G-CSF and GM-CSF are differentially released from primary human lung cells in response to pro-inflammatory cytokines

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INTRODUCTION

Granulocyte-colony stimulating factor (G-CSF) and granulocyte / macrophage-colony stimulating factor (GM-CSF) are essential for the survival, proliferation and differentiation of progenitor cells.

Both factors are known to be released from various lung cell types. In neutrophil-dominant respiratory diseases, G-CSF and GM-CSF levels are elevated. *In vitro* studies have also shown the ability of bacterial strains and certain cytokines (e.g. IL-17) to induce G-CSF and GM-CSF release from the immortalised lung cells lines A549 and BEAS-2B (Jones & Chan, 2002 *Am. J. Respir. Cell Mol. Biol.* 26; 748; Laan *et al.* 2004 *J. Immunol.* 173; 4164).

G-CSF and GM-CSF can also act as pro-inflammatory stimuli to a variety of resident lung cells including macrophages and endothelial cells. Therefore inappropriate or excessive production of these factors may contribute to chronic inflammatory disease and they may represent as attractive therapeutic targets in the treatment of inflammatory lung disease.

The aim of the present study was to quantitatively assess the release of G-CSF and GM-CSF from primary bronchial epithelial cells, smooth muscle cells and parenchymal fibroblasts isolated from human lung in response to stimulation by the pro-inflammatory cytokines TNF-α and IL-1β.

METHODS

All samples of human lung were obtained through medically qualified intermediaries with the informed consent of the donor, and with approval of the local ethics committee. Human bronchial epithelial cells (hBEC) were isolated from second generation bronchus by overnight 0.1% protease digestion. Parenchymal fibroblasts were isolated using mechanical disruption, trypsin digestion and differential plating. Bronchial smooth muscle cells (hBSMC) were purchased commercially (Cambrex, UK). Cells were seeded at $1x10^4$ cells/well and cultured to 80% confluency, then treated with TNF-α or IL-1β ($1x10^{-10}$ to $1 x10^{-4}$M) for 24 h at 37°C, 5% CO_2. Levels of G-CSF and GM-CSF in cell culture supernatants were quantified by sandwich ELISA according to the manufacturer's instructions.

RESULTS

(1) The stimulated maximal release of G-CSF was greater in hBSMC than in fibroblasts and hBEC

(2) IL-1β caused greater maximal release of both factors than TNF-α

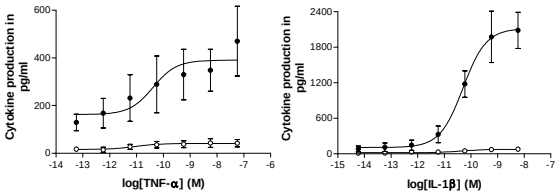
(3) IL-1β appeared ~3-27-fold more potent than TNF-α in stimulating both G-CSF and GM-CSF release from hBSMC and fibroblasts

(4) IL-1β and TNF-α stimulated ~3-30-fold greater release of G-CSF than GM-CSF from all 3 cell types

CONCLUSION

These data demonstrate that quantitatively, G-CSF is the dominant colony stimulating factor released from lung cells in response to the selected pro-inflammatory cytokines. Whether G-CSF plays a role in inflammatory lung diseases warrants further study.

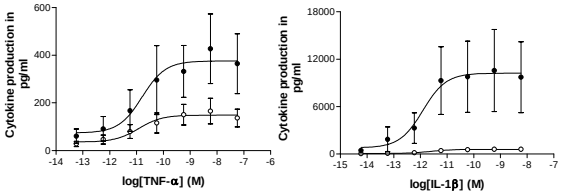
BRONCHIAL EPITHELIAL CELLS



Stimulus	CSF endpoint	pEC50 (M)	Max (pg/ml)
IL-1β	G-CSF	10.3±0.2	2087±306
	GM-CSF	10.4±0.6	77±30
TNF-α	G-CSF	10.4±0.8	470±146
	GM-CSF	11.1±1.0	42±16

Figure 1: TNF-α and IL-1β stimulation of (●) G-CSF and (o) GM-CSF release from human bronchial epithelial cells. Table shows mean ± SEM pEC50 values and and maximal pg /ml release derived from 3 donors.

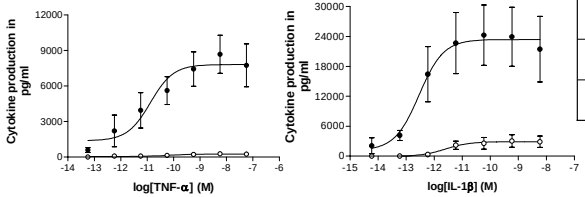
PARENCHYMAL FIBROBLASTS



Stimulus	CSF endpoint	pEC50 (M)	Max (pg/ml)
IL-1β	G-CSF	11.9±0.7	9729±4489
	GM-CSF	11.6±0.4	609±191
TNF-α	G-CSF	10.8±0.7	364±125
	GM-CSF	10.9±0.6	137±37

Figure 2: TNF-α and IL-1β stimulation of (●) G-CSF and (o) GM-CSF release from human parenchymal fibroblasts. Table shows mean ± SEM pEC50 values and and maximal pg /ml release derived from 3 donors.

BRONCHIAL SMOOTH MUSCLE CELLS



Stimulus	CSF endpoint	pEC50 (M)	Max (pg/ml)
IL-1β	G-CSF	12.5±0.7	21429±6585
	GM-CSF	11.6±0.5	2873±1131
TNF-α	G-CSF	10.9±0.4	7754±1811
	GM-CSF	10.2±0.7	241±99

Figure 3: TNF-α and IL-1β stimulation of (●) G-CSF and (o) GM-CSF release from human bronchial smooth muscle cells. Table shows mean ± SEM pEC50 values and and maximal pg /ml release derived from 3 donors.

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