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Rapid communication

Induction of E-selectin expression on vascular endothelium by digestive system cancer cells

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Introduction

The adhesion of circulating cancer cells to the vascular endothelium is an important step in the hematogenous metastasis of cancer. Carbohydrate antigens, such as sialyl Le^x and sialyl Le^a, are expressed on the surface of cancer cells, and E-selectin is expressed on the surface of endothelial cells to effect this adhesion.[1–3] The expression of E-selectin on endothelial cells is enhanced by inflammatory cytokines, such as IL-1β or TNF α.[4] In this connection, a report has been found stating that certain cytokines promote tumor cell adhesion to endothelial cells.[5] Recent evidence suggests that E-selectin is expressed on the endothelial cells of small vessels adjacent to cancer nests, and that serum E-selectin levels are elevated in patients with metastatic cancer.[6] Accordingly, it is surmised that cancer cells induce the expression of E-selectin on the endothelium either directly or indirectly. However, there are few studies of the interaction between cancer and endothelial cells.[7,8] Hence, our investigation focused on this interaction, i.e., the cancer cells of the digestive system acting upon vascular endothelium in the expression of E-selectin.

Materials and methods

Twenty-one cell lines derived from cancers of the human digestive system were examined: esophageal cancer (TE-1, TE-2, TE-3, T.Tn), gastric cancer (NUGC-3, NUGC-4, MKN74, MKN45), colon cancer (WiDR, Colo 201, SW1116, SW480, SW1083), hepatic cancer (Alexander, Hep G2, SK-Hep-1), and pancreatic cancer (SW1990, Capan-1, Capan-2, PANC-1, PaCa-2). Human umbilical vein endothelial cells (HUVECs) were obtained from Kurabou Co. (Osaka, Japan) and maintained in Daigo's T medium (Nissui Pharmaceutical Co., Tokyo, Japan) supplemented with 2 ng/ml of recombinant basic fibroblast growth factor (Takeda Pharmaceutical Co., Juso, Japan) and 10% fetal calf serum.

The cancer cells (10^5/well) were incubated on 24-well plates for 24 h, after which time, peripheral blood mononuclear cells (PBMC) (5×10^4/well), obtained from the blood sample of a healthy donor by the use of a separating solution (Nycomed Pharma AS, Oslo, Norway), were added to the supernatants of the cancer cell culture media, and cultured at 37°C. The supernatants without added PBMC were stored at 4°C. After 24-h incubation, the supernatants of the cancer cell media with and without PBMC were applied to HUVECs cultured on 12-well plates. Recombinant interleukin-1β (rIL-1β) (2 ng/ml) (kindly provided by Otsuka Pharmaceutical Co., Tokushima, Japan) was added to the culture medium of the HUVECs as a positive control. Anti-IL-1α, IL-1β, and TNFα neutralizing polyclonal antibodies (Otsuka Pharmaceutical Co.) were added to the culture media. Four h later, the HUVECs were harvested with a 0.02% EDTA-0.12% trypsin solution. Flow cytometric analysis was performed with a FACScan (Becton Dickinson Immunocytometry Systems, Mountain View, Calif.). An indirect immunofluorescence method was employed to stain the HUVECs with anti-E-selectin monoclonal antibody (British Biotechnology Ltd., Abington, UK) as the primary antibody (stained for 30 min at room temperature), followed by the addition of fluorescein isothiocyanate-labeled rabbit anti-mouse immunoglo-

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bulin (Silenus Laboratories, Hawthorn, Australia) as the secondary antibody.

Results and discussion

Unstimulated HUVECs failed to express E-selectin. When HUVECs were stimulated with 2 ng/ml of rIL-1β for 4 h, the E-selectin expression was induced on the HUVECs. The addition of the culture medium of PBMC had no detectable effect on the expression of E-selectin on HUVECs (Fig. 1). The induction of E-selectin expression on HUVECs was observed only in the culture medium of NUGC-3 cells without PBMC. This induction of E-selectin was inhibited by treatment with the anti-IL-1α antibody, but not by treatment with the anti-IL-1β or anti-TNFα antibodies. The expression of E-selectin induced by the culture medium of NUGC-3 cells with PBMC was greater than the expression of E-selectin without the medium; the expression was inhibited by treatment with anti-IL-1β antibody but not by treatment with IL-1α or anti-TNFα antibody. Thus, it appeared that cytokines acting on the induction of E-selectin on endothelial cells were different for the direct and indirect induction in NUGC-3 cells (Fig. 2). No induction of E-selectin expression was found with the culture media of the other cell lines without PBMC, except for NUGC-3 cells. However, the expression of E-selectin on HUVECs was considerable in 17 of the cell lines to which PBMCs had been added. PANC-1, Capan-1, and PaCa-2 cells were devoid of any detectable effects on the expression of E-selectin, despite the addition of PBMC to their culture medium. That is, all cell lines in which E-selectin expression was not induced were derived from pancreatic cancers. Unfortunately, from the results of this study, we cannot explain why this is so. To evaluate the contribution of cytokines to the indirect induction of E-selectin by cancer cells, we performed inhibitory experiments, using anti-cytokine neutralizing antibodies. As shown in Fig. 3, the induction of E-selectin by the culture media of cancer cells with added PBMC was inhibited by anti-IL-1β or anti-TNFα antibody. The blocking effect of the anti-IL-1β antibody on the induction of E-selectin was stronger

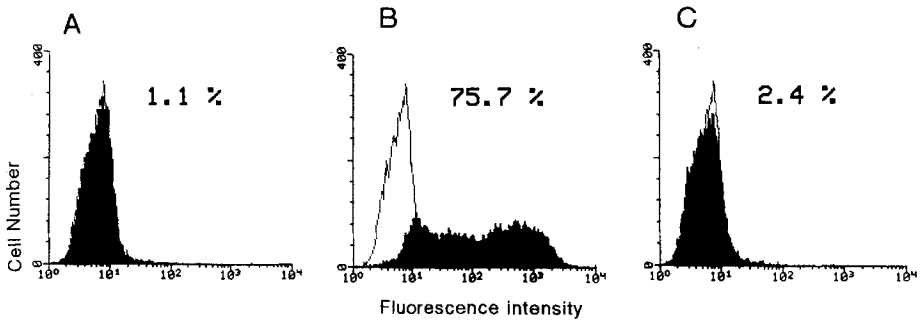

Fig. 1A–C. Expression of E-selectin on HUVECs. **A** Unstimulated HUVECs failed to express E-selectin. **B** When HUVECs were stimulated with rIL-1β, the expression of E-selectin was induced. **C** The addition of the culture medium of peripheral blood mononuclear cells (*PBMC*) did not induce the expression of E-selectin. The *percentages* indicate the rates of cells positive for E-selectin expression compared with the negative control

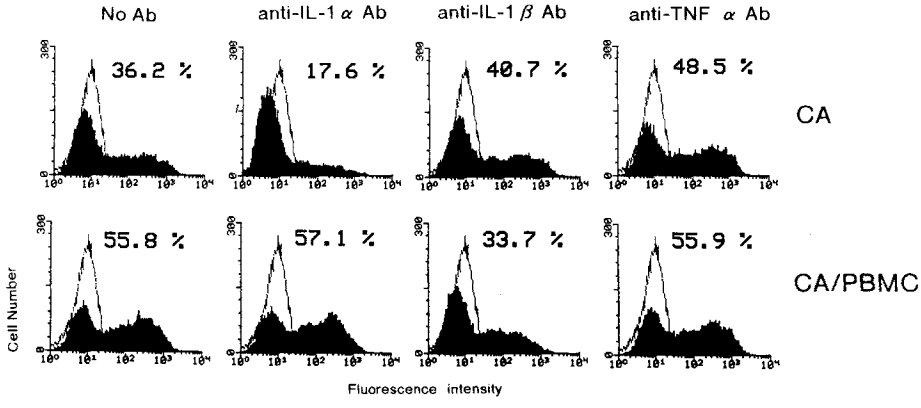

Fig. 2. E-selectin expression on HUVECs induced by the culture media of NUGC-3 cells and inhibition of this expression by anti-cytokine neutralizing antibodies. *Ab*, Antibody;, *CA*, treatment of HUVECs with culture media of cancer cells; *CA/PBMC*, treatment of HUVECs with culture media of cancer cells supplemented with PBMC

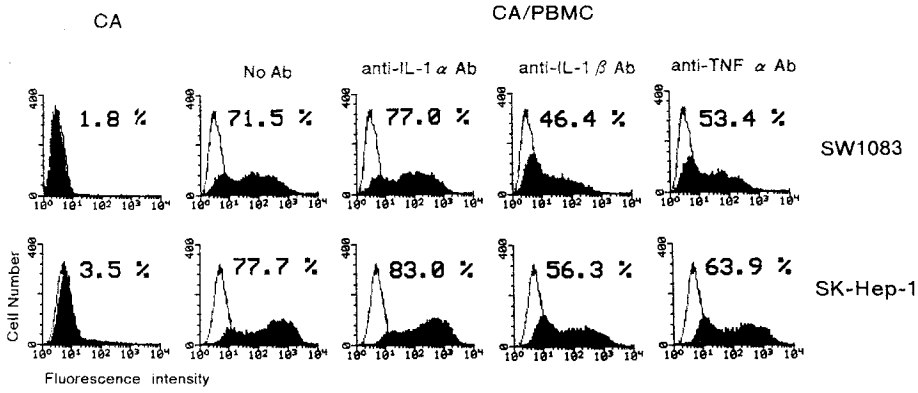

Fig. 3. E-selectin expression on HUVECs induced by culture media of SW1083 and SK-Hep-1 cells, and inhibition of this expression by anti-cytokine neutralizing antibodies

than that of the anti-TNFα antibody. No inhibitory effect was found with the anti-IL-1α antibody. These findings were also common to TE-1, MKN74, Colo 201, Capan-2, and SW1990 cells (data not shown).

Our present study has revealed that most digestive system cancer cells induce the expression of E-selectin on the vascular endothelium indirectly, through the release of IL-1β or TNFα from PBMC. In this connection, it has been reported that some types of cancer cells actually produce cytokines, such as IL-β or TNFα.[9,10] We found that only NUGC-3 cells induced the expression of E-selectin on HUVECs without PBMC, but the induction of E-selectin expression with PBMC supplementation in the culture medium of cancer cells was observed in 17 of the 20 digestive system cancer cell lines. From this finding, we conclude that cancer cells, by releasing cytokines from PBMC, induce the expression of E-selectin on the endothelium indirectly rather than directly.

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