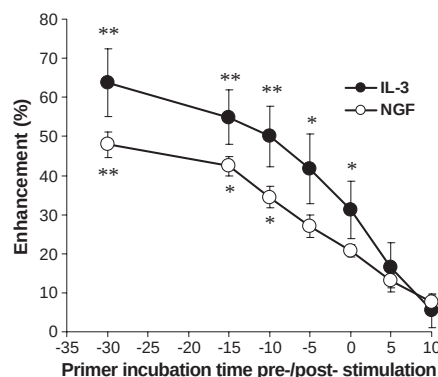


Fig. 2. Effects of different incubation times with either NGF or IL-3 (both at 10 ng/ml) prior/after stimulation of basophils with anti-IgE (1/25 000 dilution). Results are shown as a percentage enhancement of histamine release $\pm$ SEM from 7 separate experiments. All data were corrected for spontaneous secretions ($8.1 \pm 0.5\%$) and net releases caused by the priming factors themselves ($2 \pm 1\%$ for NGF, 4.1 ± 1.3 for IL-3) before calculating the percentage enhancement of release caused by anti-IgE alone (net release = $26.4 \pm 4.2\%$). * and ** denote significant differences ($p<0.05$ and $p<0.01$, respectively) of enhanced release due to the primers compared to anti-IgE alone.

min preincubation for the fulfilment of their enhancing properties which thereafter rose only slightly at 30 min. Preincubation with NGF or IL-3 for 15–30 min was also optimal for the enhancement of IL-4 and IL-13 release (not shown) and increasing these times even further (to 4 h) did not lead to any consistent change. We therefore employed a preincubation period of 15 min throughout this study. Despite the fact that NGF and IL-3 were only poor enhancers of anti-IgE when added to the cells simultaneously, a slight, though not statistically significant, priming ability remained nonetheless at 5 min after anti-IgE had been applied. This virtually disappeared at 10 min after anti-IgE stimulation, bearing in mind that the kinetics of anti-IgE induced histamine release nears completion within 15 min. This implies that NGF and IL-3 may, at least in part, be acting on intermediate IgE-signalling pathways downstream of immediate (i.e. FcεRI triggered Lyn and Syk activation) but upstream of cytoskeletal rearrangements associated with degranulation.

3.3. The enhancing abilities of NGF and IL-3, and their differences in efficacy are partly dependent on the strength of IgE-dependent activation

To further characterise the nature of IgE-dependent priming of basophil mediator release we tested the enhancing properties of NGF and IL-3 (both at 10 ng/ml) on histamine release stimulated with various titres of anti-IgE. The results in Fig. 3A show that both priming factors were capable of significantly increasing histamine release caused by anti-IgE even at optimal and super-optimal concentrations of anti-IgE. As expressed as percentage enhancement of anti-IgE induced histamine release (Fig. 3B), it is clear that NGF and IL-3 give rise to greater potentiation at lower anti-IgE concentrations than those that give maximum histamine release in the absence of the priming factors. (These data were first corrected for the small amounts of histamine released by the primers themselves.) Furthermore, the differences in enhancing efficacy between NGF and IL-3 disappeared at optimal and super-optimal anti-IgE concentrations. Similar observations were made regarding IL-4 and IL-13 secretions (not shown).

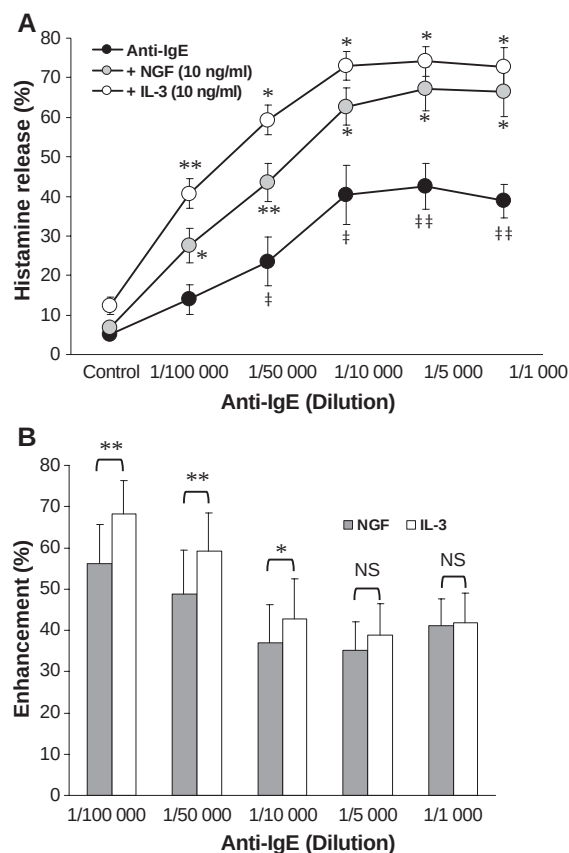


Fig. 3. (A) Effects of NGF and IL-3 (both at 10 ng/ml) on histamine release from basophils stimulated by various titres of anti-IgE. Results are expressed as mean percentage histamine release $\pm$ SEM for 5 separate experiments. † and †† denote significant ($p < 0.05$ and $p < 0.01$, respectively) release caused by anti-IgE alone compared to basal levels. * and ** denote significant ($p < 0.05$ and $p < 0.01$, respectively) differences between releases caused by anti-IgE in conjunction with either NGF or IL-3 and anti-IgE alone for each concentration point. (B) Data from the same experiment showing percentage enhancement of histamine release, caused by various titres of anti-IgE, due to either NGF or IL-3 (10 ng/ml). All data are corrected for histamine releases caused by the primers alone. * and ** indicate significant differences between the enhancement caused by NGF with that caused by IL-3.

3.4. Despite donor-dependent variations of potentiation the effects of NGF and IL-3 on histamine release are closely correlated

Another parameter governing the enhancing abilities of IL-3 and NGF was not only the strength of the anti-IgE stimulus but also the donor. We reviewed data from over 30 experiments where basophils had

been stimulated under the same conditions (i.e. anti-IgE dilution of 1/20 000–1/25 000, IL-3 and NGF concentrations of 10 ng/ml). Here, we observed that the potentiatory effects of both priming factors differed by more than two orders of magnitude between different donors (Fig. 4A–C). This difference was quite strongly dependent on the ‘releasability’ of basophils after anti-IgE stimulation alone, where the enhancement of histamine release due to either NGF (Fig. 4A) or IL-3 (Fig. 4B) negatively correlated with the release caused by anti-IgE alone. Comparing the enhancement of anti-IgE induced histamine release caused by NGF with that of IL-3 from each donor we observed a strikingly close correlation (Fig. 4C). This suggests that while IL-3 may be slightly more efficacious than NGF at potentiating histamine release at sub-optimal IgE-dependent stimulation, overall they both appear to act *via* a common mechanism because the differences between donors were much greater than the relatively minor differences of priming ability of NGF versus IL-3 within the same donor.

By grouping the above results into low (less than 10%), medium (10–30%) and high (>30%) histamine releasers with respect to anti-IgE stimulation, it is apparent that the greatest change of enhancement (up to ten-fold) due to NGF or IL-3 occurred in poor responders to anti-IgE and far less striking in medium or high releasers (Fig. 5A–B). All enhancing data were corrected for histamine release caused by the priming factors alone. Another aspect revealed by these data was that the amounts of histamine release by NGF or IL-3, albeit small, were greater in donors that were ‘high releasers’ to anti-IgE. Regarding IL-3, these differences were statistically significant comparing ‘low’ releasers to ‘high’ whereas the data for NGF were not significantly different, probably owing to the fact that this priming factor has less ability to cause direct activation at 10 ng/ml. (In contrast to the above, we could not find any such correlations regarding IL-4 or IL-13 release from basophils.)

3.5. NGF and IL-3 activate ERK1&2 and enhance IgE-dependent p38 MAPK phosphorylation

To address the question of how IL-3 and NGF potentiate IgE-dependent activation we investigated their ability to affect several signalling enzymes. In

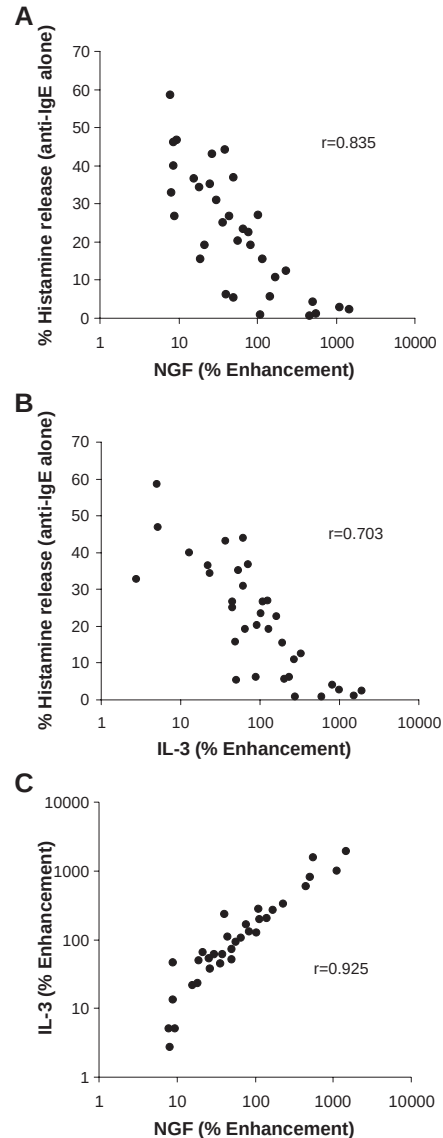


Fig. 4. Correlation between anti-IgE induced histamine release and percentage enhancement of histamine release due to either NGF (A) or IL-3 (B). (C) Correlation between percentage enhancement of anti-IgE stimulated histamine release due to NGF and IL-3 from the same experiments. Each data point represents the results from one basophil donor from a total of 34 separate donors.

Fig. 6 we show that both NGF and IL-3 dose-dependently enhance p38 MAPK phosphorylation to anti-IgE. We have previously reported that p38 MAPK plays a central role in controlling the release of all known basophil mediator types released by IgE-

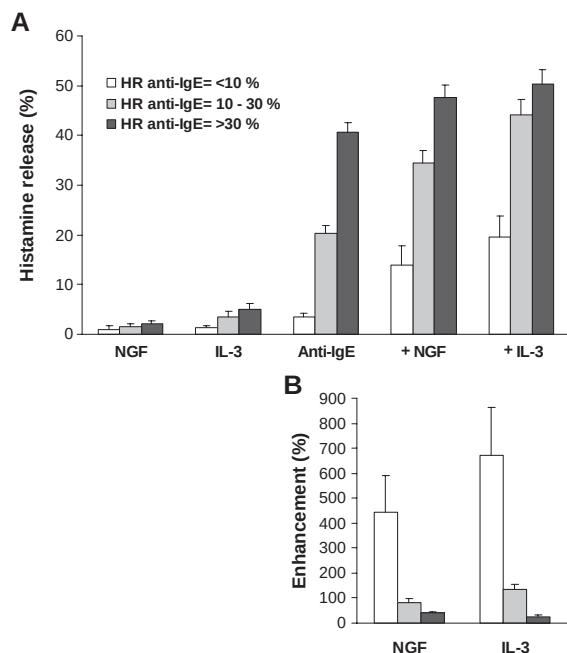


Fig. 5. Differential effects of NGF and IL-3 (either alone or in conjunction with anti-IgE) on basophil histamine release from donors grouped according to their histamine releasability to anti-IgE activation (A). The potentiatory actions of NGF and IL-3 on anti-IgE induced histamine release between these donor groups were then corrected for releases caused by the priming factors themselves and expressed as percentage enhancement in panel B.

dependent mechanisms and that IL-3 enhances the activity of this enzyme [26]. In the current report we expand on this study by showing that NGF, like IL-3, also strikingly enhances p38 MAPK phosphorylation. By itself, IL-3 was a poor inducer of p38 MAPK phosphorylation compared to anti-IgE triggering alone but nevertheless caused an increased phosphorylation above that of basal levels. In contrast, NGF failed to consistently activate p38 MAPK alone. In consent with previous reports [23,27], IL-3 was able to considerably activate ERK1&2 as well as potentiate anti-IgE induced ERK1&2 activity. NGF, on the other hand, was less able to cause ERK1&2 activation by itself in comparison with IL-3, which was also recently demonstrated by Miura et al. [23]. In the current study, however, we also show that NGF was able to markedly enhance ERK1&2 phosphorylation caused by anti-IgE, although this enhancing effect was less than that caused by IL-3. Another major member of the MAPK family, namely c-jun amino terminal

kinase, was not activated by either anti-IgE or the priming factors.

Although ERK1&2 are strikingly activated by NGF and IL-3 the MEK inhibitor PD098059, which blocks downstream ERK1&2 activation, failed to inhibit histamine release caused by either anti-IgE alone or in conjunction with the priming factors (both employed at 10 ng/ml) (Fig. 7A–C). Additionally, the inhibitor also failed to affect histamine release caused by high concentrations (100 ng/ml) of NGF or IL-3 alone (Fig. 7D–E). Conversely, in the same series of experiments, histamine release from basophils was significantly decreased ($p < 0.05$) by the p38 MAPK inhibitor SB203580 as well as antagonists of both PI 3-kinase (LY294002) and PLC (U73122), which we

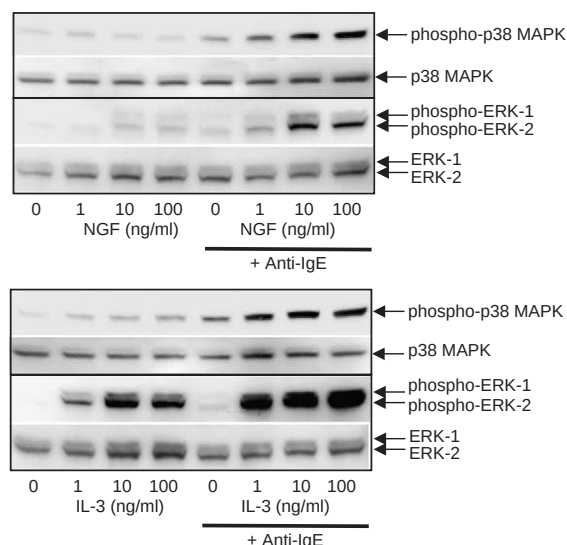


Fig. 6. Dose-dependent actions of NGF (top panel) and IL-3 (lower panel), either alone or in conjunction with anti-IgE (1/25 000 dilution), on the phosphorylation of p38 MAPK and ERK1&2. Total kinase expressions are shown as a gauge of protein loading. Highly pure basophils (>98%) were prewarmed to 37 °C and preincubated with various concentrations of NGF or IL-3 for 15 min before stimulation with anti-IgE for a further 15 min. In the absence of co-stimulation with anti-IgE, NGF and IL-3 were added to the cells 15 min before terminating the reactions on ice, followed by cell lysis. Separation of cellular proteins was performed by SDS-PAGE and Western blotting was then conducted using various phospho-antibodies against the respective proteins. After ECL detection, membranes were stripped and reprobed for the respective proteins using antibodies detecting total expression. These steps were repeated successively so that all the proteins (both phosphorylated and total expression) could be analysed for the same blot.

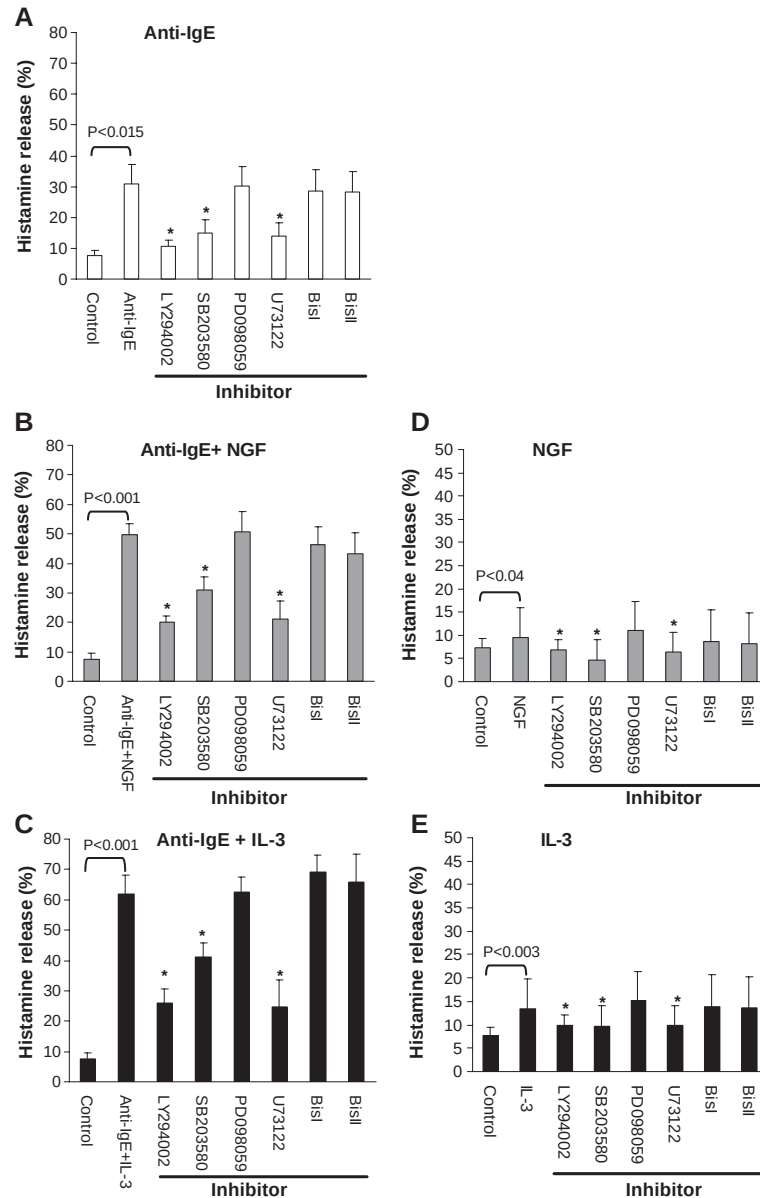


Fig. 7. Effects of LY294002 (PI 3-kinase), SB203580 (p38 MAPK), PD098059 (MEK), U73122 (PLC) and PKC (BisI&II) antagonists (5 μ M) on histamine release from purified human basophils stimulated by either anti-IgE alone (A) or in conjunction with 10 ng/ml of either NGF (B) or IL-3 (C). Panels D and E show the respective releases, and their modulation by the inhibitors, caused by NGF and IL-3 alone used at a higher concentration (100 ng/ml) than for priming of anti-IgE release. Data are shown as mean percentage releases $\pm$ SEM. * Represents a significant ($p < 0.05$) reduction of histamine release compared to basophils stimulated in the absence of the inhibitors.

have shown to be upstream regulators of p38 MAPK regarding IgE-dependent signalling [26]. Two specific PKC inhibitors (Bisindolylmaleimide I and II) failed to inhibit histamine secretion suggesting that basophil

mediator secretion caused by IgE-dependent signals and their enhancement by NGF and IL-3 is dependent on PLC, IP_3 and subsequent calcium mobilization rather than PKC.

4. Discussion

The results presented here identify p38 MAPK as a key signalling component whereby NGF and IL-3 enhance IgE-dependent basophil mediator release. We have previously shown that this kinase is crucial for IgE-dependent triggering of both histamine, eicosanoid and cytokine generation from basophils [26]. Its increased activity caused by input from signals derived from IL-3 and NGF receptor binding is therefore a likely explanation for the enhancement of basophil mediator release seen here and reported in earlier studies.

NGF binding to basophils is mediated through the high-affinity tyrosine kinase receptor A (TrkA) whereas low-affinity forms of the receptor (TrkB or TrkC), which are activated by other members of the neurotrophin family, do not appear to be expressed [20]. IL-3 binds to an unrelated receptor belonging to the haematopoietin superfamily consisting of low-affinity α subunits, which differentially bind to either IL-3, IL-5 or GM-CSF, but share a common β subunit. Unlike the NGF receptor, IL-3 receptors do not have intrinsic tyrosine kinase activity but their activation nevertheless causes downstream tyrosine kinase phosphorylation. Despite these differences, our data show that the enhancing properties of NGF and IL-3 are similar in nature and, at least with respect to histamine release, appear to be closely related. This was demonstrated by the intimate correlation between the enhancement of IgE-dependent histamine release caused by both primers comparing a large number of donors.

Explanations for the close correlation between the histamine-enhancing properties of NGF with IL-3 may reside in that either the receptor densities for both primers are controlled by common mechanisms, and/or that they share common downstream signal transduction elements. There is no evidence to support the former hypothesis since several studies have shown no significant difference in either NGF TrkA receptor or IL-3 receptor expression between basophils from atopic or non-atopic donors [18,28]. Furthermore, Sin et al. showed that, despite the lack of increased NGF receptor expression in allergic subjects, there was a significant increase in the ability of NGF to directly cause IL-13 release from basophils compared to non-allergic donors [18].

It appears that the correlative actions of NGF and IL-3 may be explained by assuming the use of common signalling pathways downstream of their respective receptors. This is at least partly true in respect to the earliest secreted basophil mediator, histamine. The effects of both factors are sensitive to blockade by the same inhibitors of several signalling enzymes (PI 3-kinase, p38 MAPK and PLC), which are also utilized by IgE-dependent signalling. A common mechanism is also suggested given the similar times required to preincubate basophils with either NGF or IL-3 for maximal enhancement of mediator release by anti-IgE.

Large donor variations were observed concerning the potentiatory effects of NGF and IL-3 on IgE-dependent histamine release. This inversely correlated with the histamine release caused by anti-IgE in the absence of the priming factors (i.e. the greater the histamine release caused by anti-IgE the less well NGF or IL-3 could potentiate it). An intrinsic donor-dependent 'releasability' appeared to be responsible for this rather the concentration of anti-IgE employed: While NGF and IL-3 enhanced more histamine release at lower anti-IgE concentrations this resulted in a maximal difference of only 30%, which is small compared to differences of enhancement between donors that had been stimulated with constant anti-IgE concentrations. Interestingly, the greater enhancing effects of IL-3 compared to NGF were no longer significant at optimal and super-optimal anti-IgE concentrations. In terms of basophil 'releasability' to IgE-receptor triggering, we observed a striking decline in the potentiatory effects of NGF and IL-3 between donors which were poor responders to anti-IgE (less than 10% histamine release) than high responders. Conversely, in the absence of co-stimulation with anti-IgE, NGF and IL-3 released more histamine in donors which were high responders to anti-IgE. However, these increased levels (which are relatively small) fail to account for the differences in enhancement of IgE-dependent release between low and high responders.

Reasons for the greater enhancing properties of NGF and IL-3 in poor histamine releasers to anti-IgE are not currently known. In high responders it can be assumed that mediator secretion cannot further be enhanced by priming due to an existing optimal interplay of IgE-dependent signalling events. It is

known that differences in basophil histamine releasability to IgE-dependent activation depends on Syk expression and that non-releasing basophils, which are Syk deficient, release histamine to IgE-receptor activation after a four day culture with IL-3 prior to activation [29]. However, this restoratory action requires longer times to occur than the marked improvement of anti-IgE induced histamine release due to IL-3 and NGF seen with low responders in the present study.

IL-3 was more efficacious than NGF at potentiating IgE-dependent histamine release but these differences were not seen at the level of cytokine release. Although all basophil mediator types are sensitive to the actions of PI 3-kinase and p38 MAPK, the differences described above clearly indicate other levels of control. No clear correlations were seen between the ability of basophils to release histamine and IL-4 or IL-13 release either to anti-IgE or in combination with the priming factors. This suggests that, while early signalling events leading to histamine release and their potentiation by NGF or IL-3 may be predictable (given the close correlation of priming effects of NGF and IL-3 between donors), downstream pathways differentially controlling IL-4 and IL-13 secretion are not. Here, the atopic status of the donor, genetic factors controlling cytokine expression and prior sensitization *in vivo* by other priming factors may underlay the lack of any correlative effects.

The enhancement of p38 MAPK by NGF and IL-3 provides a general explanation of how these factors increase IgE-dependent mediator release. IL-3 was slightly more potent than NGF at causing p38 MAPK enhancement with anti-IgE and caused a small, but noticeable, direct activation of the enzyme. These effects on the kinase also resemble the stimulatory activities with respect to mediator release, especially histamine. In contrast, the primers had far greater influence on ERK1&2 and not only potentiated IgE-dependent phosphorylation but caused considerable activation of ERK1&2 by themselves (more than that caused by anti-IgE stimulation alone). Interestingly, IL-3 caused more direct ERK1&2 activation than NGF showing that this signalling pathway, which is known to control basophil LTC₄ synthesis, is far more differentially influenced. However, ERK1&2 does not appear to play a major role in the release of histamine

or cytokines from basophils since the MEK inhibitor PD098059 fails to inhibit their secretion [27,30].

There is compelling evidence to suggest that NGF participates in allergic inflammation. Bonini et al. showed that levels of NGF are substantially enhanced in allergic disease, particularly in asthma, correlating with disease severity and IgE levels [12]. Similar increases were observed in lavage fluid following allergen provocation of airways in asthmatic patients [31] and in nasal lavage fluids of those with allergic rhinitis [32]. In comparison, IL-3, though localised to tissue sites of allergic inflammation does not appear to be increased by enhanced disease activity [33]. This indicates that, apart from the neuronal actions of NGF on airway hyperresponsiveness, the increased levels of NGF released by allergen provocation may have particular significance in governing the severity of symptoms associated with asthma and possibly other atopic diseases. Basophils are a likely target for mediating these effects since they rapidly infiltrate affected tissues in allergic individuals and, as shown in this study, their mediator secretions are strongly enhanced by the actions of NGF, in particular IL-13 release. Furthermore, in man, basophils appear to be better placed to be affected by NGF or IL-3 than mast cells which are unresponsive to the priming actions of these cytokines. Finally, unlike human mast cells, basophils are unique in their ability to rapidly release IL-4 and IL-13 following IgE-dependent stimulation (reviewed in Ref. [4]). These cytokines participate in controlling late phase allergic reactions (via upregulation of VCAM-1), cause IgE class switching in B cells and IL-4 directly supports Th2 type cell development. Thus, NGF and IL-3 may have important immunomodulatory functions governing processes which control IgE synthesis in allergic disease because of their enhancing effects on basophil cytokine generation.

Acknowledgements

The authors express their thanks to Professor Holger Kirchner (Institute of Immunology and Transfusion Medicine, University of Lübeck) for supplying Buffy coats. This project was funded in part by a grant from the Forschungsförderung der Universität zu Lübeck (Grant N07-2002).

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