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PLATELET-DERIVED GROWTH FACTOR IS A POTENT STIMULATOR OF EXPRESSION OF INTERCELLULAR ADHESION MOLECULE-1 IN HUMAN ARTERIAL SMOOTH MUSCLE CELLS

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SUMMARY- The effects of platelet-derived growth factor(PDGF) on the expression of intercellular adhesion molecule-1(ICAM-1) as an indicator of cell activation were investigated in cultured human arterial smooth muscle cells(SMC). PDGF-BB and -AB but not -AA at 2-10 ng/ml stimulated ICAM-1 expression at a subconfluent but not a confluent state in a dose-dependent manner. ICAM-1 expression was induced at 2h, reached a plateau at 4h, and continued for at least 24h after stimulation with PDGF. The maximal stimulatory effect of PDGF-BB at 10 ng/ml was comparable to that by optimal concentrations of other cytokines and inflammatory agents. These data suggested that PDGF was a potent stimulator of ICAM-1.

It is now generally accepted that leukocyte adhesion to endothelial cells(EC) is mediated by adhesion molecules. The expression of intercellular adhesion molecule-1(ICAM-1) or vascular adhesion molecule-1(VCAM-1) on EC was reported to be an important event in the formation of early atherosclerotic lesions in a number of in vivo studies(1-5). The regulation mechanism of

Abbreviations used:
EC, endothelial cells; DME, Dulbecco's modified Eagle's Medium; FBS, fetal bovine serum; ICAM-1, intercellular adhesion molecule-1; IL, interleukin; LPS, lipopolysaccharide; lysoPC, lysophosphatidylcholine; PDGF, platelet-derived growth factor; PLA2, phospholipase A2; SMC, smooth muscle cells; TNF, tumor necrosis factor; TPA, 12-O-tetra-decanoyl-phorbol 13-acetate; VCAM-1, vascular cell adhesion molecule-1.

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the expression of these adhesion molecules in relation to cytokines and hypercholesterolemia was also studied in EC in vitro(6-8). Besides EC, arterial smooth muscle cells(SMC) also expressed these adhesion molecules in vivo(3-5) and in vitro(9), although there is still little known about the role or regulation of the expression of adhesion molecules in SMC. We proposed that phospholipase A2(PLA2) reaction or its product, lysophosphatidylcholine(lysoPC), was important for the expression of these adhesion molecules in EC, and that their expression in EC by cytokines and inflammatory agents was in part through the PLA2 reaction by these agents(8). LysoPC was speculated to be a second messenger via the PLA2 reaction. The effects of LysoPC have been reported in detail in another study as well(7). Platelet-derived growth factor(PDGF) is also known as a stimulator of PLA2 reaction(10). Because EC from large vessels lack PDGF-receptors, we could not test our PLA2 hypothesis regarding the expression of adhesion molecules with PDGF in EC. However, it is well known that SMC, as opposed to EC, express sufficient amounts of PDGF-receptors. Thus we studied the effects of PDGF on the expression of adhesion molecules in SMC together with those of other cytokines and inflammatory agents.

MATERIALS AND METHODS

Chemicals The sources of materials were as follows:Lipopolysaccharide(LPS), 12-O-tetra-decanoyl-phorbol 13-acetate(TPA), PLA2 from Naja mocambique mocambique venom, Sigma(St. Louis, MO); Recombinant human interleukin(IL)-1β and -4, Collaborative Research(Bedford, MA); Human recombinant tumor necrosis factor(TNF), Genzyme Inc.(Boston, MA); Three isoforms of human recombinant PDGF, Boehringer(Mannheim, Germany). Other materials for SMC culture were reported elsewhere(11,12).

Culture of SMC SMC were explanted from intimal and medial layers of arteries obtained by shunt operations from patients subjected to dialysis. SMC were cultured as reported(11,12) and used at 5-20 passages.

Cell-ELISA assay Basically the ELISA assay was the same as in EC(8). In brief, SMC cultured in Dulbecco's modified Eagle's Medium(DME) containing 10% fetal bovine serum(FBS, Lot No. 37k0325) were seeded at 5 X 10^3/well in 96-well cell-culture

plates and cultured to subconfluency(about 10^4/well) or confluency(about 5 X 10^4/well). Cells were stimulated with various agents in DME/10% FBS for certain periods(standard 18h; time course study 1-48h) and subjected to ELISA assay using an anti-human ICAM-1 or VCAM-1 antibody.

RESULTS

Dependency of ICAM-1 expression in SMC on PDGF concentration, PDGF isomers, and cell density: Before stimulation with PDGF, SMC at subconfluency showed some absorbance of ICAM-1 by ELISA assay, suggesting that cultured SMC already expressed ICAM-1(Fig.1-a) as observed in EC(8). Eighteen hours incubation with PDGF-BB and -AB at 2-10 ng/ml stimulated expression of ICAM-1 in a dose-dependent manner, the activation rates at 10 ng/ml being 1.9- and 1.8-fold, respectively. (The cell number did not differ significantly in either the presence or absence of PDGF as cultured in 10 % FBS.) On the other hand, PDGF-AA did not significantly enhance ICAM-1 expression(Fig.1-a). At a confluent state, no isoform of PDGF stimulated the ICAM-1 expression(Fig.1-b). In the subsequent

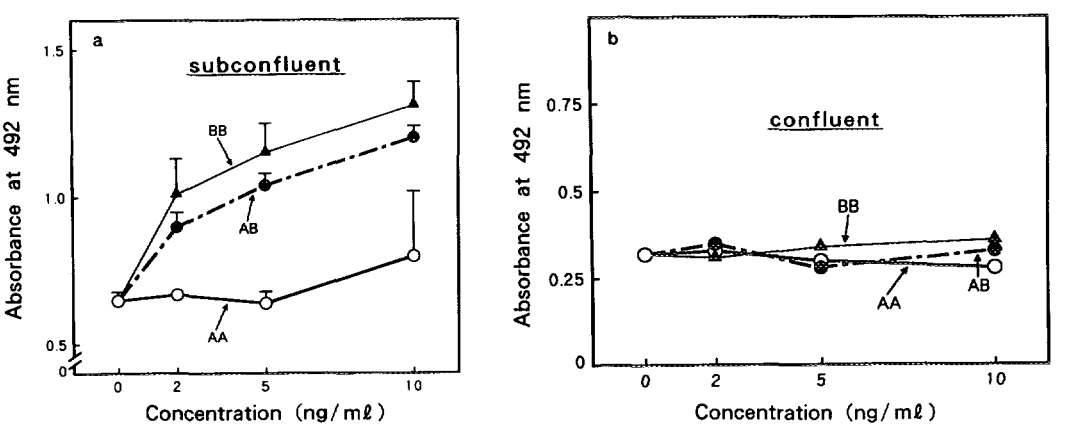

Fig.1. Effects of three isoforms of PDGF on ICAM-1 expression in cultured human SMC at subconfluency(Fig.1-a) and confluency(Fig.1-b). Human SMC were cultured to subconfluency or confluency in DME/10% FBS and stimulated with three isoforms of PDGF(-AA, open circles; -AB, closed circles; -BB, closed triangles) in DME/10% FBS for 18h. ICAM-1 expression was measured by Cell-ELISA. Each point is mean ± SD(n=3) (means only were shown in Fig.1-b).

experiments, 10 ng/ml of PDGF-BB and subconfluent states were used as the experimental conditions.

Time course of ICAM-1 expression by PDGF-BB(Fig.2) : Two hours after stimulation of SMC with 10 ng/ml of PDGF-BB, the expression of ICAM-1 had increased over the initial level,reached maxixum 4h after stimulation, and then maintained this level at least for 24h. Forty hours after stimulation, it had rather declined. The time course of ICAM-1 expression after stimulation with PDGF was similar to that with stimulators of PLA2 reaction[IL-1β (Fig.2) and TNF(9)] or as well as that of a non-stimulator[IL-4(Fig. 2)]. These data suggested that the PDGF effect on ICAM-1 expression was not an indirect one through the expression of other cytokines in SMC, which in turn induce ICAM-1 expression.

Comparison of PDGF with other cytokines and inflammatory agents: Fig. 3 shows the expressions of ICAM-1 and VCAM-1 after

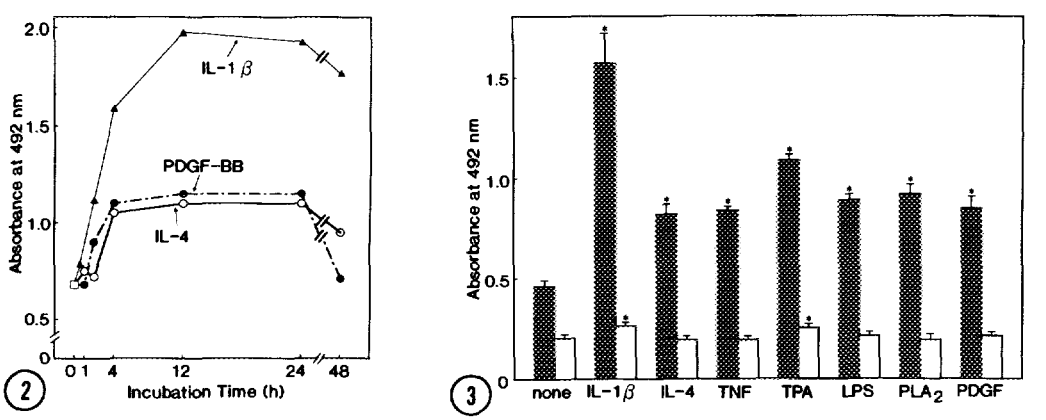

Fig.2. Time course of ICAM-1 expression after stimulation of human SMC with PDGF. Subconfluent SMC were stimulated with 10 ng/ml PDGF-BB(closed circles), 20 ng/ml IL-4(open circles) or 10 ng/ml IL-1β (closed triangles), and at each time point ICAM-1 expression was measured by a Cell-ELISA. Each point shows a mean of two separate cultures which did not differ from each other by more than 10%.

Fig.3. Effects of various substances on ICAM-1 and VCAM-1 expressions in human SMC. Subconfluent SMC were stimulated with 10 ng/ml IL-1β , 20 ng/ml IL-4, 10 ng/ml TNF, 10^{-8} M TPA, 10 μg/ml LPS, 5 U/ml venom PLA2 and 10ng/ml PDGF-BB in DME/10% FBS for 16h. Expressions of ICAM-1 and VCAM-1 were measured by Cell-ELISA. Each column shows mean ± SD(n=3). Closed columns, ICAM-1; open columns, VCAM-1. *, Significantly different from controls(none).

stimulation with various concentrations of cytokines and inflammatory agents. The concentration of each agent used was optimal in EC in terms of expression of adhesion molecules(8) except IL-4, for which optimal concentration was 20 ng/ml in SMC in other studies (data not shown). Substances such as IL-1β (10 ng/ml), IL-4(20 ng/ml), TNF(10 ng/ml), TPA(10^{-8} M), LPS(10 μg/ml) and venom PLA2(1U/ml) also stimulated ICAM-1 expression in EC at concentrations similar for SMC. The extent of effect of PDGF-BB at 10 ng/ml on ICAM-1 expression was similar to those of IL-4, TNF, LPS and PLA2, although it was less than that of IL-1β and slightly less than that of TPA. These data suggested that PDGF was also an important factor for regulating ICAM-1 expression. On the other hand, absorbance of VCAM-1 expression was very low before stimulation, and VCAM-1 expression was not at all, or only slightly enhanced(with IL-1β and TPA) by the substances mentioned above including PDGF-BB.

DISCUSSION

The substances IL-1β (13), TNF(14), TPA(15) and LPS(16), which could stimulate PLA2 reaction, or PLA2 itself enhanced ICAM-1 expression in SMC as well as in EC(8). It is of considerable interest that PDGF, which is another stimulator of PLA2 reaction(10), enhanced ICAM-1 expression in SMC. These data indicate that the PLA2 reaction is also in part involved in the ICAM-1 expression in SMC. There may be a common mechanism for ICAM-1 expression between EC and SMC. In contrast, VCAM-1 expression was hardly enhanced by the above substances that stimulated VCAM-1 expression in EC(8), and this suggests that there are probably different mechanisms for VCAM-1 expression between EC and SMC.

Among the three isoforms of PDGF, PDGF-BB and -AB, but not -AA, enhanced ICAM-1 expression in SMC, suggesting that the signal

for the stimulation of ICAM-1 expression in this case is through PDGF-β receptors, as the signaling of PDGF-AA is considered to be mediated by dimers of PDGF-α receptors and that of PDGF-BB by any combination of α and β receptors(17). The reason for this is probably not a lack of α receptors in this cell line of SMC, since the SMC used responded to the growth stimulation of PDGF-AA as well as to PDGF-BB and -AB(unpublished data). Thus, signals only through β-receptors are involved in ICAM-1 expression, and they may be related to PLA2 reaction. So far we have not seen any reports that indicated that PDGF-AA was a stimulator of PLA2 reaction.

The significance of ICAM-1 expression in SMC is not well understood. One possibility is that ICAM-1 expression is a marker of the "activated state" of SMC. We postulated that autocrine secretion of the growth factor, smooth muscle cell-derived growth factor(SDGF)(18,19), rapid growth, and the expression of scavenger receptors are characteristics of the activated phenotype of SMC(20-22). At least, stimulation of SMC with PDGF(-BB and -AB but not -AA) or TNF resulted in the expression of these features, i.e., autocrine secretion of SDGF, rapid growth and upregulation of the scavenger receptors, in SMC(20-23, and unpublished data). Therefore, it is conceivable that PDGF(-BB and -AB) and TNF are activating factors for SMC. As PDGF(-BB and -AB) or TNF induced also ICAM-1 expression in SMC in this study, it may be another marker of the "activated state" of SMC. Further studies concerning the precise role of the activation of SMC in the formation of atherosclerotic lesions are in progress.

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