Interleukin-1β inhibits PDGF-BB–induced migration by cooperating with PDGF-BB to induce cyclooxygenase-2 expression in baboon aortic smooth muscle cells

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Objective: Smooth muscle cell (SMC) migration from the media into the intima is pivotal for intimal formation after vascular injury. Platelet-derived growth factor (PDGF)-BB is a potent chemoattractant for SMCs in vitro and in vivo. We investigated whether interleukin (IL)–1β affects migration in response to PDGF-BB. Our data suggest that IL-1β is inhibitory and that this effect is mediated by cyclooxygenase (COX)-2. We further addressed the role of the mitogen-activated protein kinase p38, which is activated by PDGF-BB and by IL-1β.

Methods: Baboon aortic SMCs were prepared with the explant method. Migration was measured in a Boyden chamber assay through filters coated with monomeric collagen. COX2 expression and phosphorylation of p38 MAPK were analyzed by Western blotting.

Results: PDGF-BB (10 ng/mL) stimulates migration 3.8-fold, and IL-1β (0.1 ng/mL) reduces this response by 40%. The inhibitory effect of IL-1β is abolished by the COX inhibitor, indomethacin (10 μmol/L), the specific COX2 inhibitor, NS398 (10 μmol/L), and the p38 MAPK inhibitor SB203580 (3 μmol/L). We found that IL-1β and PDGF-BB synergize to stimulate COX2 expression. We further demonstrated that p38 MAPK is activated by IL-1β and PDGF with different kinetics and that p38 MAPK is required for maximal COX2 expression in response to IL-1β plus PDGF-BB.

Conclusion: IL-1β inhibits PDGF-BB–induced migration by cooperating with PDGF-BB to induce COX2 through activation of p38 MAPK. Whether this effect of IL-1β modulates intimal growth after vascular injury remains to be elucidated. (J Vasc Surg 2004;39:1091-6.)

Clinical Relevance: Restenosis is the cause of the unacceptably high failure rate of surgical interventions (such as vein grafts, stents, and angioplasty) to restore blood flow in occluded vessels. It is clear that inflammatory processes are critical for the development and progression of atherosclerotic lesions, and there is increasing evidence that inflammation also contributes to restenosis. Recent observations that IL-1 receptors, agonists, and antagonists are expressed upon arterial injury in various animal models strongly indicate a role for IL-1 in restenosis. This study investigates the effects of IL-1 on SMC migration, which is critical for the formation of an occluding intima. Our results suggest that IL-1 may limit the accumulation of intimal cells after injury by blocking SMC migration in a mechanism that depends on expression of cyclooxygenase-2.

Recurrent stenosis is a serious complication of arterial repair, and is caused by pathologic vessel remodeling and intimal hyperplasia. Platelet deposition and degranulation after arterial injury is a crucial process for neointimal growth, and platelet-derived growth factor (PDGF) is the major platelet-derived chemoattractant and mitogen for smooth muscle cells (SMCs). The importance of PDGF in intimal growth has been demonstrated in various animal models, including baboons. The principal effect of PDGF appears to be stimulation of SMC migration, although stimulation of SMC proliferation has also been suggested. PDGF effects in the response to vascular injury appear to be mainly mediated by PDGF receptor beta. For example, blockade of PDGF-receptor beta, but not alpha, inhibits intimal hyperplasia in the balloon-injured baboon carotid artery.

There are multiple lines of evidence that inflammatory processes contribute to recurrent stenosis after vascular injury. Proinflammatory cytokines such as interleukin (IL)–1 are potentially attractive targets to suppress intimal hyperplasia, because they are unlikely to control normal vessel functions. A role for IL-1 in recurrent stenosis has been proposed. In human beings a polymorphism in the IL receptor antagonist (IL-RA) locus has been discovered, which may protect from recurrent stenosis after coronary angioplasty and after coronary stenting. Although the consequences of this polymorphism for IL-RA expression in the vessel wall are not known, observations in IL-RA-deficient mice point to an anti-inflammatory function of IL-RA in arteries. Animal models in which IL-1 has been investigated include pigs and mice. Chronic stimulation of
healthy pig coronary arteries with IL-1 produces intimal lesions. In IL-1R1–deficient mice, compared with wild-type animals, intimal hyperplasia is reduced after carotid artery ligation. After balloon injury, expression of IL-1 is induced in the rat carotid artery and pig coronary artery. Multiple mechanisms may contribute to stimulation of intimal hyperplasia by IL-1, including direct activation of SMC proliferation.

Because PDGF and IL-1β are generated concomitantly at vascular injury, we investigated whether both factors cooperate to stimulate SMC functions. We recently reported that IL-1β potentiates PDGF-BB–induced proliferation. A likely mechanism is that IL-1β suppresses induction of cell cycle inhibitors p21 (WAF1/CIP1) and p27 (KIP1), thereby enhancing cyclin-dependent kinase 2 activation and proliferation.

Here we investigated the effects of IL-1β on PDGF-BB–induced migration. We found that IL-1β is inhibitory and that this effect relies on synergistic expression of cyclooxygenase (COX)–2 by IL-1β and PDGF-BB.

METHODS

Material. IL-1β was obtained from R & D Systems (Minneapolis, Minn). PDGF-BB was generously provided by Zymogenetics (Seattle, Wash). Indomethacin, NS398, and SB203580 were purchased from Calbiochem (La Jolla, Calif). Antibodies against COX2 and phospho-p38 MAPK were from Cayman (Ann Arbor, Mich) and Cell Signaling Technology (Beverly, Mass), respectively. All tissue culture solutions were from Gibco/Invitrogen Life Technologies (Carlsbad, Calif).

Cell culture. Baboon and human aortic SMCs were prepared with the explant method. Aortic SMCs from FVB mice were a kind gift from Michael Reidy (University of Washington, Seattle). All cells were grown in Dulbecco modified Eagle medium high glucose supplemented with 10% fetal bovine serum, 200 U/mL of penicillin, and 0.2 mg/mL of streptomycin. SMCs between 5 and 12 passages were then added back into the upper chamber. The chamber was kept for 5 hours at 37°C in a humidified incubator at 5% carbon dioxide. Cells on the bottom of the filters were fixed with methanol and stained with Diff-Quick staining solution (Baxter, Deerfield, Ill). Migration was measured as the number of cells per high-power field (100×) that migrated across the membrane between the upper and lower chambers.

Statistical analysis. All experiments were performed at least three times. Where indicated, statistical significance between control and experimental groups was calculated with adjusted Bonferroni comparisons or paired Student t test. Differences at P < .05 were considered significant.

RESULTS

IL-1β inhibits PDGF-BB–induced migration in a COX2 and p38 mitogen-activated protein kinase (MAPK)–dependent manner. In a Boyden chamber assay, PDGF-BB (10 ng/mL) typically stimulates SMC migration by three to four times. This response is inhibited by 40% when IL-1β (0.1 ng/mL) is added (Fig 1). Doses of PDGF-BB and IL-1β used have been recently established as optimal in these cells for IL-1β and PDGF-BB with respect to growth stimulatory effects of IL-1β on PDGF-BB–induced proliferation. To test the possibility that COX2 mediates the inhibitory effect of IL-1β, we used the general COX inhibitor indomethacin (10 μmol/L) and the COX2 specific inhibitor NS398 (10 μmol/L). Given previous observations that p38 MAPK is critical for COX2 expression by IL-1β in airway SMCs, we also investigated the effect of the p38 MAPK inhibitor SB203580 (3-10 μmol/L). Indomethacin (Fig 1, A) and SB203580 (Fig 1, C) have no effect on PDGF-BB–induced migration, whereas NS398 may be slightly inhibitory (Fig 1, B). The migratory response to PDGF-BB in the presence of either compound (indomethacin, NS-398, SB203580) was higher than the response to PDGF-BB in the presence of IL-1β (Fig 1). Addition of either drug significantly stimulates migration by IL-1β plus PDGF-BB (Fig 1). Together these data indicate that IL-1β inhibits PDGF-BB–induced migration in a mechanism that depends on COX2 and p38 MAPK.
IL-1β synergizes with PDGF-BB to stimulate COX2 expression; role of p38 MAPK. To investigate COX2 expression, we determined COX2 protein levels with Western blotting after 5-hour stimulation with PDGF-BB in the absence or presence of IL-1β. The 5-hour time point was chosen because Boyden chambers were also incubated for 5 hours after PDGF-BB stimulation. IL-1β (0.1 ng/mL) alone induces COX2 expression twofold over untreated controls, whereas PDGF-BB has no significant effect. COX2 expression markedly increases in cells on costimulation with IL-1β and PDGF-BB (Fig 2). Because such synergism has not yet been reported, we addressed the question of whether it is specific for baboon SMCs or is of more general validity. We found synergistic expression of COX2 by PDGF-BB and IL-1β in murine and human SMCs (Fig 3).

To demonstrate that p38 MAPK is involved in COX2 expression by IL-1β and PDGF-BB, we measured COX2 in SMCs treated with SB203580. The inhibitor blocks expression of COX2 under all conditions. Significant is an inhibition of 50% after stimulation with IL-1β plus PDGF-BB (Fig 2). We next examined the extent to which IL-1β and PDGF-BB contribute to p38 MAPK activation. Activity of p38 MAPK was determined by assessing its phosphorylation status with a phospho-specific antibody. Both factors transiently stimulate p38 MAPK, but the kinetics is different. PDGF-BB activation of p38 MAPK peaks at 10 minutes, whereas p38 MAPK activation by IL-1β is maximal at 30 minutes (Fig 4). Costimulation of SMCs with IL-1β plus PDGF-BB results in higher p38 MAPK activity at each time point when compared with cells stimulated with either factor alone (Fig 4). After 60 minutes p38 MAPK phosphorylation is absent, regardless of stimulants used (Fig 4; data for 3-hour, 4-hour, and 5-hour time points not shown).
DISCUSSION

Both IL-1β and PDGF have been described as stimulating COX2 expression in SMCs. What has not yet been appreciated is that both factors strongly cooperate to induce COX2. This mechanism is present in human, baboon, and murine SMCs, whereas the potency of PDGF-BB or IL-1β alone to stimulate COX2 expression may vary between species (Figs 2 and 3). The reason for this apparent difference between species is not known. One major consequence of the synergistic expression of COX2

Fig 3. Cyclooxygenase-2 (COX2) expression in murine and human smooth muscle cells. Quiescent smooth muscle cells from mouse (black bars) and human being (gray bars) were stimulated with interleukin-1β (IL-1β; 0.1 ng/mL) and platelet-derived growth factor-BB (PDGF-BB; 10 ng/mL), as indicated. Total cell lysates were prepared 5 hours after stimulation. COX2 expression was analyzed after Western blotting with densitometry of the films. Data (mean ± SD; n = 4 for mouse, n = 2 for human) are presented as percent COX2 expression, with 100% the strongest band per experiment. In all six experiments, strongest COX2 expression was obtained in response to IL-1β plus PDGF-BB.

Fig 4. Phosphorylation of p38 mitogen-activated protein kinase (MAPK) in response to interleukin-1β (IL-1β) and platelet-derived growth factor-BB (PDGF-BB). Quiescent baboon smooth muscle cells were stimulated with IL-1β (IL, 0.1 ng/mL) and PDGF-BB (P, 10 ng/mL) for 10, 30, 60, and 120 minutes, as indicated. Total cell lysates were prepared and analyzed with Western blotting for phosphorylated p38 MAPK. A, Representative blot. B, Data (mean ± SEM, n = 3) are presented as percent p38 MAPK phosphorylation, with 100% the strongest band per experiment. For all three experiments, p38 MAPK phosphorylation was strongest in response to IL-1β plus PDGF-BB at 30 minutes after stimulation.

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by IL-1β and PDGF-BB is an inhibition of PDGF-BB–
induced migration (Fig 1). The molecular basis for the
synergism between PDGF-BB and IL-1β in expressing
COX2 remains to be elucidated. Our data suggest that p38
MAPK, which is activated by both factors, with differing
kinetics (Fig 4), has a crucial role in maximal COX2 expres-

sion in response to IL-1β plus PDGF-BB. Blockade of p38
MAPK decreases COX2 expression to an extent that the
enzyme no longer inhibits PDGF-BB–induced migration
(Figs 1, C and 2). Given recent findings in other cell
types,27–29 it is likely that p38 MAPK is required for mes-
seg stabi lization. Blockade of p38 MAPK does not abolish
COX2 expression by PDGF-BB plus IL-1β, suggesting
additional cooperation of the two factors at the transcrip-
tion level. Our observation that PDGF-BB and IL-1β
stimulate p38 MAPK with different kinetics may indicate
that both factors use different signaling pathways for p38
MAPK activation.

The role of COX2 in recurrent stenosis remains to be
clarified. COX2 is expressed at arterial injury, but blockade
of COX2 in the balloon-injured rat carotid artery had no
effect on lesion development.30 It is likely that conse-
quences of COX2 expression in the developing intima are
complex, and have positive and negative influence on neo-
ontimal formation. COX2–mediated synthesis of inflamma-
tory prostaglandins may promote intimal growth by stim-
ulating recruitment of macrophages, which in turn release
SMC mitogens and chemotacticants. In addition, prosta-
glandins may stimulate the growth of new blood vessels,
which supply the growing intima. On the other hand,
prostaglandin E₂ and prostacyclin both stimulate cyclic
adenosine monophosphate production in SMCs, which
inhibits proliferation. Consistent with this mechanism are
recent observations in prostacyclin receptor–deficient
mice, in which injury-induced vascular proliferation and
platelet activation are enhanced, compared with in
controls.31

Our observation that IL-1β inhibits SMC migration
suggests that IL-1β may attenuate intimal formation at
early stages after injury. We have recently reported that
IL-1β potentiates PDGF-BB–induced growth by inhibiting
expression of cell cycle–dependent kinase inhibitors
p21 and p27. It may be speculated that the immediate
release of IL-1β after injury restricts intimal formation by
inhibiting SMC migration, but when present over the long
term IL-1β promotes intimal growth by stimulation of
intimal SMC proliferation.

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REFERENCES

4. Ferns GA, Raines EW, Sprugel KH, Motani AS, Reidy MA, Ross R. Inhibition of neointimal smooth muscle accumulation after angioplasty
5. Jackson CL, Raines EW, Ross R, Reidy MA. Role of endogenous platelet-derived growth factor in arterial smooth muscle cell migration

11. Francis SE, Camp NJ, Burton AJ, Dewberry GM, Gunn J, Stephens-
15. Rectenwald JE, Moldawer LL, Huber TS, Seger JM, Ozaki CK. Direct evidence for cytokine involvement in neointimal hyperplasia. Circula-

16. Wang X, Romanic AM, Yue TL, Feusterer GZ, Olstein EH. Expression
of interleukin-1beta, interleukin-1 receptor, and interleukin-1 receptor antagonist mRNAs in rat carotid artery after balloon angioplasty.
18. Nathe TJ, Decou J, Walsh B, Bourns B, Cloves AW, Daum G. Interleu-
kine-1beta inhibitor expression of p21(WAF1/CIP1) and p27(KIP1) and enhances proliferation in response to platelet-derived growth factor BB
19. Ross R, Kariya B. Morphogenesis of vascular smooth muscle cells in
20. Koyama N, Hart CE, Cloves AW. Different functions of the platelet-
derived growth factor-alpha and -beta receptors for the migration and
21. Jaccard J, Wan CK. LISREL approaches to interaction effects in multi-
22. Laporte JD, Moore PE, Lahiri T, Schwartzman IN, Panettieri RA Jr,
Shore SA. p38 MAP kinase regulates IL-1 beta responses in cultured


