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INTERLEUKIN 1 INCREASES THE PRODUCTION OF ENDOTHELIN-1 BY CULTURED ENDOTHELIAL CELLS

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SUMMARY: We examined the effect of human recombinant interleukin 1 (IL-1) on the production of endothelin-1 by cultured porcine endothelial cells. The induction of endothelin-1 mRNA began within 1 hr of exposure to IL-1, showed twin peaks at 4 and 24 hr, and declined thereafter. Enzyme-linked immunosorbent assay revealed that the amount of endothelin-1 peptide in conditioned media was also increased by IL-1 in a dose- and time-dependent manner. Our results suggested that IL-1, a macrophage-derived cytokine, may affect the contraction and proliferation of vascular smooth muscle cells by stimulating the production of endothelin by endothelial cells.

Recently, it has become recognized that the subendothelial migration and localization of macrophages are the earliest events in atherogenesis (1). Among the many secretory products of macrophages, interleukin 1 (IL-1) has been shown to have a broad range of biological effects on various target cells, including leukocytes, fibroblasts (2), vascular smooth muscle cells (3), and endothelial cells (4). In particular, IL-1 acts on the vascular endothelium to influence the synthesis of various vasoactive substances, which can modulate the inflammatory response, the coagulation system, and the contractile functions of the vascular wall (4).

The newly discovered endothelium-derived peptide, endothelin, has a potent contractile effect on vascular smooth muscle (5), and may contribute to the vasospasm (6) which is thought to be associated with atherosclerosis (7). Moreover, endothelin may be involved in the pathogenesis of atherosclerosis through its proliferative effect on vascular smooth muscle

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ABBREVIATIONS: interleukin 1 (IL-1); Dulbecco's modified Eagle's medium (DMEM); platelet activating factor (PAF).

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(8). In this study, to clarify the relationship between macrophage function and endothelin production, we investigated the effect of human recombinant IL-1 on the production of endothelin-1 by cultured vascular endothelial cells.

MATERIALS AND METHODS

Reagents: Human recombinant IL-1 was obtained from Otsuka Pharmaceutical Co. (Tokushima, Japan) (9). The platelet activating factor (PAF) antagonist, CV-6209, was supplied by Takeda Chemical Industries (Osaka, Japan) (10). Indomethacin was purchased from Sigma Chemical Co. (St. Louis, MO).

Cell Culture: Endothelial cells were obtained from adult porcine thoracic aorta and cultured in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum at 37°C in an atmosphere of 5 % CO_2 / 95 % air. Cultures were determined to contain endothelial cells on the basis of their typical 'cobble-stone' morphology. Cells from the 5th to the 10th passages were grown to confluence in 28.3 cm^2 culture dishes. The cells were washed twice with DMEM and maintained in 2.5 ml of DMEM containing 1% fetal bovine serum for 12-24 h before experiments.

RNA Extraction and Northern Blot Analysis: The total cellular RNA content was extracted from endothelial cells by the LiCl-urea method and quantitated by determining the absorbance at a wavelength of 260 nm. RNA samples were electrophoresed in 1.2% agarose/formaldehyde gel, transferred to a nylon membrane (Hybond-N, Amersham), and fixed by ultraviolet irradiation. The endothelin-1-specific probe, pET4, a λgt10 clone encoding porcine endothelin-1 cDNA (5), was labeled by a random-primed labeling method using [α-^{32}P]dCTP. Membranes were hybridized with ^{32}P-labeled endothelin-1 cDNA and exposed to X-ray film for autoradiography. The membranes were subsequently rehybridized with myosin regulatory light chain cDNA (pGMRL7E1; Masaki et al., unpublished observations) as an internal standard.

Enzyme-linked Immunosorbent Assay (ELISA): An ELISA method for the measurement of endothelin-1 has been described elsewhere (11). In brief, we developed two monoclonal antibodies (1C10 and 8H10) which recognized the N- and C- terminal sequences of endothelin-1, respectively, and used a 'sandwich method' for the measurement of endothelin-1 concentrations in the culture medium.

RESULTS and DISCUSSION

Endothelial cells grown to confluence were stimulated at time 0 with 50 ng/ml of either IL-1α or IL-1β. Fig. 1 shows data from a kinetic study of

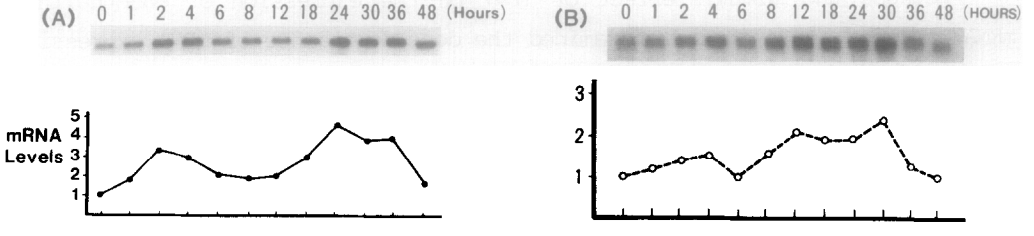

Figure 1: Time course of endothelin-1 mRNA expression by IL-1-treated endothelial cells. The insets show Northern blot analysis of endothelin-1 mRNA expression, whereas the figures show the corresponding densitometry of the above autoradiographs. The control level of mRNA in the series was assigned the number 1.0 and all other values were calculated relative to that value. Endothelial cells were treated with 50 ng/ml of IL-1α (A) or 50 ng/ml of IL-1β (B) for the indicated times.

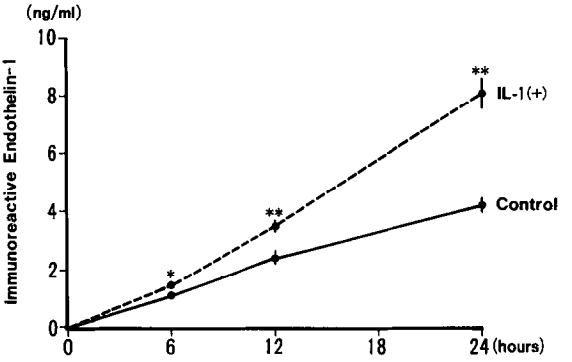

Figure 2: Time course of endothelin-1 production induced by IL-1. Endothelial cells were incubated with 100 ng/ml of IL-1β for the indicated times. After incubation, the levels of immunoreactive endothelin-1 in the culture supernatant were measured. The data are shown as the mean±SE, n=5. *p<0.05, **p<0.01 VS. control.

the expression of endothelin-1 mRNA by IL-1-treated endothelial cells. Induction of endothelin-1 mRNA began within 1 hr of exposure to IL-1α, peaked once at 4 hr and again after 24 hr, and then declined thereafter. A similar pattern was seen in IL-1β-treated endothelial cells. Previously, we have shown that endothelin-1 mRNA could be induced by either transforming growth factor-β (11) or low shear stress (12). However, the duration of mRNA induction by each of these stimuli was significantly shorter than that produced by IL-1.

We determined the level of immunoreactive endothelin-1 in the culture medium, to confirm that the induction of endothelin-1 mRNA resulted in increased synthesis and release of endothelin-1 peptide (Fig. 2). IL-1 stimulated the production of endothelin-1 peptide in a time-dependent manner; the increase was significant at 6 h and a further increase was noted after 12 and 24 h.

Because the maximal effect of IL-1 on the expression of endothelin-1 mRNA was seen at 24-30 h, we examined the dose-dependence of mRNA expression at 24 h. Both IL-1α and IL-1β stimulated endothelin-1 mRNA expression in a dose-dependent manner (Fig. 3). The IL-1α dose causing 50 % of the maximal response (ED_{50}) was approximately 10 ng/ml, which was lower than the ED_{50} for IL-1β (approximately 50 ng/ml). Although IL-1β was about 5-fold less potent than IL-1α, the maximum level of endothelin-1 mRNA induction produced by IL-1β did not differ from that due to IL-1α (data not shown).

A similar dose-response relationship for IL-1 was obtained from the ELISA assay data (Fig. 4). The concentration of IL-1α required to stimulate

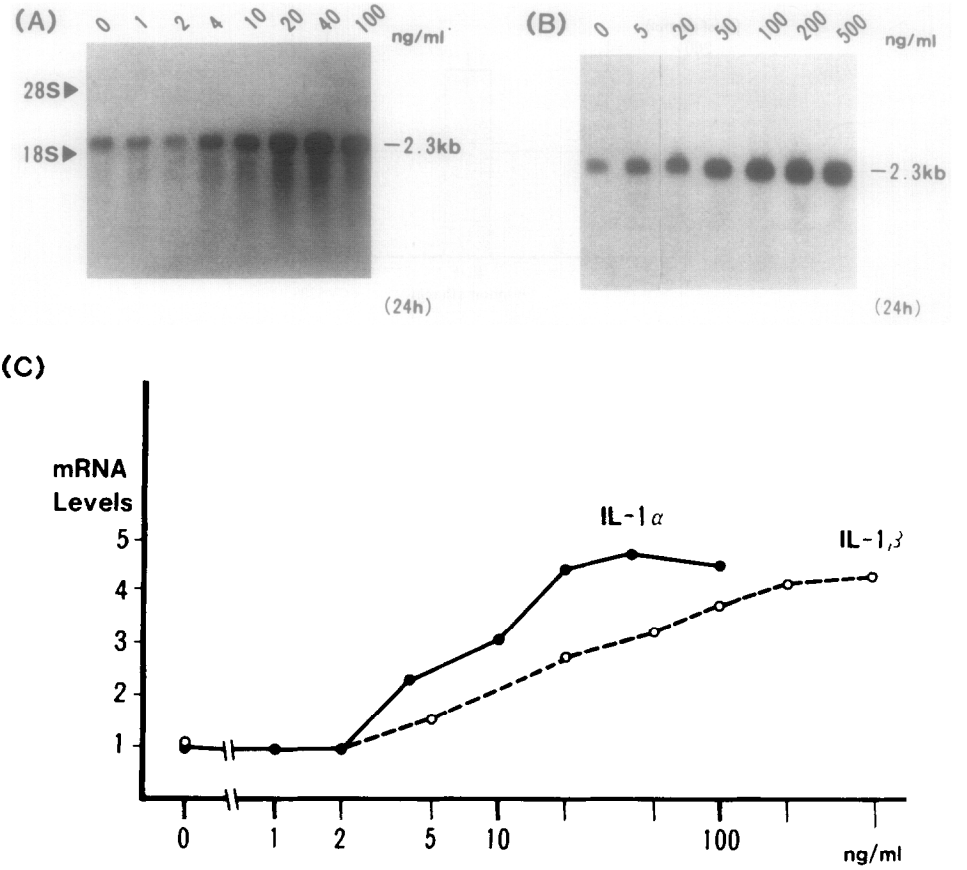

Figure 3: Expression of endothelin-1 mRNA in response to graded doses of IL-1. Endothelial cells were incubated with various concentrations of IL-1α (A) or IL-1β (B) for 24 h. Data are representative values of three individual experiments. The figure (C) shows the quantitative results of densitometry of the autographs.

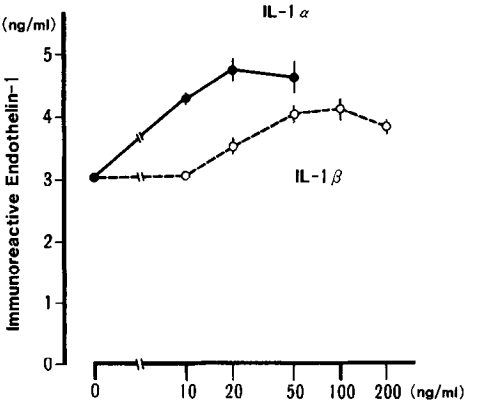

Figure 4: Effect of various concentrations of IL-1 on endothelin-1 production by endothelial cells. After incubation for 24 h, the levels of immunoreactive endothelin-1 in the supernatant were measured. The data are shown as the mean±SE, n=4.

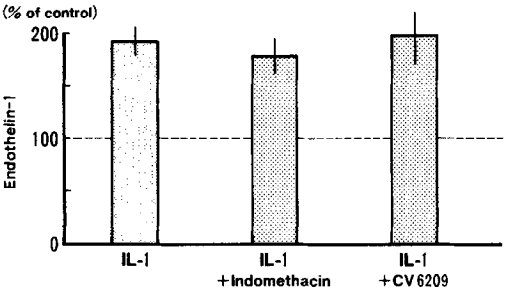

Figure 5: The effects of indomethacin and CV6209 on IL-1-induced endothelin-1 production. The effects of indomethacin ($3x10^{-6}$ M) or CV6209 (10^{-6} M) on the endothelin-1 production of endothelial cells induced by IL-1β (100 ng/ml) were examined. After incubation for 24 h, the levels of immunoreactive endothelin-1 in the supernatant were measured. The data are shown as the mean±SE, n=5.

maximum endothelin-1 production was significantly lower (20 ng/ml) than that for IL-1β (100 ng/ml). Treatment with higher concentrations (>100 ng/ml) of IL-1α resulted in endothelial cellular morphological changes (13) and decreased the production of immunoreactive endothelin-1.

Dejana et al. have examined the effects of different molecular species of IL-1 on the functioning of human umbilical vein endothelial cells (14). In agreement with our results, they reported that human recombinant IL-1β was less potent than IL-1α when calculated on a ng/ml basis.

As it is known that IL-1 can affect various cellular functions through the induction of prostaglandin or PAF synthesis (3,15), we examined the effects of indomethacin and CV-6209 (a PAF antagonist) on IL-1-induced endothelin-1 production. The concentration of CV-6209 used has been reported to be adequate for the suppression of PAF synthesis (10). Neither indomethacin nor CV-6209 suppressed IL-1-stimulated immunoreactive endothelin-1 production (Fig. 5), which suggested that it could be independent of both prostaglandin and PAF synthesis.

Although it is impossible to directly extrapolate these results to the *in vivo* situation, our study suggests that IL-1 produced by macrophages in vessel wall lesions may affect the contraction and proliferation of vascular smooth muscle cells by stimulating endothelin-1 production by endothelial cells. Thus, there may exist a new connection, "an IL-1-endothelin axis", between macrophages and smooth muscle cells. Further investigations are required into the relationship between this "IL-1-endothelin axis" and the pathogenesis of vasospasm or atherosclerosis.

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