Role of the endogenous prostaglandin E2 in human lung fibroblast interleukin-11 production

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Interleukin-11 (IL-11) is known to be a member of the interleukin-6 (IL-6)-type cytokine family. IL-11 is likely to be a major determinant of immune regulation in acute and chronic inflammatory lung diseases, although it is not directly linked with specific disease processes. It has already been shown that unstimulated human lung fibroblasts did not produce significant amounts of IL-11, the addition of interleukin-1α (IL-1α) and/or transforming growth factor-β (TGF-β) stimulated fibroblasts dose-dependently to produce IL-11. Northern blot analysis showed that these stimulators also upregulated IL-11 mRNA expression. As it has been previously reported that IL-1 and TGF-β stimulate prostaglandin E2 (PGE2) release from lung fibroblasts, we investigate here the role of endogenous PGE2 and the direct effects of the two inhibitors of prostaglandin synthesis, indomethacin and dexamethasone, on IL-11 production by human lung fibroblasts. The addition of indomethacin, a cyclo-oxygenase inhibitor, resulted in significant suppression of IL-11 production and mRNA expression in lung fibroblasts. There was no detectable effect of PGE2 alone on IL-11 levels; however, the suppression of IL-11 production by indomethacin was almost completely reversed by addition of PGE2. In contrast, suppression of IL-11 production by indomethacin was not reversed by addition of thromboxane B2 and carbocyclic thromboxane A2. In addition, dexamethasone completely suppressed IL-11 production and downregulated IL-11 mRNA. These results suggest that endogenous PGE2 acts as an autocrine stimulus for IL-11 production by human lung fibroblasts activated by IL-1α and TGF-β.

Introduction

Macrophage/monocyte-derived cytokines, including interleukin-1 (IL-1), tumour necrosis factor (TNF) and transforming growth factor-β (TGF-β), have been shown to be important contributors to the inflammatory and fibrotic processes of the lung (1,2). Recent data have suggested that the local stromal cell population, such as lung fibroblasts, is also a significant contributor to the local cytokine pool which causes inflammatory and fibrotic responses in airway disorders (3). Interleukin-6 (IL-6), interleukin-8 (IL-8), granulocyte macrophage colony stimulating factor (GM-CSF) and granulocyte-CSF (G-CSF) are all major products of lung fibroblasts and are found in various inflamed tissues (3–5).

Interleukin-11 (IL-11) was originally identified from the primate bone marrow-derived stromal cell line, PU-34, based on its ability to stimulate the proliferation of IL-6-dependent cells (6). In accordance with this finding, most studies on IL-11 have focused on its roles in haematopoiesis (7–9). However, IL-11 has other bioactivities such as the induction of acute phase protein production (10), B-cell activation (11) and augmentation of the production of metalloproteinase inhibitor (12). Recent studies have also demonstrated that IL-11 has protective effects in a number of animal models of acute and chronic inflammation or injury (13–15). This protection is due, at least in part, to the ability of IL-11 to regulate the production of pro-inflammatory cytokines and nitric oxide by macrophages (13, 15). Since lung stromal cells have the ability to produce IL-11 in response to various stimuli (16, 17), IL-11 may have an important role in the modulation of inflammatory and fibrotic responses in human lung disorders.

The products of the cyclo-oxygenase pathway, such as prostaglandin E2 (PGE2), also participate in inflammatory responses (18). An inhibitory effect of PGE2 on immune cell function has been documented. PGE2 can inhibit the release of IL-1 and TNF from myeloid cells (19). In addition, Hamilton et al. showed that fibroblast-derived colony stimulating factors were modulated by endogenous and exogenous prostanooids (20). Therefore, a link between PGE2 and stromal cell cytokine production appears to be
effects of the two inhibitors of prostaglandin synthesis, indomethacin and dexamethasone, on IL-11 production by human lung fibroblasts has not been clarified. Thus, in this study, we investigated the role of endogenous PGE2 and the direct effects of the two inhibitors of prostaglandin synthesis, indomethacin and dexamethasone, on IL-11 production by human lung fibroblasts.

Materials and Methods

Materials

Human recombinant IL-1α was kindly provided by Dainippon Pharmaceutical Co. (Osaka, Japan). Recombinant TGF-β1 was purchased from R&D Systems (Minneapolis, MN, U.S.A.). PGE2, thromboxane B2 and carbocyclic thromboxane A2 were purchased from Cayman Chemical Co. (Ann Arbor, MI, U.S.A.). Indomethacin and dexamethasone were purchased from Wako Pure Chemical Industries Ltd. (Osaka, Japan). Dulbecco’s modified Eagle’s medium (DMEM) and phosphate-buffered saline (PBS(-)) were purchased from Nissui Pharmaceutical Co. (Tokyo, Japan).

Cell Preparation

Human lung tissues were obtained from lungs of patients undergoing lobectomy because of lung cancer. The normal part of the lobe was washed with PBS and the pleural tissues and bronchi were removed after resection. Human lung fibroblasts were prepared as previously described (22). Briefly, specimens were minced and enzymatically digested with 0.5-1.0 mg ml⁻¹ clostridium collagenase (Wako Pure Chemical Industries) and 5-10 mg ml⁻¹ deoxyribonuclease 1 (Sigma Chemical Co. St. Louis, MO, U.S.A.) for 2-3 h. After digestion, the single cells were filtered through gauze, washed three times with DMEM and filtered through nylon mesh. The cells were resuspended in complete medium [DMEM supplemented with 10% heat-inactivated fetal calf serum (FCS), 100 U ml⁻¹ penicillin G, 50 mg ml⁻¹ streptomycin, 2 mM L-glutamine and non-essential amino acids (GIBCO BRL, Gland Island, NY, U.S.A.)]. The cells were cultured overnight to adhere to a plastic dish. The dish was washed to remove non-adherent cells. After three to four passages, fibroblasts were used as freshly isolated human lung fibroblasts and grown to confluence in 5% CO₂/95% air in complete medium.

Lung Fibroblast Culture

Lung fibroblasts were treated with trypsin and plated at a density of 2 x 10⁵ cells per well in 24-well flat-bottom plates. To examine whether treatment of freshly isolated human lung fibroblasts with IL-1α and/or TGF-β might modulate their production of IL-11 or PGE2, the cells were incubated with various concentrations of IL-1α and/or TGF-β for the time indicated. After incubation, the cell-free culture supernatants were stored at -20°C until assay for IL-11 or PGE2.

To examine the role of endogenous PGE2 on IL-1α and TGF-β-induced IL-11 production, lung fibroblasts were incubated with IL-1α and TGF-β in the presence and absence of 10⁻⁵ M indomethacin or 10⁻⁶ M dexamethasone for 24 h. After incubation, the cell-free culture supernatants were stored at -20°C until assay for IL-11.

Measurement of IL-11 Protein and Prostaglandin E₂

IL-11 protein was measured with an ELISA assay kit (Quantikine TM human IL-11 ELISA kit; R&D Systems). PGE2 was measured with ELISA assay kit purchased from Neogen TM corporation (Lexington, KY, U.S.A.).

mRNA Isolation and Analysis

Lung fibroblasts were incubated with IL-1α and/or TGF-β for 24 h in the presence and absence of 10⁻² M indomethacin or 10⁻⁶ M dexamethasone. Total cellular RNA in the treated cells was extracted by acid guanidinium thiocyanate-phenol-chloroform extraction method using ISOGEN (Nippon Gene, Tokyo, Japan), and Northern blot analysis was carried out as previously described (22). Fifteen micrograms of total RNA from each experimental condition was size fractionated by electrophoresis through 1% agarose/17% formaldehyde gels, transferred to nylon membranes (Amersham, Tokyo, Japan), and hybridized with digoxigenin-labelled cDNA probes (Boehringer Mannheim, Germany). IL-11 cDNA was a gift of Dr Paul Schendel (Genetics Institute, Cambridge, MA, U.S.A.). The intensity of the band was analysed by National Institute of Health (NIH) Image software.

Statistical Analysis

Values are presented as the mean ± (SEM). Data were analysed by a Student’s t-test. P-values less than 0.05 were considered as statistically significant.

Results

As shown in Fig. 1, lung fibroblasts produced a trace amount of IL-11 without stimuli. IL-1α or TGF-β stimulated lung fibroblasts to produce IL-11 in a dose-dependent manner [Fig. 1(a,b)]. Incubation with both cytokines resulted in higher IL-11 production than with either cytokine alone [Fig. 1(c)].

As shown in Fig. 2, the amounts of IL-11 production in response to IL-1α and TGF-β increased in a time-dependent manner, and reached a maximum at 24 h.

As shown in Fig. 3, unstimulated lung fibroblasts produced a trace amount of PGE2. A low level of PGE2 production was detectable under TGF-β stimulation, while
Prostaglandin E2 and Interleukin-11 Production

Fig. 1. Effect of IL-1α and TGF-β on IL-11 production by freshly isolated human lung fibroblasts. Cells were incubated with various concentrations of IL-1α (a), TGF-β (b) or 1.0 ng ml⁻¹ IL-1α plus 5.0 ng ml⁻¹ TGF-β (c) for 24 h. IL-11 was measured by ELISA in the supernatant. Data are expressed as mean ±SEM of at least three independent experiments. *P < 0.05 vs. the medium control; **P < 0.01 vs. the medium control; #P < 0.005 vs. the medium control; ##P < 0.001 vs. the medium control.

Fig. 2. Time course of IL-1α and TGF-β-induced IL-11 production by freshly isolated human lung fibroblasts. Cells were incubated with 1.0 ng ml⁻¹ IL-1α plus 5.0 ng ml⁻¹ TGF-β for the time indicated, and IL-11 was measured by ELISA in the supernatants. Data are expressed as mean ±SEM of three independent experiments.

Fig. 3. Effect of IL-1α and TGF-β on PGE2 production by freshly isolated human lung fibroblasts. Cells were incubated with 1.0 ng ml⁻¹ IL-1α and/or 5.0 ng ml⁻¹ TGF-β for 24 h. PGE2 was measured by ELISA in the supernatants. Data are expressed as mean ±SEM of four independent experiments. *P < 0.05 vs. the medium control; **P < 0.01 vs. the medium control.

A significant increase in PGE2 level was detectable after IL-1α stimulation. Incubation with both cytokines resulted in higher PGE2 production than with either cytokine alone (Fig. 3).

As shown in Fig. 4, the production of IL-11 was significantly inhibited by indomethacin. In addition, dexamethasone completely inhibited IL-11 production (Fig. 4).

The levels of IL-11 mRNA expressed by unstimulated cells were extremely low, while a significant increase in IL-11 mRNA levels was observed upon stimulation with 1.0 ng ml⁻¹ IL-1α or 5.0 ng ml⁻¹ TGF-β (Fig. 5). Lung fibroblasts incubated with IL-1α and TGF-β in combination contained significantly higher levels of IL-11 mRNA than fibroblasts incubated with the cytokines individually. Furthermore, indomethacin inhibited IL-11 mRNA in

1.0 ng ml⁻¹ IL-1α and 5.0 ng ml⁻¹ TGF-β-stimulated lung fibroblasts (Fig. 5). In addition, dexamethasone suppressed IL-11 mRNA almost completely (Fig. 5).

There were no detectable effects of PGE2 alone (data not shown) however, suppression of IL-11 production by indomethacin was reversed almost completely by addition of exogenous PGE2 (Fig. 6). In contrast, the suppression of IL-11 production by indomethacin was not reversed by addition of thromboxane B₂ and carbocyclic thromboxane
Inhibitory effects of indomethacin or dexamethasone on IL-1α and TGF-β induced IL-11 production by freshly isolated human lung fibroblasts. Cells were incubated with 1.0 ng ml⁻¹ IL-1α and 5.0 ng ml⁻¹ TGF-β in the presence and absence of 10⁻⁵ M indomethacin (IND) or 10⁻⁶ M dexamethasone (DEX) for 24 h. IL-11 was measured by ELISA in the supernatants. Data are expressed as mean ± SEM of three independent experiments. #P<0.005 vs. the IL-1α and TGF-β stimulation; **P<0.001 vs. the IL-1α and TGF-β stimulation.

AZ (data not shown). Furthermore, suppression of IL-11 production by dexamethasone was not reversed by the addition of PGE₂ (data not shown).

Discussion

IL-11 was initially reported as a haematopoietic cytokine with thrombopoietic activity (7). In addition to this haematopoietic effects, however, it has been demonstrated that IL-11 protects a variety of models of tissue from inflammation and injury (13–15). This protection is due to the ability of IL-11 to regulate macrophage production of pro-inflammatory cytokines such as TNF and IL-1. Recently, Leng and Elias reported that IL-11 can also inhibit interleukin-12 (IL-12) production by macrophages (23). Excessive IL-12 production has been implicated in the pathogenesis of a number of diseases including sarcoidosis, sepsis and Sjögren’s syndrome (24–26). Lung stromal cells have the ability to produce IL-11 in response to cytokines, such as IL-1α and TGF-β (16), and infection with some respiratory viruses (17). Thus IL-11 may have an important role in the modulation of inflammatory responses in lung disorders.

In agreement with previous results on lung fibroblast and epithelial cell lines (16,17), we demonstrated that freshly isolated human lung fibroblasts produce immunoreactive IL-11 in response to IL-1α and TGF-β. The stimulatory effect of IL-1α and TGF-β on IL-11 production was detected at the concentrations of 1.0 ng ml⁻¹ and 5.0 ng ml⁻¹, respectively.

Prostaglandin E₂ is a product of the arachidonic acid metabolism cascade and can be synthesized by a wide range of cell types. Lung fibroblasts are also a source of PGE₂ during inflammatory process (27). It has been reported that IL-1α and TGF-β stimulate PGE₂ synthesis in lung fibroblasts (28). Furthermore, PGE₂ is reported to regulate IL-11 production positively in osteoblasts and synovial cells (21, 29). The present study showed that indomethacin blocked IL-1α and TGF-β induction of PGE₂ by human lung fibroblasts. It also suppressed IL-11 production and its gene expression in human lung fibroblasts. The suppression of IL-11 production by indomethacin was almost completely reversed by addition of exogenous PGE₂. In contrast, the suppression of IL-11 production by indomethacin was not reversed by the addition of thromboxane B₂ and carbocyclic thromboxane A₂. Therefore, endogenous PGE₂ appeared to act as an autocrine stimulus for IL-11 production. Our data also showed that dexamethasone inhibited IL-11 production and mRNA expression almost completely in IL-1α and TGF-β stimulated lung fibroblasts. However, the suppression of IL-11 production by dexamethasone was not reversed by the addition of PGE₂. The
mechanisms of inhibition by dexamethasone remain to be clarified. It has been shown that glucocorticoids inhibit the induction of the gene coding for inducible cyclooxygenase (COX-2) in monocytes and epithelial cells (30). However, glucocorticoids may also block COX-2, but also block the synthesis of cytokines in several ways. Glucocorticoids may inhibit the synthesis of cytokine receptors, such as the IL-2 receptor (31). Several cytokines produce their cellular effects by activating transcription factors, such as AP-1 and NF-κB, which activate or repress target genes that are regulated in an opposing manner by glucocorticoid receptor (32). This activation is strongly inhibited by glucocorticoids. As the IL-11 gene contains the AP-I site, which is known to be important for IL-11 transcription, in the 5' flanking lesion (33,34), dexamethasone may inhibit the production of IL-11 via the inhibition of AP-1 activity.

In summary, we have demonstrated that IL-1α and TGF-β stimulated freshly isolated human lung fibroblasts to produce IL-11 in a dose- and time-dependent manner along with increased IL-11 mRNA, and that endogenous PGE2 appeared to act as an autocrine stimulus for IL-1α and TGF-β-induced IL-11 production by lung fibroblasts. The lung is constantly exposed to various environmental stimuli, including micro-organisms and chemical compounds. Alveolar macrophages, mast cells and eosinophils stimulated with external and endogenous stimuli release many cytokines and arachidonic acid metabolites including IL-1, TGF and PGE2. IL-11 production by lung fibroblasts is regulated by these molecules. It is important to examine the IL-11 production and regulation mechanisms in normal human lung tissue in order to clarify the pathogenesis of the inflammation process.

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References

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