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Short communication

Release of nerve growth factor by human pulmonary epithelial cells: role in airway inflammatory diseases

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Abstract

Elevated levels of nerve growth factor (NGF) have been detected in the bronchoalveolar lavage fluid of patients with asthma. However, the source of this enhanced mediator production is not known. Here, we investigate the production of NGF from a human airway epithelial cell line (A549). Under basal conditions, A549 cells generated NGF in a time-dependent fashion. However, basal release was significantly augmented in a concentration-dependent manner in cells treated with interleukin-1β (IL-1β) or tumour necrosis factor-α (TNF-α) and inhibited by dexamethasone. These data suggest that NGF released from structural cells may be an important target for the anti-inflammatory effects of steroids in asthma therapy.

Keywords: Airway epithelium; Neurotrophin; Asthma

1. Introduction

Autonomic nerves, and in particular, sensory nerves, regulate many aspects of airway function including smooth muscle tone, blood flow, airway secretions, microvascular leakage and the release of mediators from inflammatory cells. There is now abundant evidence to suggest that neural control of the airway may be abnormal in inflammatory lung diseases such as asthma and that neurogenic mechanisms may contribute to the pathophysiology and/or the symptomatology of asthma (Barnes et al., 1991).

Nerve growth factor (NGF) is one of a family of neurotrophins essential for the maintenance and growth of sensory and sympathetic neurones, and some central neurones (Lewin and Barde, 1996). Under physiological conditions, neurotrophins are produced by nerve-associated cells like glia or Schwann cells and by nerves themselves (Lindholm et al., 1987; Meyer et al., 1992). It is now recognised that NGF may also play an important role in the inflammatory process and it has been demonstrated that fibroblasts (Hattori et al., 1993), mast cells (Leon et al., 1994) macrophages and T and B lymphocytes (Sant'Ambrogio et al., 1994) can also produce and release NGF. In a recent report, high serum levels of NGF were detected in patients with severe allergic bronchial asthma (Bonini et al., 1996) and another study has demonstrated the local upregulation of neurotrophin production in bronchoalveolar lavage fluid from allergic subjects following allergen provocation (Virchow et al., 1998). However, the source of this increased NGF is not known.

The airway epithelium acts as a physical barrier between the environment and the delicate structures of the lung. However, it is increasingly recognised that the epithelium is a rich source of lipid and peptide mediators that may modulate airway inflammation. In this study, we have investigated whether an airway epithelial cell line (A549) cell releases NGF and whether this can be modulated by pro-inflammatory cytokines and corticosteroids.

2. Materials and methods

The human pulmonary epithelial cancer cell line (A549), derived from lung alveolar adenocarcinoma and representative of airway epithelial cells, was purchased from American Type Culture Collection (Rockville, MD). In previous studies comparing A549 with primary human airway ep-

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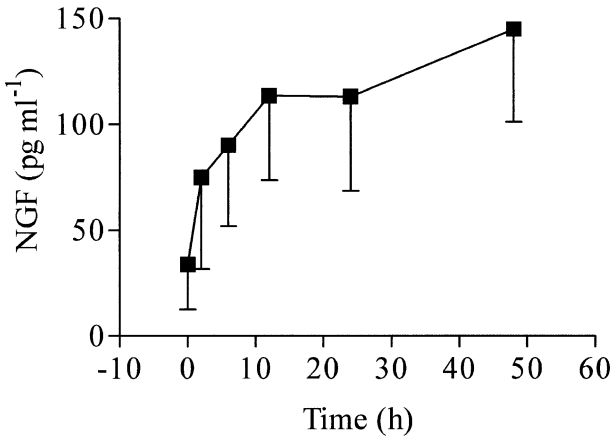

Fig. 1. The time-dependent release of nerve growth factor (NGF) from the A549 human epithelial cell line. Results are shown as the mean ± S.E.M. of four separate determinations.

ithelial cells, both cell types have been shown to exhibit similar characteristics in culture in terms of the secretion of inflammatory mediators (Kwon et al., 1994; Mitchell et al., 1994). A549 cells were grown on 35-mm 6-well culture plates and cultured as previously outlined (Mitchell et al., 1994). Cells were deprived of serum 24 h prior to the start of all the experiments. Following 24-h incubation in serum-free media, media was replaced together with test compounds and the time-dependent basal release of NGF was determined in the supernatant by a specific enzyme-linked immunosorbent assay (ELISA). In separate experiments, cells were treated with interleukin-1β (IL-1β, 0.1–100 ng ml^{-1}), tumour necrosis factor-α (TNF-α, 0.1–100 ng ml^{-1}) or vehicle (phosphate-buffered saline, PBS) and NGF levels in the supernatant were determined 24 h later. The effect of corticosteroids on NGF release was examined by addition of dexamethasone (1 μM) 30 min prior to the addition of interlukin-1β or TNF-α (10 ng ml^{-1}) or vehicle. We have previously found that dexamethasone, at this concentration and with this pre-incubation time, has an inhibitory action on mediator release from this cell type (Mitchell et al., 1994). Cell viability, assessed by mitochondrial-dependent reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide to formazan, was not affected by any of the agents added.

2.1. Drugs, chemicals and analytical reagents

NGF was determined in the supernatant by a specific ELISA (Promega, Madison, WI, USA). IL-1β and TNF-α were obtained from R&D Systems Europe (Abingdon, Oxfordshire, UK). All other materials were purchased from Sigma (Poole, Dorset, UK).

2.2. Statistical analysis

Results are shown as mean ± S.E.M. of *n* separate experiments. Where appropriate, data were analysed by Kruskal–Wallis non-parametric analysis of variance followed by the Dunn's multiple comparison test. All treatments were compared with control values and $P < 0.05$ was considered to be significant.

3. Results

Under basal conditions, A549 cells generated NGF in a time-dependent fashion (Fig. 1). However, basal NGF release (133.15 ± 26.1 pg ml^{-1}) was significantly augmented in a concentration-dependent manner in cells treated with IL-1β or TNF-α for 24 h (Fig. 2), with NGF levels of

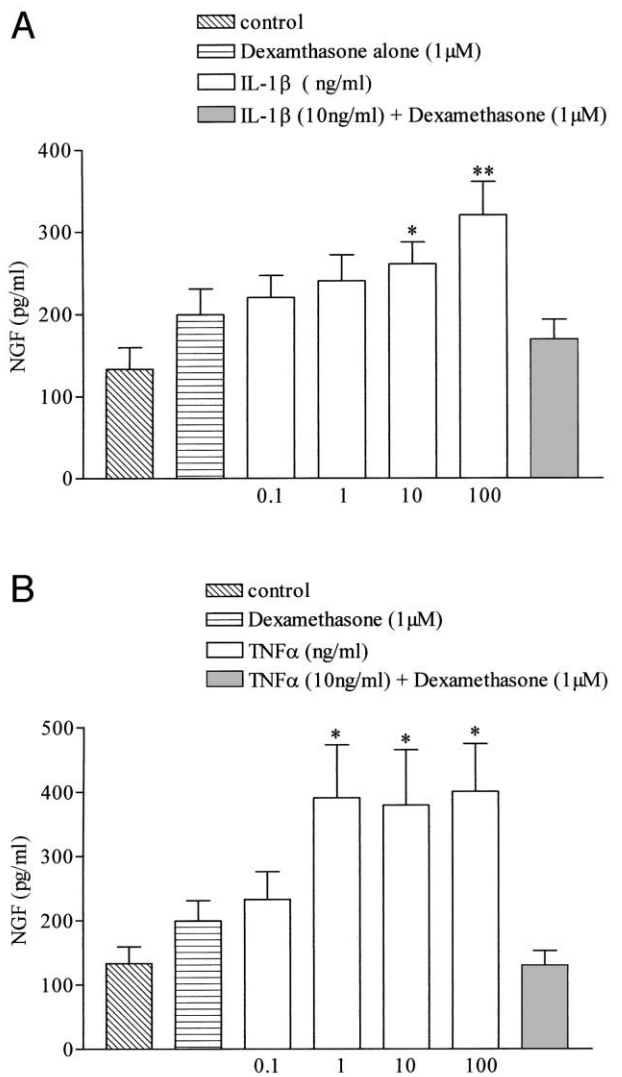

Fig. 2. The release of nerve growth factor (NGF) from the A549 human epithelial cell line in response to interleukin-1β (IL-1β) (A) and tumour necrosis factor-α (TNF-α) (B) for 24 h. Results are shown as the mean ± S.E.M. of eight separate determinations. Furthermore, the effect of dexamethasone (1 μM), pre-treatment (30 min prior to the cytokine) on NGF release from A549 cells evoked by each cytokine [IL-1β (A) and TNF-α (B), each at 10 ng ml^{-1} for 24 h] is described. Treatment groups were compared by the Kruskal–Wallis test followed by the Dunn's multiple comparison test ($^{*}P < 0.05$, $^{**}P < 0.01$).

260.8 ± 26.5 pg ml^{-1} and 379.0 ± 85.9 pg ml^{-1}, detected in supernatant following incubation with 10 ng ml-1 IL-1β and TNF-α, respectively. Dexamethasone (1 μM) alone did not affect the basal release of NGF (199.4 ± 31.7 pg ml^{-1}). However, the release of NGF elicited in the presence of the cytokines IL-1β and TNF-α (each at 10 ng ml^{-1}) was inhibited by pre-treatment with dexamethasone (72% and 100% inhibition, respectively, Fig. 2A and B).

4. Discussion

In addition to its well-known effects on neuronal survival and phenotype, considerable evidence suggests that NGF may also affect immune cell activity. Thus, as well as being released from a number of different inflammatory cell types including mast cells, macrophages and lymphocytes, it has been shown to promote inflammatory mediator release from basophils (Burgi et al., 1996), mast cells (Tal and Liberman, 1997), T- and B- lymphocyte (Lambiase et al., 1997; Sant'Ambrogio et al., 1994) cells, and macrophages (Susaki et al., 1996). Here, we show that airway epithelial cells may also release NGF and that this release is enhanced under inflammatory conditions. This data is consistent with previous reports that have shown an increase in both NGF mRNA and peptide levels in fibroblasts following treatment with TNF-α and in antigen-stimulated T lymphocytes (Hattori et al., 1993; Sant'Ambrogio et al., 1994).

The function of NGF released from the airway epithelium is not clear, although evidence suggests that it may contribute to the inflammation and hyperresponsiveness associated with asthmatic disease via effects on inflammatory cells, as described above, but also by affecting sensory nerve function. For example, neurotrophins are able to increase tachykinin expression in airway sensory neurons in the guinea-pig (Hunter et al., 2000), and NGF has been shown to induce a neurokinin-1 receptor-mediated airway hyperresponsiveness in guinea-pigs (De Vries et al., 1999). Such an increase in tachykinin expression in the airways in response to neurotrophins would support the evidence indicating a role for tachykinins in the development of airways hyperrresponsiveness (Kraneveld et al., 1997).

If neurotrophins do in fact alter the function of airway sensory nerves under inflammatory conditions, then the epithelium would be an attractive candidate as a source of the growth factors as they lie in close proximity to the nerve endings. In this study, we have demonstrated for the first time that airway epithelial cells can produce NGF and that this can be increased under pro-inflammatory conditions. Moreover, this enhanced release may be inhibited completely by glucocorticoids. These data suggest that the release of this growth factor from these structural cells may be an important target for the anti-inflammatory effects of steroids in asthma therapy.

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