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Short communication

Cytokine modulation of granulocyte macrophage-CSF and granulocyte-CSF release from stimulated vascular smooth muscle cells

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Abstract

Cytokine-stimulated vascular smooth muscle cells release the colony-stimulating factors (CSFs) granulocyte macrophage-CSF and granulocyte-CSF. We have investigated the effects of a range of cytokines on the release of CSFs from human vascular smooth muscle cells stimulated with interleukin-1β. Interleukin-4 suppressed granulocyte macrophage-CSF release but potentiated granulocyte-CSF release; interferon-γ inhibited the release of both, whilst interleukin-5 had no effect. Both interleukin-10 and interleukin-13 inhibited granulocyte macrophage-CSF release but did not affect granulocyte-CSF release. The ability of individual cytokines to differentially modulate CSFs has profound consequences for the populations of leukocytes present at the site of inflammation. © 2002 Published by Elsevier Science B.V.

Keywords: Interleukin-4; Interferon-γ; Interleukin-10; Interleukin-13; Colony-stimulating factor; Smooth muscle cell, vascular

1. Introduction

In addition to their traditional contractile and structural function, vascular smooth muscle cells are now recognised as being an important source of biological mediators in diseases such as septic shock, pulmonary hypertension and atherosclerosis. Specifically, we have shown that human vascular smooth muscle cells cultured from a range of human blood vessels can be stimulated to release interleukin-8 (Jordan et al., 1999), prostaglandins (Bishop-Bailey et al., 1998) or nitric oxide (Chester et al., 1998). However, most recently, we have described pathways by which human vascular smooth muscle cells can be stimulated to release two important CSFs: granulocyte macrophage-CSF and granulocyte-CSF (Stanford et al., 2000a).

CSFs are responsible for the proliferation and differentiation of cells in the bone marrow and can also modulate the function of mature leukocytes, promoting their activation and survival (Lopez et al., 1986). Thus, studying how human cells release CSFs is of importance to our understanding of the inflammatory process. We have recently shown that prostacyclin differentially modulates the release of granulocyte macrophage-CSF (reducing release) and granulocyte-CSF (increasing release) by human vascular smooth muscle cells (Stanford et al., 2000a) and thereby identified a key divergence in their release pattern, which is associated with changes in leukocyte survival (Stanford et al., 2001).

In our initial study, we found that the presence of the pro-inflammatory ('Th1-type') cytokine interleukin-1β was a crucial stimulant for vascular cells to release CSFs. However, other cytokines, including those broadly described as 'Th2-type' cytokines, will be present at different stages of the inflammatory response. Thus, the purpose of this study was to investigate how a range of cytokines specifically affects the release of granulocyte macrophage-CSF vs. granulocyte-CSF.

2. Materials and methods

2.1. Cell culture

Internal mammary artery and saphenous vein was obtained from patients undergoing coronary artery bypass surgery at the Royal Brompton and Harefield N.H.S. Trust. Arterial and venous smooth muscle cells were cultured as

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described previously (Stanford et al., 2000b). Confluent cells (passage numbers 2–9 only) were plated onto 96 well plates for use in experiments. Serum was withdrawn from cells 24 h prior to treatments.

2.2. Cell treatments

At the beginning of all experiments, new supplemented medium (Dulbecco's Modified Eagles Medium plus 10% foetal calf serum, penicillin, streptomycin, glutamine, amphotericin B and MEM non-essential amino acids) was added to smooth muscle cells. Arterial and venous cells were treated with increasing concentrations of interleukin-4 (0.01–10 ng/ml) or interferon-γ (0.01–100 ng/ml) in the presence or absence of interleukin-1β (1 ng/ml). In other experiments, arterial cells were treated with increasing concentrations of interleukin-5 (0.01–10 ng/ml), interleukin-10 (0.01–10 ng/ml) or interleukin-13 (0.01–10 ng/ml) in the presence or absence of interleukin-1β (1 ng/ml). After 24 h, the supernatant was removed from cells and granulocyte macrophage-CSF and granulocyte-CSF release were measured by enzyme-linked immunosorbent assay (ELISA). Cell viability was assessed by mitochondrial-dependent reduction of 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) to formazan. None of the treatments used affected smooth muscle cell viability.

2.3. Materials

Human recombinant cytokines were purchased from R&D Systems. MTT was from Sigma. Matched ELISA reagents for human granulocyte macrophage-CSF were from Pharmingen and matched for granulocyte-CSF were from R&D Systems.

2.4. Statistical analysis

All data are represented as the mean ± the standard error of the mean (S.E.M) and given as $n=3$ using cells cultured from one individual. All experiments were carried out on cells obtained from a further 2–3 patients and similar results were obtained. Data were analysed by one-way analysis of variants (ANOVA), post-test Dunnett. $P<0.05$ was considered to be statistically significant.

3. Results

As observed previously (Stanford et al., 2000a), basal release of granulocyte macrophage-CSF and granulocyte-CSF from arterial and venous smooth muscle cells was undetectable. Neither interleukin-4 nor interferon-γ alone stimulated CSF release from either cell type. However, interleukin-1β stimulated the release of granulocyte macrophage-CSF and granulocyte-CSF from both cell types, the release of granulocyte-CSF being higher than that of granulocyte macrophage-CSF. Interleukin-4 potentiated granulocyte-CSF, but inhibited granulocyte macrophage-CSF, release from both arterial and venous smooth muscle cells stimulated with interleukin-1β (Fig. 1). By contrast, interferon-γ inhibited the release of both CSFs, in a concentration-dependent fashion, by stimulated arterial and venous smooth muscle cells (Fig. 2).

Interleukin-5, interleukin-10 or interleukin-13 alone had no effect on basal release of granulocyte macrophage-CSF or granulocyte-CSF from arterial smooth muscle cells. Similarly, no effect of interleukin-5 on granulocyte macrophage-CSF or granulocyte-CSF release was observed in the presence of interleukin-1β. Interleukin-10, at concentrations of 0.1, 1.0 and 10 ng/ml, significantly inhibited (by one-way ANOVA) granulocyte macrophage-CSF release from interleukin-1β stimulated arterial smooth muscle cells (control vs. interleukin-10 at 10 ng/ml: 122.8 ± 6.5 vs. 86.9 ± 5.4, $n=3$). Similarly, interleukin-13 at concentrations of 1.0 and 10 ng/ml significantly inhibited (by one-way ANOVA) granulocyte macrophage-CSF release from interleukin-1β stimulated arterial smooth muscle cells (control vs. inter-

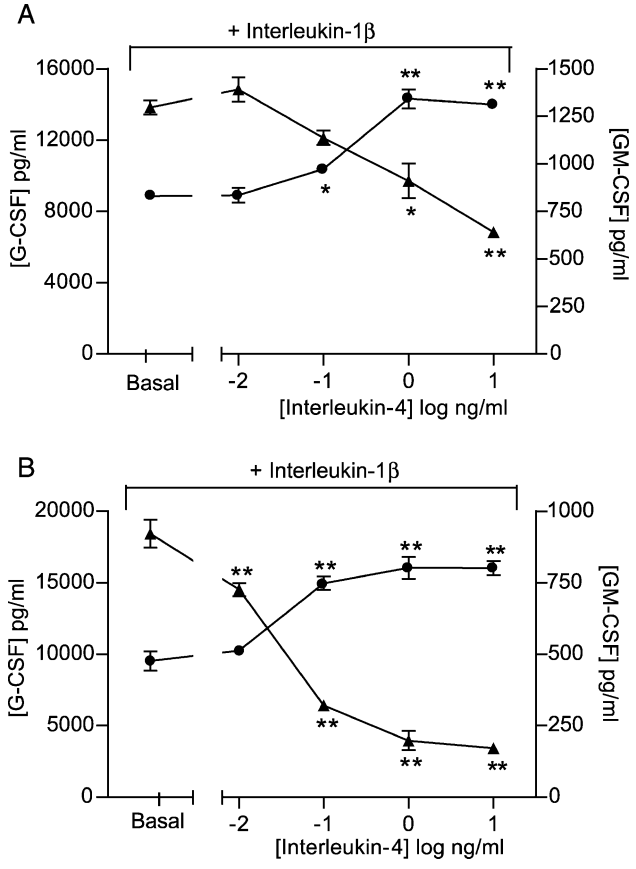

Fig. 1. Effect of interleukin-4 (0.01–10 ng/ml) on granulocyte-CSF (G-CSF) ● and granulocyte macrophage-CSF (GM-CSF) ▲ release from human cultured (A) arterial and (B) venous smooth muscle cells stimulated for 24 h with interleukin-1β (1 ng/ml). Figure represents $n=3$ using cells cultured from one patient. Similar results were obtained using cells cultured from two other patients. One-way ANOVA vs. basal (post-test Dunnett): * $P<0.05$, ** $P<0.01$.

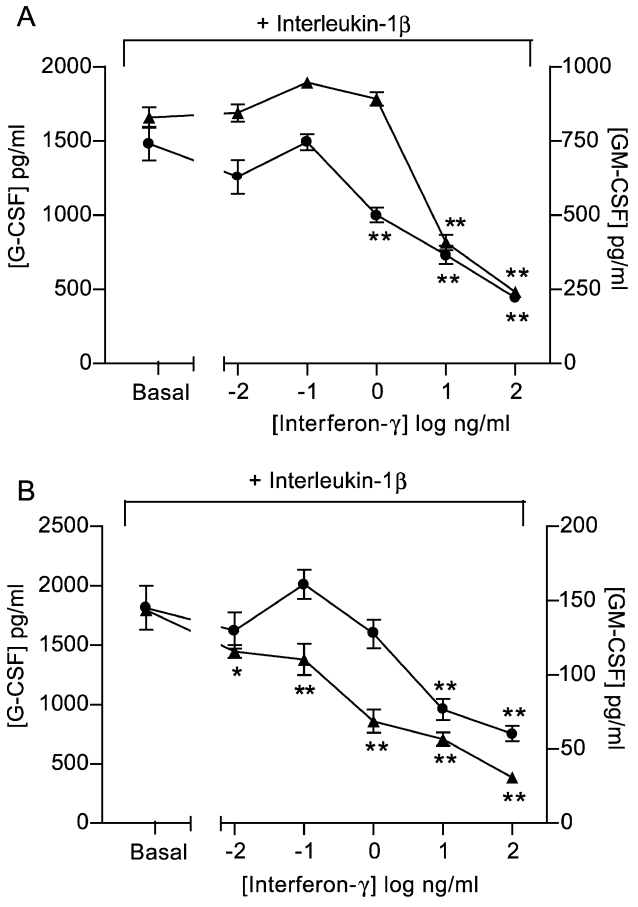

Fig. 2. Effect of interferon-γ (0.01–100 ng/ml) on granulocyte-CSF (G-CSF) ● and granulocyte macrophage (GM-CSF) ▲ release from human cultured (A) arterial and (B) venous smooth muscle cells stimulated for 24 h with interleukin-1β (1 ng/ml). Figure represents $n=3$ using cells cultured from one patient. Similar results were obtained using cells cultured from two other patients. One-way ANOVA vs. basal (post-test Dunnett): * $P<0.05$, * * $P<0.01$.

leukin-13 at 10 ng/ml: 148.1 ± 4.3 vs. 66.0 ± 8.5, $n=3$). Neither interleukin-10 nor interleukin-13 influenced granulocyte-CSF release from arterial smooth muscle cells.

4. Discussion

In vascular diseases such as atherosclerosis, damage to the endothelium leads to the exposure of the underlying smooth muscle cells. We have previously shown that under inflammatory conditions vascular smooth muscle cells are capable of releasing granulocyte macrophage-CSF and granulocyte-CSF (Stanford et al., 2000a). Atherosclerosis is an inflammatory disease and the presence of both Th1 and Th2 cells within the atherosclerotic lesion has been demonstrated (Ross, 1999). Th1 cells evoke cell-mediated immunity and phagocyte-dependent inflammation. Th2 cells evoke strong antibody responses and eosinophil accumulation. Because of the emerging evidence for a role of vascular smooth muscle cells in the immune response, we have investigated the effects of a range of Th1/Th2 cytokines on CSF release from these cells.

Granulocyte-CSF and granulocyte-CSF, in addition to their haemopoietic role, modulate the function of mature leukocytes promoting their activation and survival (Lopez et al., 1986). Interleukin-4 is a Th2 cytokine, strongly implicated in allergic reactions. Th2 cytokines are known to suppress Th1 cell-mediated inflammation. We found that interleukin-4 suppressed granulocyte macrophage-CSF release whilst promoting granulocyte-CSF release from stimulated arterial and venous smooth muscle cells. This finding is supported by another study in the literature carried out using human synovial fibroblasts (Hamilton et al., 1992). In contrast to the differential effect of interleukin-4 on CSF release reported here, Doucet et al. (1998) found that in human lung, fibroblasts interleukin-4 promotes the release of both granulocyte macrophage-CSF and granulocyte-CSF. In monocytes (Lenhoff et al., 1998) and human umbilical cord vein endothelial cells (Lenhoff and Olofsson, 1996), interleukin-4 inhibits the release of both granulocyte-CSF and granulocyte-CSF. The result of differential effects of interleukin-4 on CSF release from smooth muscle cells (this study) and synovial fibroblasts (Hamilton et al., 1992) is clearly not ubiquitous and the signal transduction pathways have yet to be elucidated. Interestingly, in keeping with our observation that interleukin-4 suppresses granulocyte macrophage-CSF release, interleukin-4 is reported to down-regulate granulocyte macrophage-CSF promoter activity in human lung epithelial cells (Bergmann et al., 2000).

The Th1 cytokine interferon-γ, like interleukin-4, inhibited the release of granulocyte macrophage-CSF from stimulated arterial and venous smooth muscle cells. However, by contrast to our observations with interleukin-4, interferon-γ also suppressed granulocyte-CSF release from both cell types. This is in line with other studies carried out in human umbilical cord vein endothelial cells (Lenhoff and Olofsson, 1996), human thymic epithelial cells (Galy and Spits, 1991) and human synovial fibroblasts (Hamilton et al., 1992). In the setting of the atherosclerotic plaque, interferon-γ is regarded as being a pro-inflammatory cytokine, particularly when present in combination with other pro-inflammatory cytokines such as interleukin-1β and tumour necrosis factor-α. However, there are also conditions, such as in Th2 cell-mediated allergic airway inflammation, where interferon-γ displays anti-inflammatory properties (Cohn et al., 2001). The inhibitory action of interferon-γ on CSF release seems to be common to all a wide range of cell types and may represent an anti-inflammatory effect of this cytokine.

Interleukin-10 and interleukin-13 are Th2 type cytokines whose effects, like those of interleukin-4, are generally considered inhibitory to the inflammatory response. In line with this, we found that both interleukin-10 and interleukin-13 inhibited granulocyte macrophage-CSF release from arterial smooth muscle cells. These results are supported in the literature by a study carried out on monocytes (Lenhoff

et al., 1998) but are in contrast to a study using human bronchial epithelial cells, where interleukin-13 promoted and interleukin-10 had no effect on granulocyte macrophage-CSF production (Nakamura et al., 1996). In our study, we found that in arterial smooth muscle cells, neither cytokine had any effect on granulocyte-CSF release. The literature reports both interleukin-10 and interleukin-13 to have inhibitory effects on granulocyte-CSF release by monocytes (Lenhoff et al., 1998) and interleukin-13 to augment both granulocyte macrophage-CSF and granulocyte-CSF release by human lung fibroblasts (Doucet et al., 1998). As noted for interleukin-4, the modulatory effects of both interleukin-10 and interleukin-13 on CSF release do not appear to be ubiquitous and the signal transduction pathways involved have yet to be described. Interleukin-5, another Th2 type cytokine, had no effect on the release of either CSF.

Granulocyte macrophage-CSF and granulocyte-CSF preferentially activate different populations of leukocytes, granulocyte macrophage-CSF acting on a wider range of leukocytes than granulocyte-CSF. Both CSFs act similarly on populations of neutrophils (Stanford et al., 2001). However, granulocyte macrophage-CSF is likely to be more important than granulocyte-CSF at stimulating the survival and differentiation of monocytes to macrophages. Macrophages are the precursor for foam cells the formation of which is central to the development and propagation of the atherosclerotic process. Thus, pathways that increase the release of one CSF and inhibit the release of another will have profound effects on the populations of leukocyte present at sites of inflammation such as the atherosclerotic plaque. We have identified cyclooxygenase-2 (Stanford et al., 2000a) and interleukin-4 (this study) as such pathways. Other Th1/Th2 cytokines including interferon-γ, interleukin-10 and interleukin-13 are also able to modulate CSF release from vascular smooth muscle cells and thus may act indirectly to influence leukocyte survival. Our data, using vascular smooth muscle cells, reveal pathways that are not present in all cell types and may help to explain the complex and specific nature of diseases that specifically affect the vessel wall such as atherosclerosis. In addition, this study adds to our understanding of the interactions that occur between cytokines at the site of inflammation.

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