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Cyclo-oxygenase-2 Regulates Inducible ICAM-1 and VCAM-1 Expression in Human Vascular Smooth Muscle Cells

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Prostaglandins are well characterised inflammatory mediators, whose formation is regulated by constitutive (COX-1) or inducible (COX-2) isoforms of cyclo-oxygenase. We have previously demonstrated that IL-1β causes an induction of COX-2 in human vascular smooth muscle (1). This present study investigates the ability of different cytokines to induce ICAM-1 and VCAM-1 on human vascular smooth muscle, and tests whether co-induced COX-2 would regulate their expression. IL-1β induced ICAM-1, and COX activity, while it had no affect on VCAM-1. Conversely, IL-4 induced VCAM-1, while it had no effect on PGE_2 release or ICAM-1 expression. Inhibition of IL-1β induced COX-2 and elevated ICAM-1 expression, an effect reversed by exogenous PGE_2. Furthermore, IL-1β inhibited IL-4 induced VCAM-1 expression, which was also reversed by COX-2 inhibition. These results demonstrate that COX-2 limits adhesion molecule expression on human vascular smooth muscle cells and suggest that COX-2 can play a protective role in cardiovascular and inflammatory diseases.

Adhesion of cells to each other, or to the extracellular matrix, is regulated by cell surface adhesion molecules. This important ability of cells to interact with their surroundings, is an important components in a number of physiological and pathological settings, including growth and differentiation, wound healing, cell migration, immune responses, and tumour invasion, (2-4). Although, the best characterised system to study adhesion molecules is the endothelium, (2), vascular diseases, such as atherosclerosis, involve the migration of vascular smooth muscle, and the accumulation of monocytes and lymphocytes into the intimal layer. Cell to cell interaction within the smooth muscle layer, mediated by adhesion molecules is therefore also likely to play an important role in this process (5,6).

Vascular SMC can express intercellular adhesion molecule (ICAM)-1 (7), and vascular cell adhesion molecule (VCAM)-1 (8). Although ICAM-1 is often expressed basally on cells (9), both ICAM-1 (9,10), and VCAM-1 (11) can be induced by pro-inflammatory cytokines, and are present on neointimal SMC of the atherosclerotic plaques (12-14). Agents, which elevate intracellular cAMP, can have anti-inflammatory effects on many cellular functions, including the expression of adhesion molecule expression on human airway SMC (15). Indeed, recently the prostacyclin mimetic cicaprost, and forskolin, have also been demonstrated to inhibit ICAM-1 and VCAM-1 induction in human vascular SMC (16).

We have previously demonstrated that cyclo-oxygenase (COX-2) can be highly induced in human vessel segments (1), and vascular SMC (17) in culture by different cytokines, which have also been demonstrated to induce ICAM-1 and VCAM-1 expression in a number of different cell types (2-4). The main prostanoids formed by COX-2 under these conditions were prostacyclin and PGE_2. In animal studies (18) and man (19) COX-2 is elevated during the inflammatory response, and is considered responsible for the high levels of detrimental prostanoid produced. This study shows the ability of different cytokines to regulate the induction of ICAM-1 and VCAM-1 on human SMC, and implicates a role for co-induced COX-2 in limiting their expression.

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Abbreviations used: COX, cyclo-oxygenase; SMC, smooth muscle cell.

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METHODS

Materials. Human recombinant interleukin (IL)-1β , -4, -13, and tumour necrosis factor (TNF)-α were from Boehringer-Mannheim U.K., Lewes, East Sussex, U.K.; interferon (IFN)-γ was from R&D Systems; tritiated prostanoids were from Amersham, Slough, Berks., U.K.; all cell culture media and supplements were supplied by Gibco BRL, Paisley, Renfewshire, Scotland; L-745,337 (5-methanesulfonamido-6- (2,4-difluorothiophenyl) -1-indanone) and selective COX-1 and COX-2 antibodies were a gift from Merck Frosst, Montreal, Canada; mouse anti-human ICAM-1 (RR1/1) IgG_1 monoclonal antibody was provided by Dr. R. Rothlein, Boehringer Inglheim Pharmaceuticals, Ridgefield, CT; monoclonal antibody 4B2 (anti-VCAM-1) was a generous gift from Dr. R. Pigott, British Biotech, Oxford, UK; all other reagents were from Sigma Chemical Co., Poole, Dorset, U.K.

Culture of human vascular smooth muscle. SMC (passages 2-6) were grown from segments of undistended saphenous vein, obtained from patients undergoing coronary artery bypass surgery, as previously described (Bishop-Bailey et al., 1998; submitted). Ethical permission was obtained from the Ethical Committee of the Royal Brompton National Heart and Lung Hospital. Cells were explanted, and cultured using Dulbecco's modified Eagle medium (DMEM) containing 1mM sodium pyruvate and phenol red, supplemented with penicllin (100 IU/mL), streptomycin (0.1 mg/mL), 2 mM glutamine, and 20% foetal calf serum (37°C; 5% CO_2; 95% air) throughout. PGE_2 measurement by RIA, and COX-2 protein determination by Western blot analysis, were performed as previously described (17,20).

Measurement of ICAM-1 and vascular cell adhesion molecule VCAM-1 expression on SMC. ICAM-1, and VCAM-1 were detected on confluent monolayers by an ELISA method (21) using mouse anti-human ICAM-1 (RR1/1), or anti-human VCAM-1 (4B2) primary monoclonal antibodies, and a peroxide-linked goat anti-mouse secondary antibody. SMC were grown to confluence in 96-well plates, and treated for 24h with a number of cytokines and/ or drug combinations. Chromophore development was determined by measuring optical density at 405nm (OD_{405}) using a Titretec MCC/340 Multiscan microplate reader. Background absorbance was determined from monolayers incubated without primary antibody and this value was then subtracted from the absorbance readings. Adhesion molecule expression is given as mOD_{405}.

The expression of ICAM-1 or VCAM-1 was studied in SMC treated in the presence or absence of IL-1β (10 ng ml^{-1}), TNF-α (10 ng ml^{-1}), IFN-γ (1000 U ml^{-1}), LPS (10 μg ml^{-1}), IL-4 (10 ng ml^{-1}) or IL-13 (10 ng ml^{-1}). To assess whether COX-2 or PGE_2 regulated ICAM-1 or VCAM-1 expression, control or cytokine treated SMC were incubated in the presence or absence of the selective COX-2 inhibitor L-745,337 (22), at a concentration of 10μM, which gives consistently a >85% inhibition of COX-2 in these cells (17), and/ or PGE_2 (1μM).

RESULTS

Effect of different cytokines on the expression of ICAM-1 or VCAM-1 on human SMC. Under control culture conditions, SMC constitutively expressed ICAM-1. Addition of IFN-γ alone, stimulated a large significant increases in ICAM-1 expression. IL-1β or TNF-α alone, also induced ICAM-1 expression, but to a lesser extent than seen with IFN-γ (figure 1a). IL-4 or IL-13 had no affect on the basal expression of ICAM-1 (figure 1a).

VCAM-1 expression was undetectable under control culture conditions. Incubation of SMC with IL-1β or TNF-α had no affect on VCAM-1 expression (figure 1b). A small, but significant induction of VCAM-1 was however stimulated by IFN-γ. Incubation of SMC with IL-4, or IL-13 alone caused a large significant induction of VCAM-1 (figure 1b).

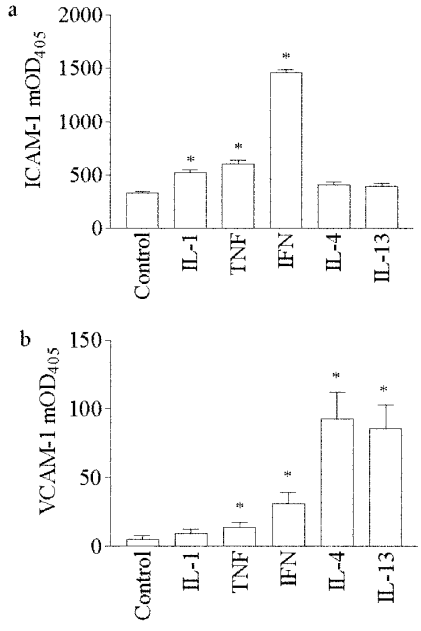

FIG. 1. The effect of different cytokines on ICAM-1 (1a) or VCAM-1 (1b) expression. SMC were treated with IL-1β (10 ng ml^{-1}), TNF-α (10 ng ml^{-1}), IFN-γ (1000 U ml^{-1}), IL-4 (10 ng ml^{-1}), or IL-13 (10 ng ml^{-1}). The data represent the mean ± s.e. mean for 9–21 determinations from 3–7 patients. * denotes significant ($P<0.05$; Bonferroni comparison following one-way analysis of variance) between control and cytokine induced ICAM-1 expression.

Effects of IL-1 and IL-4 on induction of COX-2 activity in human SMC. Under control culture conditions the release of PGE_2 by SMC was 9 ± 2 ng ml^{-1}. Incubation with IL-1β significantly induced PGE_2 released in SMC (84 ± 22 ng ml^{-1}) which was completely blocked (1 ± 1 ng ml^{-1}) if the selective COX-2 inhibitor L-745,337 was included. As IL-1β alone induced both COX-2 and ICAM-1 expression, this was used to further study the ability of COX-2 to regulate ICAM-1 expression.

IL-4 alone (10 ± 2 ng ml^{-1}) had no affect on PGE_2 release compared to control culture conditions (9 ± 2 ng ml^{-1}). However, when used in combination, IL-4 significantly inhibited IL-1β induced PGE_2 release (IL-4 and IL-1β 52 ± 5 ng ml^{-1}; IL-1β alone 84 ± 22 ng ml^{-}; $p<0.05$ Mann-Whitney U-test), again a combination which was completely blocked (2 ± 1 ng ml^{-1}) when L-745,337 was included. Although, IL-4 significantly reduced COX-2 activity in combination with IL-1β, PGE_2 release was still greatly elevated compared to control culture conditions. Therefore, IL-1β (which alone did not induce VCAM-1), was used to study the effects of COX-2 induction on IL-4 induced VCAM-1 expression. PGE_2 release was measured after 24h accumulation. The data represents n=9 determinations from 3 separate patients.

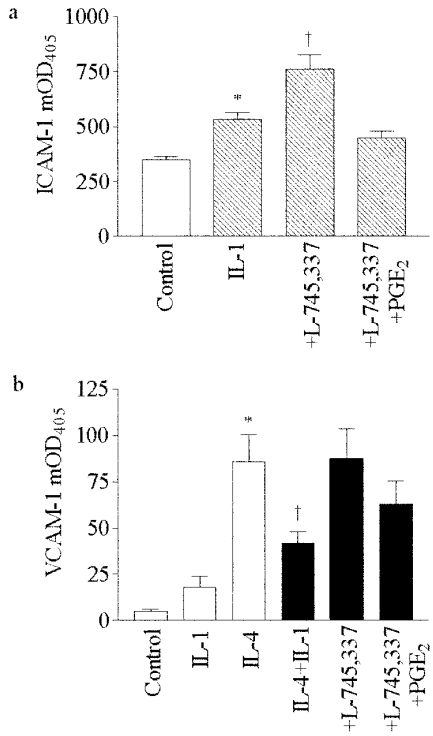

FIG. 2. COX-2 regulation of ICAM-1 and VCAM-1 expression on human vascular SMC. (**a**) The effect of the selective COX-2 inhibitor L-745,337 (10 μM) or exogenous PGE_2 (1μM) in the presence of L-745,337, on the IL-1β induced ICAM-1 expression (hatched bars). The data represent the mean ± s.e. mean for 8–24 determinations from 3–8 patients. * denotes $P<0.05$, one-way ANOVA, followed by Bonferroni comparison between control and IL-1β induced ICAM-1 expression. † denotes $P<0.05$, one-way ANOVA, followed by Bonferroni comparison between ICAM-1 expression induced by IL-1β in the presence of L-745,337 and IL-1β in the presence of L-745,337 with PGE_2. (**b**) The effect of the selective COX-2 inhibitor L-745,337 (10 μM) or exogenous PGE_2 (1μM) in the presence of L-745,337, on the IL-4 and IL-1β induced VCAM-1 expression (solid bars). The data represent the mean ± s.e. mean for 5–10 determinations from 3–5 patients. * denotes $P<0.05$, one-way ANOVA, followed by Bonferroni comparison between control and IL-1β induced VCAM-1 expression. † denotes $P<0.05$, one-way ANOVA, followed by Bonferroni comparison between VCAM-1 expression induced by IL-1β in the presence of L-745,337 and IL-1β in the presence of L-745,337 with PGE_2.

Effect of COX-2 inhibition and exogenous PGE_2 on the IL-1β-induced expression of ICAM-1 on human SMC. Under control culture conditions, L-745,337 had no effect on basal ICAM-1 (mOD_{405}; control 240 ± 11; with L-745,337, 239 ± 3; n=9 determinations 3 from patients). By contrast, inhibition of COX-2 by L-745,337 significantly increased ICAM-1 expression in IL-1β treated cells (figure 2a), an affect which was reversed by PGE_2 (figure 2a). Under control culture conditions, PGE_2 had no effect on basal ICAM-1 (mOD_{405}; control 240 ± 11; with PGE_2, 219 ± 15).

Effect of COX-2 inhibition and exogenous PGE_2 on the IL-4 and IL-1β-induced expression of VCAM-1 on human SMC. Under control culture conditions, L-745,337 had no effect on basal VCAM-1 expression (control, 2 ± 1; L-745,337, 4 ± 1; n=9 determinations 3 from patients). Co-incubation of IL-1β with IL-4 significantly inhibited IL-4 induced VCAM-1 expression (figure 2b), an affect which was reversed by co-incubation with the selective COX-2 inhibitor L-745,337. Furthermore, addition of PGE_2, tended to reverse the effect seen by L-745,337 (figure 2b). Under control culture conditions, PGE_2 had no effect on basal VCAM-1 (mOD_{405}; SV control, 2 ± 1; PGE_2, 6 ± 2).

DISCUSSION

The adhesion receptors ICAM-1 and VCAM-1 play an important and well defined role in the adherence of leukocytes to damaged blood vessels and may also mediate cell-cell interactions of stromal cells within the vessel wall. Here we show that under control culture conditions human SMC express ICAM-1 and not VCAM-1. Moreover, the stimulation of ICAM-1 and VCAM-1 expression on these cells appeared to be regulated by different cytokines. Indeed, ICAM-1, but not VCAM-1, was increased when cells were stimulated with the 'inflammatory' cytokines TNF-α, or IL-1β. Conversely, VCAM-1, but not ICAM-1, was increased when cells were treated with the 'inhibitory' cytokines IL-4 and IL-13, which can both act through a common receptor (23). However, the multi-functional cytokine IFN-γ stimulated the expression of both ICAM-1 and VCAM-1. Some of these observations are in keeping with recent studies showing IL-4 and IFN-γ induce VCAM-1 (24), and TNF-α and IL-1β to induce ICAM-1 (16) on cultured human SMC.

We have previously shown that certain cytokines, including IL-1β, induce the expression of COX-2 in human vascular segments (1) or the human SMC used in this study (17). In the current study, we confirm that IL-1β stimulates the release of COX-2 derived PGE_2, and show that this results in the suppression of adhesion receptor expression on human vascular SMC. The IL-1β induced expression of ICAM-1 was doubled when COX-2 activity was blocked with L-745,337. VCAM-1 expression stimulated by IL-4, was not accompanied by COX-2 induction. However, when cells were co-stimulated with IL-4 (to induce VCAM-1) and IL-1β (to induce COX-2), VCAM-1 expression was reduced, an affect that was reversed by L-745,337. PGE_2, the primary COX-2 metabolite released by these cells (17) reversed the L-745,337 induced increase in ICAM-1 expression, and tended to do so also on VCAM-1 expression. These data suggest that the inhibitory actions of COX-2 on adhesion receptor expression in human vascular SMC is mediated, at least in part by the release of PGE_2. These observations are in keeping with others showing that *exogenous* prostanoids can inhibit the expression of ICAM-1 and VCAM-1 in a range of SMC cultures

(15,24), as well as astrocytes (25) via activation of adenylate cyclase and elevation of cAMP.

Interestingly, we found that IL-4 inhibited the IL-1β induced release of PGE_2. This is consistent with others showing the ability of IL-4 to inhibit COX-2 induction in isolated syniovocytes (26), osteoblasts (27) or U937 cells (28), and in contrast to observations in human full term amnion (29).

Both ICAM-1 (12,13) and VCAM-1 (14), which like COX-2 have been implicated in 'general' inflammatory responses, are also expressed in human atherosclerotic lesions, as are a number of cytokines (30) known to induce COX-2 and adhesion molecules. Thus, there is considerable evidence to suggest that COX-2 which is expressed in damaged vessels (31,32), as well as inflammatory sites, would be co-expressed with adhesion receptors, and could potentially modulate there expression in man. Since COX-2 activity inhibits adhesion receptor expression, inhibition of COX-2 by selective or 'traditional' non-steroidal anti-inflammatory drugs may contribute to inflammation at some sites, such as the gastric mucosa, or during wound healing (33).

Our results show the potential for COX-2 to have an important regulatory role in limiting inflammatory responses, and suggest prolonged use of COX-2 inhibitors may prolong or exacerbate stages of the disease processes by regulating the level of adhesion receptor expression.

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