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SPECIAL REPORT

Release of granulocyte-macrophage colony stimulating factor by human cultured airway smooth muscle cells: suppression by dexamethasone

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Smooth muscle cells represent a significant percentage of the total cells in the airway but their contribution to the inflammatory response seen in airway disease has not been studied. Hence, we have looked at the release of the cytokine granulocyte-macrophage colony stimulating factor (GM-CSF) in response to bacterial lipopolysaccharide (LPS) and the pro-inflammatory cytokines interleukin-1β (IL-1β), tumour necrosis factor α (TNFα) and interferon γ (IFNγ). Human airway smooth muscle (HASM) cells released GM-CSF under basal conditions (45.4 ± 13.1 pg ml^{-1}) that was significantly enhanced by IL-1β and TNFα with a maximal effect seen at 10 ng ml^{-1} (1.31 ± 0.07 ng ml^{-1} and 0.72 ± 0.16 ng ml^{-1}, respectively). In contrast, neither LPS nor IFNγ produced a significant increase in GM-CSF release. However, HASM cells exposed to IL-1β, TNFα and IFNγ generated more GM-CSF than that evoked by any cytokine alone (2.2 ± 0.15 ng ml^{-1}). The release of GM-CSF elicited by the cytokine mixture was inhibited by cycloheximide and dexamethasone. These data suggest that HASM cells might play an active part in initiating and/or perpetuating airway inflammation in addition to controlling airway calibre.

Keywords: Airway smooth muscle; glucocorticosteroid; cytokines

Introduction Chronic inflammatory diseases of the airway, such as asthma, are characterized by reversible airway obstruction and non-specific airway hyperresponsiveness. The histopathology of such airways shows infiltration of large numbers of inflammatory cells, a marked increase in the smooth muscle layer due to cellular hypertrophy and hyperplasia, and significant desquamation of the epithelium. The loss of epithelial cells exposes the underlying smooth muscle to a plethora of noxious airborne agents and inflammatory cytokines released by both resident and infiltrating inflammatory cells. If human airway smooth muscle (HASM) cells respond to such stimuli they could potentially be a rich source of mediators in the diseased lung and, as such, play a pivotal role in perpetuating airway dysfunction. Indeed, preliminary data suggests that HASM cells could participate in the inflammatory response as evinced from their ability to express the inflammatory form of the enzyme cyclo-oxygenase-2 (COX-2) (Saunders *et al*., 1996) and release both interleukin-8 (IL-8) (Watson *et al*., 1996) and RANTES (John *et al*., 1996) in response to pro-inflammatory mediators. To investigate further the potential pro-inflammatory role of HASM cells their ability to generate granulocyte-macrophage colony stimulating factor (GM-CSF) in response to lipopolysaccharide (LPS) and the pro-inflammatory cytokines IL-1β, tumour necrosis factor α (TNFα) and interferon γ (IFNγ), alone and in combination was assessed. GM-CSF was selected as this cytokine has been implicated in the activation, proliferation and subsequent survival of inflammatory cells such as neutrophils and eosinophils (Lopez *et al*., 1986; Akagawa *et al*., 1988) and may thus be implicated in the pathogenesis of allergic airway disease. Since, glucocorticoids inhibit cytokine release from inflammatory and immune cells, the effect of dexamethasone on GM-CSF release from HASM cells was also evaluated.

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Methods HASM cells were isolated from tracheal rings obtained from heart or heart and lung transplantation (2 male, 1 female 27–44 years) and cultured as outlined previously (Hirst *et al*., 1992). Cell viability, assessed by mitochondrial-dependent reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide to formazan, was not affected by any of the agents studied.

GM-CSF was measured by use of a specific sandwich ELISA. Cells were treated with either IL-1β (0.01–10 ng ml^{-1}), TNFα (0.01–10 ng ml^{-1}), IFNγ (0.01–10 ng ml^{-1}), LPS (0.01–10 μg ml^{-1}) or the cytokine mixture (IL-1β, TNFα, and IFNγ, each 10 ng ml^{-1}). In separate experiments dexamethasone (1 μM) or the protein synthesis inhibitor, cycloheximide (10 μg ml^{-1}), was added 30 min before the addition of the cytokine mixture. In addition, cells were exposed to the cytokine mixture for 0, 2, 4, 6, 12 and 24 h before the medium was removed to determine the kinetics of GM-CSF release.

Results HASM cells spontaneously generated GM-CSF (45.4 ± 13.1 pg ml^{-1}) that was augmented in a concentration-dependent manner by IL-1β and TNFα with a maximal effect seen at 10 ng ml^{-1} (1.3 ± 0.07 and 0.72 ± 0.15 ng ml^{-1}, respectively). IFNγ and LPS did not increase the elaboration of GM-CSF above basal release. HASM cells exposed to the cytokine mixture produced more GM-CSF than any of the cytokines alone (2.2 ± 0.15 ng ml^{-1}) (Figure 1a). The release of GM-CSF in response to the cytokine mixture was time-dependent reaching significance at 12 h ($t = 0$, 19.6 ± 17.3 pg ml^{-1}; $t = 12$ h, 2.5 ± 0.51 ng ml^{-1}; $t = 24$ h, 4.1 ± 0.9 ng ml^{-1}).

The release of GM-CSF from HASM cells elicited by the cytokine mixture was inhibited by pretreatment with cycloheximide ($98.7 \pm 0.58\%$) and dexamethasone ($84 \pm 1.25\%$) (Figure 1b). Cycloheximide and dexamethasone treatment alone did not affect basal GM-CSF release from HASM cells.

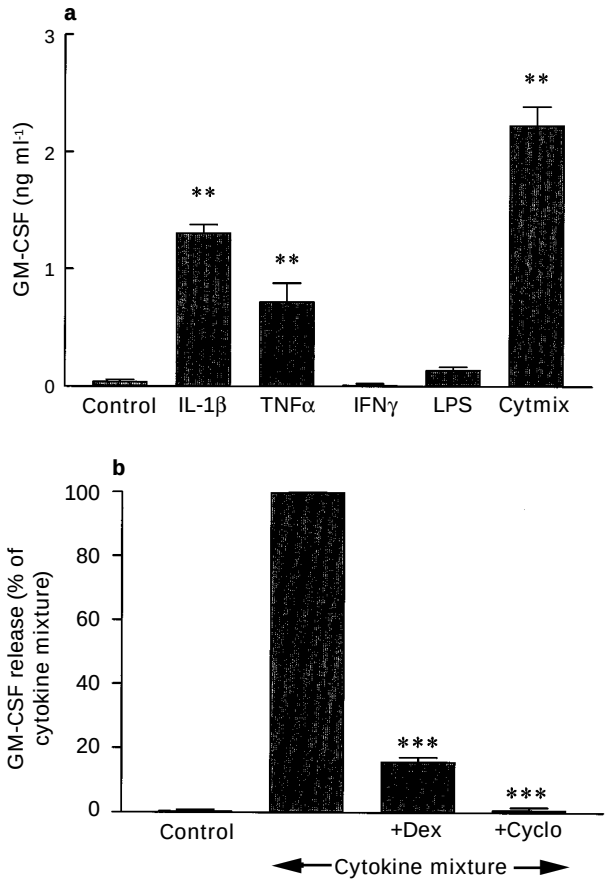

Figure 1 (a) The release of GM-CSF from HASM in response to various cytokines independently and all together (Cytmix; IL-1β, TNFα and IFNγ each at 10 ng ml^{-1}) and LPS (10 μg ml^{-1}). Results are shown as the mean $\pm$ s.e.mean of 9 determinations from 3 patients. Treatment groups were compared by ANOVA and Dunn's Multiple comparison test (**$P<0.01$). (b) The effect of cycloheximide (cyclo, 10 μg ml^{-1}) and dexamethasone (Dex, 1 μM), pretreatment (30 min before cytokine mixture) on GM-CSF release from HASM cells evoked by the cytokine mixture (IL-1β, TNFα, IFNγ, each at 10 ng ml^{-1} for 24 h). Results are shown as the mean $\pm$ s.e.mean of 8 determinations from 3 patients. Treatment groups were compared by the one sample t test. The null hypothesis was rejected when $P<0.05$ (***$P<0.001$).

Discussion Although the mechanisms underlying chronic inflammation of the airways remain unclear, evidence is available implicating cytokines in both the induction and perpetuation of these pathologies (Barnes, 1994). Until now the potential for HASM cells to release such mediators has not been explored. Data presented here would indicate that these cells are capable of releasing cytokines and, as such, are likely to be a major source of such mediators in the inflamed airway. GM-CSF is a ubiquitous cytokine which is known to promote inflammatory cell activation, proliferation and subsequent cell survival (Lopez *et al*., 1986; Akagawa *et al*., 1988). Therefore, it is tempting to hypothesize that activation of HASM cells and the release of GM-CSF (and theoretically other cytokines) may lead to infiltration, activation and enhanced survival of a number of pro-inflammatory and immune cells. As such, this GM-CSF-orchestrated exacerbation of the normal inflammatory response may go some way to explain the sustained inflammation seen in chronic airway disease. However, it is unlikely that a single cytokine such as GM-CSF is responsible for the perpetuation of the inflammatory responses seen in airway dysfunction. Therefore, it is important to determine the profile of cytokines and other putative mediators of inflammation released from these cells which may help to elucidate the exact mechanisms which lead to sustained chronic airway disease.

Glucocorticoids inhibit the release of GM-CSF and other cytokines from inflammatory cells, but their effects on cytokine release from HASM cells have not yet been explored. We have demonstrated for the first time that glucocorticoids inhibit the release of a cytokine from HASM cells, which suggests that these, previously considered, structural cells may be an important target cell for the anti-inflammatory effects of steroids in asthma therapy.

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