Nerve Growth Factor Modifies the Expression of Inflammatory Cytokines by Mast Cells Via a Prostanoid-Dependent Mechanism

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Nerve Growth Factor Modifies the Expression of Inflammatory Cytokines by Mast Cells Via a Prostanoid-Dependent Mechanism

Jean S. Marshall,* Kaede Gomi,* Michael G. Blennerhassett,† and John Bienenstock†

Nerve growth factor (NGF) is well recognized to have a number of potent effects on mast cells, including increasing mast cell numbers in vivo and inducing mast cell degranulation in vitro. More recently, NGF has been demonstrated to induce PGD₂ production by mast cells through the induction of mast cell cyclooxygenase expression. We have observed that NGF at doses as low as 10 ng/ml will induce IL-6 production and inhibit TNF-α release from rat peritoneal mast cells in the presence of lysophosphatidylserine as a cofactor. NGF synergizes with LPS treatment of peritoneal mast cells (PMC) for the induction of IL-6.

Examination of the mechanism of this phenomenon has revealed that NGF can induce both rat PMC and mouse bone marrow-derived cultured mast cells to produce substantial levels of PGE₂. This response is maximal at later time points 18–24 h after NGF activation. The ability of NGF to induce PGE₂ is not dependent on mast cell degranulation. Other stimuli capable of inducing IL-6, such as LPS, do not induce production of this prostanoi. Inhibition of cyclooxygenase activity by PMC using either flurbiprofen or indomethacin inhibited both the NGF-induced PGE₂ synthesis and the NGF-induced alterations in TNF-α and IL-6 production. These results suggest a role for mast cell-derived prostanoids in the regulation of local inflammatory responses and neuronal degeneration after tissue injury involving induction of NGF production.

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Mast cells are most frequently found at body sites that interface with the external environment such as the skin, airways, and gastrointestinal mucosa. Several studies have demonstrated that mast cells are often found in close contact nerves (1, 2) and that there may be a functional interaction between mast cells and the nervous system (3–6). In the context of tissue injury or chronic inflammation, the coordinated actions of nerves and mast cells are an important and often overlooked aspect of the host response (7). Mast cells have been demonstrated to be a potent source of a wide variety of inflammatory and immunomodulatory cytokines and chemokines including TNF-α, IL-6, granulocyte-macrophage-CSF (8, 9), IL-8 (10), IL-13 (11), and leukemia-inhibitory factor (12). Certain of these mast cell derived cytokines have been demonstrated to have effects on nerve development and differentiation. For example, leukemia-inhibitory factor is known to be a cholinergic neuronal differentiation factor (13). IL-6 has been shown to induce neurite outgrowth in the PC12 cell line (14) and to have a range of anti-inflammatory effects (15).

In contrast, TNF-α has been suggested to induce neuronal damage (16, 17) following tissue damage and is considered a major initiator of inflammation.

A number of neurally derived factors have been demonstrated to alter both mast cell development and function. These include neuropeptides such as substance P and growth factors including 2.5S nerve growth factor (NGF) (3). NGF is essential for the survival of sensory and sympathetic neurones (18) and has a wide range of other effects (19). In rodents, NGF induces degranulation of peritoneal and skin mast cells (20, 21). NGF can be produced by a variety of cell types including fibroblasts and Schwann cells. This neurotrophin will also induce a hyperplasia of mast cells in both connective tissue and mucosal sites when injected into neonatal rats (22). Mast cells have been demonstrated to possess receptors for NGF (23, 24), and NGF has been demonstrated to prevent mast cell apoptosis (19). In the human, NGF has been demonstrated to enhance the development of basophil-containing colonies in culture (25). More recently, it has been demonstrated that NGF can induce the production of cyclooxygenase-2 which has a role in the long term production of PG D₂ from rat peritoneal mast cells (PMC) (26).

Previous studies have demonstrated that the production of the cytokines IL-6 and TNF-α by rat PMC can be modulated by other mediators found at inflammatory sites such as IFN-γ (27, 28), IL-10 (29), and PGE₂ (30). In some cases, the modulation of cytokine production is selective. For example, PGE₂ will selectively enhance IL-6 while decreasing TNF-α production from PMC (30). PGE₂ has also been demonstrated to act as a growth factor for mast cells (31) and to ablate both early and late asthmatic responses (32–34). A wide range of cell types are known to synthesize PGE₂ including macrophages, neutrophils, fibroblasts, and follicular dendritic cells. Rat PMC produce mainly PGD₂ and low levels of PGE₂ following IgE-mediated activation (35, 36). Mast cell products such as histamine have also been shown to induce PGE₂ production by other cells (37, 38). In the current study, we have examined NGF modulation of TNF-α and IL-6 in freshly isolated rat PMC and the role of PGE₂ in mediating the effects of NGF treatment.

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1 Abbreviations used in this paper: NGF, 2.5S nerve growth factor; DSCG, disodium cromoglycate; PMC, rat peritoneal mast cells; mBBMC, mouse bone marrow-derived cultured mast cells; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium.
NGF INDUCES PGE\textsubscript{2} FROM MAST CELLS

Materials and Methods

**PMC source and activation**

Lewis rats (Harlan Sprague-Dawley, Indianapolis, IN) were housed in the central animal facility at McMaster University in filter-hooded cages and allowed food and water ad libitum. All experimental procedures were approved by the Animal Research Ethics Board of McMaster University. These animals were used as a source of PMC which were obtained by lavage with a 0.1% solution of BSA in PBS. PMC were purified on a 30%/80% discontinuous Percoll gradient (Pharmacia, Uppsala, Sweden) to >95% purity. The predominant contaminating cells were neutrophils. Macrophage contamination was not observed in any experiment. One million cells/ml were resuspended in RPMI 1640 without phenol red (Life Technologies, Grand Island, NY) supplemented with 5% FCS (v/v), penicillin (50 U/ml), streptomycin (50 µg/ml) 1.4 mM CaCl\textsubscript{2}, and 100 µg/ml soybean trypsin inhibitor (Sigma Chemical, St. Louis, MO). PMC were treated with NGF (Boehringer Mannheim, Indianapolis, IN) at a range of concentrations, LPS (Escherichia coli serotype 055:B5; Sigma) or with calcium ionophore A23187 as positive control for degranulation. In some experiments examining immediate histamine release, the cells were initially activated for 10 min at 37°C with NGF or other agents, centrifuged at 150 × g for 15 min, and then resuspended in supplemented RPMI (as above) including activating agents. Other inhibitors of cyclooxygenase or cytokine expression such as flurbiprofen (Sigma), indomethacin (Sigma), or disodium cromoglycate (DSCG) (Sigma) were added to cells concurrent to the addition of NGF unless otherwise stated.

**Mediator release**

Histamine release by PMC was assessed by a fluorometric method as previously described (39). Briefly, after 10 min of treatment with potential secretagogues (or controls) PMC were centrifuged at 150 × g for 10 min at 4°C, and supernatants were removed. The cell pellet was resuspended in the original volume to give a cell concentration of 1 × 10\textsuperscript{6}/ml. Samples of pellet and supernatant were diluted 1:50 (v/v) in HEPES-Tyrode’s buffer and boiled for 5 min to inactivate histaminase. The buffer composition (mM) was NaCl 137, glucose 5.6, KCl 2.7, Na\textsubscript{2}PO\textsubscript{4} 0.4, CaCl\textsubscript{2} 1, HEPES 10, supplemented with BSA (0.1%), pH 7.3. After precipitation of proteins with TCA (25%), fluorescent products from the o-phthalaldehyde reaction were measured by using a Shimadzu (Columbia, MD) CR15 fluorescence spectrometer. Histamine release was expressed as the percentage of the total histamine content (histamine in supernatant/histamine in supernatant + histamine in pellet) × 100). Histamine release was also measured in supernatants and cell pellets after 24 h in some experiments. PGE\textsubscript{2} release by both mouse bone marrow-derived cultured mast cells (mBMMC), and rat PMC was measured by ELISA assay of snap-frozen (unboiled) cell supernatants taken at time points from 10 min to 24 h after treatment of the cells with 5 ng/ml NGF or other agents using a commercial kit according to manufacturer’s instructions (Biotrak ELISA, Amersham, Cambridge, U.K.). All experiments were performed in the presence of lysophosphatidylserine (10 µg/ml) unless otherwise stated. None of the cell-activating agents had any significant effect on the detection of PGE\textsubscript{2} by this assay method.

**B9 bioassay**

The bioactivity of IL-6 was measured by a previously described B9 hybridoma proliferation assay (40). Briefly, B9 cells were cultured in MEM-F11 (Life Technologies), supplemented with 5% FCS, 2-ME (5 × 10\textsuperscript{−7} M), and a supernatant source of IL-6. The IL-6 assay was performed in triplicate for each sample or standard in microtitrator plates (Nunc, Roskilde, Denmark). B9 cells (initially 2500/well) were cultured in the described medium in either the presence or the absence of putative IL-6-containing samples. After a 72-h culture of cells with samples or standard, 10 µl/well of 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium (MTT) (Sigma) was added, and the plates were incubated for 4 h at 37°C. Fifty µl/well 10% Triton-HCl were then added, and a further 18 h of incubation at room temperature in the dark was conducted. The colored reaction product was assayed at 570 nm by an ELISA reader. Supernatant samples from mast cells were stored at −20°C before analysis. The samples were compared with a standard IL-6 supernatant prepared from human fibroblasts which had been standardized by comparison with recombinant human IL-6 at several dilutions to assess IL-6 content. Results are expressed as units of IL-6 per ml on the basis that 1 U is equivalent to the amount of sample required to induce a half-maximal response. One unit is equivalent to ~0.45 pg of IL-6 according to the National Biological Standard Board. None of the other known mast cell-derived cytokines have been shown to induce proliferation of this cell line under similar assay conditions. IL-6 standard curves were run in the presence of NGF, A23187, flurbiprofen, indomethacin, and DSCG at the highest concentrations used in this study.

None of these agents significantly changed the response of the B9 cells to standard IL-6 preparations.

**TNF-α bioactivity assay**

TNF-α in cell-free supernatants was measured by a cytotoxicity bioassay with the use of the TNF-α-sensitive L929 mouse fibroblast cell line, using a modification of the method previously described (41, 42). Briefly, 50 µl/well 1 × 10\textsuperscript{4} L929 cells/ml in RPMI medium supplemented with 5% FCS and 1% penicillin/streptomycin solution (Life Technologies) were plated onto 96-well flat bottom plates (NuncIon) and incubated for 16–20 h at 37°C. Medium was discarded by suction and replaced with fresh medium containing 20 µg/ml cycloheximide and 100 µg/ml soybean trypsin inhibitor. Recombinant mouse TNF-α (Genzyme, Cambridge, MA) was diluted in the same medium and used as a standard. Seven 10-fold serial dilutions starting from 20,000 pg/ml were used to establish the standard curve. Sample (50 µl/well) or standard (50 µl/well) was then added. The plates were then incubated at 37°C for 18 h before assessment of cell number. MTT (10 µl/well; 5 mg/ml) was added to the plate, and a further 4 h of incubation was conducted. Then 50 µl of a solution of 50% N,N-dimethylformamide (Caledon Laboratories, Edmonton, Canada) and 20% SDS (Bio-Rad, Mississauga, Canada), pH 7.4, were used to dissolve the MTT. After an 18-h incubation at 37°C, the plates were read at 570 nm. Results were read off the standard curve to obtain the concentration of TNF-α present in the samples. Multiple samples and supernatants from unactivated cells served as negative controls. The addition of NGF, LPS, prostanoids, indomethacin, or flurbiprofen or DSCG, at the range of concentrations used in this study did not significantly alter the standard TNF-α curve.

**mBMMC culture and treatment**

mBMMC were cultured from bone marrow cells flushed from the femurs of male C57BL/6 mice. Cells were cultured in RPMI medium supplemented with 10% FCS, 1% penicillin/streptomycin solution (Life Technologies), 1% HEPES solution (Life Technologies) (supplemented RPMI), and the further addition of WEHI-3B supernatant as a source of IL-3 (10% v/v of a 10× concentrate). Cells were used after 7 wk of culture and had a purity of >95% as assessed by metachromatic staining of cytosome centrifuged preparations using toluidine blue. Washed cells were incubated at a concentration of 2 × 10\textsuperscript{4}/ml and resuspended in supplemented RPMI as above with the further addition of 100 µg/ml of soybean trypsin inhibitor (Sigma).

**Statistical analysis**

IL-6 and TNF-α data were compared by the nonparametric Wilcoxon signed rank test, in view of the data distribution. PGE\textsubscript{2} and histamine responses were compared using a paired Student t test for data derived from PMC from individual rats, and an unpaired test was used for mBMMC experiments.

**Results**

Initial experiments examined the ability of NGF to modify the production of the inflammatory cytokines IL-6 and TNF-α by rat PMC. We observed that NGF in the presence of lysophosphatidylserine induced a dose-dependent increase in IL-6 production over that of cells cultured in medium alone (Fig. 1A). This increase was significant (p < 0.05) at a concentration of NGF of 10 ng/ml but was more substantial at higher NGF concentrations. A concurrent decrease in spontaneous TNF-α production by PMC was observed in NGF plus lysophosphatidylserine-treated cells (Fig. 1B).

In this case, however, the NGF-induced inhibition of TNF-α release was maximal and highly (p < 0.001) significant at an NGF concentration of 1 ng/ml.

Under these culture conditions, NGF induced little mast cell degranulation as assessed by histamine release after 10 min (mean spontaneous release, 5 ± 2%; mean release in response to 100 ng/ml NGF, 11 ± 4%; n = 8/group). NGF in the presence of lysophosphatidylserine did not induce detectable production of IFN-γ from PMC under similar conditions (data not shown). Treatment of rat PMC with NGF in the absence of lysophosphatidylserine also resulted in a significant (p < 0.05) increase in the production of IL-6 but only when very high concentrations of NGF (>100 ng/ml) were used (control, 257 ± 201; 1000 ng/ml NGF;
A concentration of 1 × 10^6/ml were incubated for 24 h with supplemented RPMI containing additional protease inhibitor alone (media) or with the further addition of a range of concentrations of NGF with 10 μg/ml of lysophosphatidylserine as a potential cofactor for NGF action. A significant increase in IL-6 expression was observed at 10 and 100 ng/ml concentrations of NGF (A). A significant decrease in TNF-α was observed at the highest four concentrations of NGF used. n = 12 per group for media and for 1, 10, and 100 ng/ml NGF conditions, n = 4 per group for 0.1 and 0.01 ng/ml NGF conditions (B). *, p < 0.05, ***, p < 0.001 Wilcoxon signed rank test. Values are mean ± SEM.

636 ± 271 U/ml IL-6). No significant alterations in TNF-α were observed following NGF treatment in the absence of lysophosphatidylserine. Treatment of mast cells with lysophosphatidylserine alone had no effect on TNF-α or IL-6 production.

In view of our previous observations of enhancement of IL-6 expression and reduction of TNF-α expression following PGE_2 treatment of PMC, we considered the possibility that a prostanoid intermediate might be responsible for the changes in cytokine expression. We therefore examined the ability of NGF to induce PGE_2 production by PMC during the 18-h time course used for the cytokine experiments (Fig. 2). We observed a significant induction of PGE_2 production by PMC as early as 30 min after NGF treatment. A large, dose-dependent PGE_2 response was observed after 18 h of incubation with significant PGE_2 induced by as little as 1 ng/ml of NGF. LPS, which we have shown to be capable of inducing both TNF-α and IL-6 expression by rat PMC (29, 42), did not induce PGE_2 production.

Although our rat PMC preparations were highly purified (mean purity, 98.3%), we considered the possibility that contaminating cells from the peritoneal cavity could be responsible for the observed PGE_2 response. No significant correlation was observed between the number of contaminating cells observed in each preparation and the amount of PGE_2 production induced by NGF. In four PMC preparations, purified to homogeneity for mast cells, a mean of 225 ± 32 pg/ml of PGE_2 was induced by treatment of cells with 100 ng/ml NGF following 18 h of incubation.

To confirm that prostanoid expression induced by NGF was responsible for the observed changes in cytokine expression in rat PMC, we examined the ability of two cyclooxygenase inhibitors, indomethacin and flurbiprofen, to modify both the production of PGE_2 and the alterations in cytokine expression by NGF-treated mast cells (Table I). We observed that treatment with both agents, at doses we have previously demonstrated to be nontoxic to the cells and which do not alter the degree of histamine release, significantly abrogated both the NGF-induced enhancement of IL-6 production and reduction of TNF-α expression. Indomethacin but not flurbiprofen significantly enhanced TNF-α production over

### Table I. Cytokine production by PMC in response to NGF: examination of the effects of DSCG and Indomethacin

<table>
<thead>
<tr>
<th>Stimulus</th>
<th>IL-6 (U/ml)</th>
<th>TNF-α (pg/ml)</th>
<th>PGE_2 (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Media</td>
<td>1408 ± 192</td>
<td>367 ± 67</td>
<td>32 ± 5</td>
</tr>
<tr>
<td>NGF alone (100 ng/ml)</td>
<td>2635 ± 393*</td>
<td>183 ± 91*</td>
<td>186 ± 36</td>
</tr>
<tr>
<td>Boiled NGF (100 ng/ml)</td>
<td>1656 ± 557</td>
<td>361 ± 151</td>
<td>34 ± 12</td>
</tr>
<tr>
<td>NGF (100 ng/ml) + DSCG (10^-6 M)</td>
<td>2514 ± 492*</td>
<td>482 ± 263</td>
<td>147 ± 42</td>
</tr>
<tr>
<td>NGF (100 ng/ml) + indomethacin (10^-6 M)</td>
<td>1188 ± 104</td>
<td>971 ± 251*</td>
<td>17 ± 6</td>
</tr>
<tr>
<td>NGF (100 ng/ml) + flurbiprofen (10^-6 M)</td>
<td>1223 ± 241</td>
<td>523 ± 109</td>
<td>37 ± 6</td>
</tr>
<tr>
<td>NGF (100 ng/ml) + flurbiprofen (10^-6 M) + PGE_2 (10^-6 M)</td>
<td>2945 ± 254</td>
<td>153 ± 87</td>
<td>ND</td>
</tr>
</tbody>
</table>

* *Mean ± SEM; n = 4/group except media group (n = 8).
* *. Significant (p < 0.05) difference between test group and media controls.
media control values. When cells were treated with PGE₂ concurrently with NGF and flurbiprofen, IL-6 expression was again enhanced, and TNF-α production was inhibited (Table I). In contrast, use of the mast cell-stabilizing agent DSCG in the presence of NGF had no effect on the expression of these cytokines. To exclude the possibility that very low/undetectable levels of endotoxin, which might remain in the NGF preparations, were responsible for the observed effects on cytokine expression, we examined the effects of treating PMC for 18 h with boiled NGF. No significant induction of IL-6, inhibition of TNF-α expression, or PGE₂ induction was observed by boiled NGF at a concentration of 100 ng/ml in the presence of lysophosphatidylserine.

Our previous studies have demonstrated that LPS and PGE₂, when used in combination, induce a synergistic IL-6 response from PMC. Other prostanoids such as PGE₁ do not exhibit such synergistic effects. We predicted, therefore, that if NGF was inducing IL-6 expression by PMC via PGE₂ or a similar intermediate, it should show a synergistic response when combined with LPS treatment. Our experiments (Fig. 3) confirmed that this was the case and also demonstrated that PGE₂ had only an additive effect on IL-6 production when used in combination with NGF.

To examine the effects of NGF in the context of a degranulating stimulus, we treated PMC in the presence of lysophosphatidylserine with substance P at either of two doses (10⁻⁵ or 10⁻⁸ M) in combination with NGF, 100 ng/ml. At a concentration of 10⁻⁵ M, substance P treatment alone induced >30% degranulation; concurrent treatment with NGF had no significant effect on this short term histamine release response. The higher dose of substance P alone also induced a significant TNF-α response at 18 h. This response was completely abrogated by the addition of NGF. In contrast, substance P did not induce a significant IL-6 response, and the response to NGF treatment appeared unchanged in the presence of substance P at either of the doses used (Table II).

There are many examples of heterogeneity in mast cell responses to activating agents. Unlike rat PMC, mBMMC have not been demonstrated to degranulate in response to NGF. In contrast to rat PMC, they do not produce PGD₂ following NGF treatment. In additional experiments, we therefore examined the ability of cultured mouse bone marrow mast cells to produce PGE₂ in response to NGF or LPS stimulation (Fig. 4). We observed that mBMMC also produced substantial amounts of PGE₂ in response to NGF treatment over 24 h. As was observed with the rat PMC preparations, NGF (100 ng/ml) induced a substantial PGE₂ response. This response was significant as early as 1 h after activation and was approximately fourfold background levels. LPS did not induce any PGE₂ response in mBMMC at 1 h, and the trend toward a small PGE₂ response at 24 h was not statistically significant. Examination of the effects of NGF treatment on cytokine production by mBMMC revealed that NGF treatment at doses up to 1000 ng/ml did not enhance IL-6 production (data not shown). Separate studies of the effects of PGE₂ treatment have revealed that much higher levels of PGE₂ (10⁻⁷ M) than those induced by NGF treatment are required to significantly elevate IL-6 production in this cell type (43). We were unable to assess the ability of NGF to inhibit endogenous TNF-α production since the levels of TNF-α produced by unstimulated mBMMC were very low (<2 pg/ml) and below the limit of detection of our assay system.

Discussion

NGF has been described to have a number of potentially proinflammatory effects on mast cell and basophil development and function. In the current study, we demonstrate that NGF can also induce mast cells to produce significant amounts of PGE₂, which is considered to have predominantly antiinflammatory effects at mucosal sites, and provide evidence that such prostanoid production is responsible for the autocrine modulation of mast cell IL-6 and TNF-α production.

We have previously reported that PGE₁, PGE₂, and cholera toxin will induce IL-6 release and inhibit TNF-α production (30, 44). Our initial observations of dose-dependent, NGF-induced, regulation of IL-6 and TNF-α were very similar to these cyclic AMP-dependent effects. However, NGF-dependent signaling is not thought to be predominantly cyclic AMP mediated (26, 45). Therefore, we considered the possibility that a prostanoid, such as

Table II. Effect of NGF in combination with substance P on mediator production by rat peritoneal mast cells

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Histamine* (µg/ml)</th>
<th>TNF-α (pg/ml)</th>
<th>IL-6 (U/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Media</td>
<td>0</td>
<td>142 ± 58</td>
<td>955 ± 545</td>
</tr>
<tr>
<td>Substance P alone</td>
<td>10⁻⁵ M</td>
<td>34.4 ± 3.4*</td>
<td>232 ± 27*</td>
</tr>
<tr>
<td>Substance P alone</td>
<td>10⁻⁸ M</td>
<td>0.8 ± 0.6</td>
<td>163 ± 25</td>
</tr>
<tr>
<td>NGF alone</td>
<td>100 ng/ml</td>
<td>4.1 ± 1.0</td>
<td>81 ± 34*</td>
</tr>
<tr>
<td>NGF + substance P</td>
<td>10⁻⁵ M</td>
<td>36.7 ± 3.8*</td>
<td>77 ± 27*</td>
</tr>
<tr>
<td>NGF + substance P</td>
<td>10⁻⁸ M</td>
<td>2.1 ± 1.1</td>
<td>71 ± 17*</td>
</tr>
</tbody>
</table>

* Histamine release measured at 10 min. cytokine production after 18 h of incubation. All experiments performed in the presence of 10 µg/ml lysophosphatidylserine. Spontaneous histamine release (<5% in all experiments) subtracted from values shown. Mean values ± SEM. n = 4/group. *, significant (p < 0.05) increase compared with media control; **, significant (p < 0.01) decrease compared with media control.
PGE₂, might be induced by NGF and regulate mast cell cytokine expression via an autocrine mechanism.

In rodents, low concentrations of NGF have been demonstrated to induce mast cell degranulation, an effect that is dependent on the presence of a phospholipid cofactor such as phosphatidylserine or lyso phosphatidylserine (20). In the current study, we observed no significant induction of histamine release from mast cells, except at very high NGF concentrations. We believe this difference in response to that previously reported may be due to the high concentrations of cells used (1 × 10⁶ cells/ml as compared with 2 × 10⁶/ml for previous histamine release studies (21, 22)) or the presence of protease inhibitors within the media. Under alternate conditions, with lower cell concentrations, the batches of NGF used for these studies were active as degranulating agents for mast cells in the presence of lyso phosphatidylserine (data not shown). Possible explanations for the apparent inhibition of degranulation at higher cell concentrations include a cell contact-mediated inhibitory mechanism or reduced NGF receptor occupancy on a per cell basis.

In this and other similar studies, we have noted that freshly isolated rat PMC appear to spontaneously produce TNF-α within our culture system despite the use of strict endotoxin-free conditions. We do not know whether this reflects activation of mast cells during the purification process or whether mast cells in vivo will spontaneously produce this cytokine. Studies of normal rat peritoneal lavage fluid demonstrate that low but detectable levels of TNF-α are present in the absence of exogenous stimuli (data not shown). However, it is not known whether the source of such TNF-α is the mast cell or other cells such as macrophages found at this site in rodents.

The time course of PGE₂ induction in this study had some similarities to the induction of PGD₂ by NGF treatment of PMC reported by Murakami et al. (26). Most notably, the major PGE₂ response was observed at later time points 18–24 h after treatment. However, the initiation of detectable PGE₂ induction was delayed relative to the reported induction of PGD₂ (26), with a response first detectable, with higher doses of NGF, 30 min postactivation. In contrast, the early PGD₂ response to NGF was reported to be maximal by 10 min postactivation. These observations are in keeping with earlier studies which suggest that PMC are not a good source of PGE₂ following short term activation with Ag or ionophore (36). It is likely that the major PGE₂ response is dependent on the induction of new enzymes such as the cyclooxygenase-2 which has been shown to be induced following NGF activation of PMC (26) and enhanced by interaction of mast cells with fibroblasts (46). However, the doses of NGF required to induce substantial changes in mast cell cytokine production are, in several cases, much lower than those reported to induce PGD₂ by rat PMC (26). mMBCM produced a substantial amount of PGE₂ following NGF activation despite a reported lack of a detectable PGD₂ response to NGF in these cells.

LPS activation of neither the PMC nor the mMBCM induced significant levels of PGE₂(α), although such induction has previously been noted in other cell types. The undetectable levels of endotoxin in the NGF that we used for these studies and the absence of an effect once the NGF was denatured by boiling provide firm evidence that LPS contamination is not the cause of the observed PGE₂ response.

It is unlikely that PGD₂ or other prostanoids are responsible for the observed changes in IL-6 and TNF-α by NGF-treated mast cells. We have previously demonstrated that PGD₂, at concentrations up to 1 μM, has no effect on the production of either of these cytokines in unstimulated or activated PMC (30). In previous studies, PGE₂ has been demonstrated to have a unique ability, compared with other prostanoids, to synergistically induce IL-6 by LPS-activated PMC (30). NGF treatment of the PMC was shown to have a similar effect in the current study. The ability of PGE₂, in the presence of NGF and flurbiprofen treatment, to enhance IL-6 and inhibit TNF-α production further supports the concept that PGE₂ is the mediator responsible for NGF-induced alterations in cytokine expression.

NGF induction of PGE₂ might provide a mechanism for some of the observations of NGF enhancement of mast cell growth. Studies by Hu et al. (31) have demonstrated that PGE₂ enhances the development of mMBCM, a phenomenon that can be demonstrated only when low endotoxin culture conditions are used to minimize endogenous prostanoid production. NGF has been shown by some groups (47) but not others (48) to enhance mast cell growth and differentiation in similar systems. However, the potential for an important role for PGE₂ as an intermediate in this process has not yet been addressed experimentally.

The ability of NGF to modulate mast cell cytokine expression via PGE₂ induction has some important implications. Under conditions of neuronal damage and repair, the long term mast cell PGE₂ response to NGF could provide a mechanism by which local harmful cytokine responses might be limited. An antiinflammatory role for NGF has been suggested by previous studies (49), and our data demonstrate that NGF can inhibit mast cell TNF-α production even in the context of the degranulatory stimulus of high doses of substance P. TNF-α has been demonstrated to be toxic to neuronal tissues (16, 17) while, in contrast, IL-6 has been demonstrated to have the ability to promote neurite outgrowth from the PC-12 cell line (14, 50). Therefore, the overall effect of NGF, through effects on mast cells, may be to limit neuronal damage and enhance repair mechanisms. In body sites such as the intestine, where close association between mast cells and nerves is well documented (1, 2), PGE₂ is known to have an antiinflammatory and cytoprotective effect. NGF induction of PGE₂ production by mast cells might contribute to such a generalized antiinflammatory effect on the tissue. The ability of other NGF receptor-bearing cells to produce PGE₂ in response to NGF remains to be determined but could have important implications within the central nervous system or at other sites following nerve damage.

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References


