Effect of Cytokines on the Expression of Cell Adhesion Molecule and on the Adhesion of Melanoma Cells to Endothelial Cells

Se Jong Kim, M.D., Nam Soo Kim, M.D., and Jung Lim Lee, M.D.

Department of Microbiology, Yonsei University College of Medicine, Seoul, Korea

We examined the role of cell adhesion molecules (CAM) by which tumor cells bind to the endothelial cells using human umbilical vein endothelial cells (HUVEC) and cultured melanoma cells. Endothelial cells from human umbilical veins were isolated and examined for CAM expression and its modulation by tumor necrosis factor- α (TNF- α), interleukin-1 (IL-1), interleukin-6 (IL-6) or interferon- γ (IFN- γ). The expression of intercellular adhesion molecule 1 (ICAM-1) on HUVEC was increased by TNF- α , IL-1 and IFN- γ when measured by ELISA or flow cytometric (FACS) analysis. IL-6 did not increase ICAM-1 expression on HUVEC. Two melanoma cell lines, Malme-3M and SK-Mel-28, showed increased expression of ICAM-1 after treatment with TNF- α , IL-1 and IFN- γ in FACS analysis. IFN- γ induced increased expression of HLA-DR only in SK-Mel-28 melanoma cells, not in Malme-3M melanoma cells. Neither HUVEC nor melanoma cells expressed lymphocyte function-associated antigen 1 (LFA-1) in either the basal (i.e., cytokine untreated) condition or the cytokine treated condition. Melanoma cells showed minimal increment in adhesion to TNF- α or IL-1 treated HUVEC than to cytokine untreated HUVEC. HUVEC and melanoma cells did not express LFA-1 and increased ICAM-1 expression by TNF- α , IL-1 and IFN- γ treatment in FACS analysis did not coincide with minimal increase of melanoma cells adhesion to cytokine treated HUVEC. These results suggest that adhesion between melanoma cells and HUVEC is probably mediated by molecular interaction other than ICAM-1/LFA-1.

Key Words: HUVEC-melanoma cell adhesion, ICAM-1, LFA-1, TNF- α , IL-1, IFN- γ

INTRODUCTION

The majority of deaths in cancer patients might be caused not by the primary tumor but by its metastasis. Understanding the process by which malignant tumor cells spread throughout the body has therefore become a major goal of cancer research. Metastasis

is a multistep process involving the release of cancer cells from a primary tumor and their entry into circulation, where they are disseminated to distant organ sites to form secondary tumors. Interaction of tumor cells with endothelial cells has been considered an important initial process of complex metastatic cascade. It has been postulated that specific recognition mechanisms might exist for the adhesion of endothelial cells and tumor cells (Nicolson et al., 1988). However, it remains unclear what adhesion mechanism between tumor cells and endothelial cells is involved in the metastatic process. Even though several endothelial cell-derived cell adhesion molecules (CAMs), such as intercellular adhesion molecule 1 (ICAM-1) (Springer, 1990), endothelial leukocyte adhesion molecule 1 (ELAM-1) (Bevilacqua et al., 1989), and vascular cell

Address for correspondence: Se Jong Kim, Department of Microbiology, Yonsei University College of Medicine, 134 Shinchon-dong Seodaemoon-ku, Seoul 120-752, Korea. Telephone: (02) 361-5280 This work was supported by a research grant (1991) from the Sam Mi Cultural Foundation on behalf of the Korean Medical Association and in part by CMB-YUHAN (1989-4).

adhesion molecule 1 (VCAM-1) (Osborn et al., 1989), seem to be related in part of the adhesion between endothelial cells and tumor cells, none of these cell adhesion molecules have been proven to be related to the metastatic process (Lee et al., 1992).

In vivo and *in vitro* comparisons between normal cells or benign tumor cells and malignant tumor cells suggest that alterations of surface molecules accompany neoplastic transformation (Christos et al., 1990), and there is marked phenotypic heterogeneity among primary and metastatic tumors, even among different metastatic lesions in the same patient (Donatella et al., 1986). Many attempts have been made to clarify the correlation between these phenotypic changes in tumor cells and their metastasizing properties, tumor progression, and the host immune response (Ruiter et al., 1984; Christos et al., 1990). It is likely that the tumor cell's repertoire of surface molecules may influence not only the host immune response but also the metastatic potential of tumor cells.

Cytokines such as tumor necrosis factor- α (TNF- α), interleukin-1 (IL-1) or interferon- γ (IFN- γ) are important modulators in endothelial cell functions such as hemostasis (Bevilacqua et al., 1984; Bussolino et al., 1986), vascular tone and permeability (Brett et al., 1989), and circulating cell recruitment (Carlos and Harlan, 1990; Springer, 1990). They could also modulate antigenic profiles of cancer cells (Christos et al., 1990; Roberta et al., 1990) and enhance the metastatic potentials of cancer cells (Pramila and Frances, 1987). Here we have examined the changes of CAM expression on both endothelial cells from human umbilical vein (HUVEC) and human melanoma cells after treatment with cytokines (TNF- α , IL-1, and IFN- γ), and we have evaluated the effect of TNF- α , IL-1, and IFN- γ on the adhesion of melanoma cells to HUVEC.

MATERIALS AND METHODS

Preparation and culture of endothelial cells

HUVEC was isolated from fresh umbilical cords by collagenase treatment and maintained in tissue culture as described previously (Jaffe et al., 1973; Kim and Kim, 1991). Briefly, the umbilical cord veins from normal placenta was perfused with warm basal medium consisting of RPMI 1640 (Hazleton, Lenexa, KS) supplemented with N-2-hydroxyethylpiperazine-N-2-ethane sulfonic acid (HEPES, Hazleton, Lenexa, KS) 15 mM/L, penicillin (120 mg/L), streptomycin (270 mg/L) and fungizone (2 mg/L) to wash out the blood and was allowed to drain. Its one end was clamped with a nylon tie and 0.1% type IV collagenase (Sig-

ma, St. Louis, MO) was then infused into the vein. The umbilical cord, whose both ends were clamped, was incubated at 37°C for 15 min. Collected cells were sedimented and resuspended in basal medium containing 20% fetal calf serum (Gibco, Grand Island, NY). The cells were plated into 1% gelatin-coated culture plates and maintained in 37°C, 5% CO₂ condition. Endothelial cells in culture were identified by their characteristic cellular morphology, growth pattern, and the presence of a factor VIII-related antigen. Factor VIII-related antigen was demonstrated by commercially available kits of the biotin-streptavidin system (BioGenex laboratories, San Ramon, CA), as described previously (Kim and Kim, 1991).

Melanoma cell lines

Human melanoma cell lines, Malme-3M and SK-Mel-28, were obtained from the American Type Culture Collection (ATCC, Rockville, MD) and maintained in the media recommended by ATCC. Malme-3M cells were maintained in the medium L-15 (Sigma, St. Louis, MO) containing 15% fetal calf serum, and SK-Mel-28 cells were maintained in the basal medium containing 10% fetal calf serum.

ELISA for ICAM-1 expression

HUVEC was plated on 96-well tissue culture plates and allowed to grow to confluence. They were then preincubated with culture media alone or with various concentrations of TNF- α (50-100 u/ml), IL-1 (5-100 u/ml) or IL-6 (5-100 u/ml) for 2 hours. Then the cells were fixed in 2% paraformaldehyde for 10 min at room temperature. They were then washed with phosphate buffered saline (PBS), incubated with 3% peroxide and then 0.1% bovine serum albumin (BSA) solution respectively for 1 hour at 37°C. The monoclonal antibody MAb 84H10 to ICAM-1 or an irrelevant control antibody was added to each well and the mixture was incubated for additional one hour. They were then washed with PBS and incubated for 1 hour with peroxidase-conjugated goat anti-mouse IgG. The wells were then washed again with PBS and 0.4% ortho-phenylenediamine substrate was added. The optical densities were measured by a Titertek ELISA reader (Flow Laboratories, Rickmansworth, Herts, UK) at 492 nm.

Flow cytometric analysis

HUVEC was grown to confluence and then stimulated with TNF- α , IL-1, and IFN- γ in a concentration of 200 u/ml. Added to cultures was only the fresh media without cytokines for the control cultures. After incubation for 20 hours, cytokine-treated or

cytokine-untreated cells were detached by incubation with 0.125% trypsin and phenotyped by indirect immunofluorescence (Anichini et al., 1986). The primary antibody was added and the cells were incubated on ice for 30 min, and washed with PBS. The FITC-conjugated secondary antibody was added. After another 30 min incubation, the cells were washed three times and resuspended in 1ml of PBS. Flow cytometric analysis of cell surface molecules was carried out using a FACStar (Becton Dickinson, Mountainview, CA). The following monoclonal antibodies were used as primary antibodies: MAb 84H10 (mouse IgG1) to ICAM-1 (Makgoba et al., 1988), MAb B8.12.2 (mouse IgG2b kappa) to HLA-DR (Hansen et al., 1978), MAb 25.3.1 (mouse IgG1 kappa) to LFA-1 (Fisher et al., 1986). MAbs were obtained from Immunotech S.A. (Marseille Cedex, France).

Melanoma cells were also phenotyped and analyzed in the same manner as the above procedures.

Melanoma cell-Endothelial cell adhesion assay

HUVEC was plated on gelatin-coated 96-well culture plates and allowed to grow to monolayer. They were preincubated with either culture medium alone or different cytokines at various concentrations for 4 hours and then washed three times with basal medium. Tumor cells were labeled with ^{51}Cr (New England Nuclear, Boston, MA) by incubating for 1.5 hours at 37°C. After labeling, tumor cells were washed three times with basal medium containing 12mM HEPES and 1% BSA, resuspended in basal medium and added to cytokine-treated or untreated endothelial cell monolayers. After incubation for 30 min at 37°C, the plates were carefully washed three times to remove nonadherent cells. Remaining adherent cells were then lysed with 1% Triton X-100 (Sigma, St. Louis, MO), and harvested supernatants were counted in a gamma counter.

Results were expressed as follows

% adhesion =

$$\frac{\text{experimental release} - \text{spontaneous release}}{\text{maximum release} - \text{spontaneous release}} \times 100$$

where spontaneous release is assessed by incubating tumor cells in the absence of endothelial cells and maximum release is evaluated by incubating tumor cells in the presence of 1% Triton X-100.

RESULTS

Endothelial cell culture from human umbilical vein

Endothelial cells from the human umbilical cord were isolated by treatment with collagenase. When the

isolated cells were seeded on gelatin coated culture plates, clumps of 15-30 round cells could be seen. Proliferation of endothelial cells started at the periphery of isolated clumps of cells, and directed radially away from their center. After 7 days in primary culture, the cells reached to confluent monolayers. The cells were homogenous, closely opposed, flat and polygonal with round or oval nuclei containing several prominent nucleoli (Fig. 1-A). They were identified by their morphological cobblestoned appearance typical to endothelial cells and by demonstrating factor VIII-related antigen (Fig. 1-B). Namely, they showed a dot staining pattern localized in cytoplasmic granules, especially at the perinuclear region. The monolayered cells were detached with 0.125% trypsin treatment and were maintained in the basal medium containing 20% fetal calf serum. The cells were also grown to monolayer within 5-7 days. Experiments were performed with HUVEC after 2-6 passages to minimize the experimental variations.

Cytokine effects on the proliferation of HUVEC

To determine the effects of cytokines on HUVEC proliferation, HUVEC was seeded onto 1% gelatin-coated culture plates and cultured in the medium alone or in the medium containing TNF- α or IL-1 for 72 hours. There were no provoked proliferations of endothelial cells from gelatin coated culture plates by TNF- α or IL-1 as measured by MTT assay (Fig. 2-A & B). However, the cells with no gelatin coating plastic culture plates showed provoked proliferation by TNF- α or IL-1 in dose-dependent manner. After stimulation with cytokines, the endothelial cells were transformed to spindle shapes (data not shown). To clarify whether these changes were results from morphologic changes of endothelial cells or from proliferation of fibroblasts, examination of the factor VIII-related antigen in these cells was done and it could be observed that all of the cells were stained positively for the factor VIII-related antigen. Thus it was concluded that the shape of endothelial cells was changed to spindles by cytokines.

Cytokine effects on ICAM-1 expression of HUVEC

Since cytokine inducible CAM on endothelial cells has been implicated as adherence proteins mediating tumor cell binding to endothelial cells, the cell-surface expression of ICAM-1 and LFA-1 on HUVEC was measured in parallel with HLA-DR expression using ELISA and FACS analysis before and after cytokine treatment.

Treatment for 2 hours with TNF- α or IL-1 induced

Fig. 1-A Monolayered endothelial cells from human umbilical vein. The characteristic morphology of endothelial cells was shown. Seven days after plating. One percent gelatin coated culture plate and basal medium containing 20% fetal calf serum were used. $\times 320$

Fig. 1-B. Immunohistochemical test for factor VIII-related antigen in HUVEC. Cultured cells expressed factor VIII-related antigen in virtually 100% of cells. Note the cytoplasmic color change. $\times 320$

Fig. 2. Cytokine effect on HUVEC proliferation. Values are mean $\pm$ SEM of 4 independent experiments (A; TNF- α treatment) or mean of 1 triplicated experiment (B; IL-1 treatment).

Fig. 3. The effect of TNF- α on ICAM-1 expression of HUVEC. HUVEC was treated with various doses of TNF- α for 2 hours and ICAM-1 was measured by ELISA. Values are mean $\pm$ SEM of 9 independent experiments.

increased ICAM-1 expression by dose dependent manner, as measured by ELISA (Fig. 3 & 4). In contrast, IL-6 did not affect ICAM-1 expression on the HUVEC (Fig. 5).

For the FACS analysis, HUVEC was treated for 20 hours with a fixed dose (200 u/ml) of TNF- α , IL-1, or IFN- γ . This concentration was chosen on the basis of the results of ELISA. Since IL-6 showed no effect on the ICAM-1 expression of HUVEC, it was replaced to IFN- γ for further experiment. IFN- γ is known as a potent inducer of CAM and MHC molecules on various tumor cells (Michele et al., 1989; Roberta et al., 1990), and also known to enhance the metastatic potential of cancer cells in experimental animals (Pramila and

Frances, 1987). While TNF- α significantly increased the expression of ICAM-1 of HUVEC, IFN- γ induced little increment of ICAM-1 expression on HUVEC (Fig. 6-A). TNF- α appeared to be a more potent inducer of ICAM-1 than IFN- γ or IL-1. Expression of LFA-1 and HLA-DR was not induced on HUVEC by either unstimulated condition (medium alone) or the stimulation with various cytokines (Fig. 6-B & C).

Cytokine effects on expression of cell surface molecules on melanoma cells

In order to determine the effects of TNF- α , IL-1 and IFN- γ on the modulation of CAM on the melanoma cells, Malme-3M and SK-Mel-28 were treated for 20

Fig. 4. The effect of IL-1 on ICAM-1 expression of HUVEC. HUVEC was treated with various doses of IL-1 for 2 hours and ICAM-1 was measured by ELISA. Values are mean±SEM of 5 independent experiments

Fig. 5. The effect of IL-6 on ICAM-1 expression of HUVEC. HUVEC was treated with various doses of IL-6 for 2 hours and ICAM-1 was measured by ELISA. Values are mean±SEM of 3 independent experiments.

hours with a fixed dose (200 u/ml) of each cytokine, and CAM expression was measured by flow cytometry (Fig. 7, 8 & 9).

There was no significant difference in ICAM-1 expression between Malme-3M and SK-Mel-28 under the cytokine untreated state, and LFA-1 and HLA-DR were not expressed on both cell lines (Fig. 9). ICAM-1 expression on both cell lines was increased by treatment with TNF- α and IFN- γ , but not significantly by

IL-1 (Fig. 7). HLA-DR expression was increased only in the SK-Mel-28 cell line with 20 hour IFN- γ treatment, but not in the Malme-3M (Fig. 8). IFN- γ appeared to be the most active cytokine in modulating ICAM-1 expression of the melanoma cells and acted as an inducer of HLA-DR expression only in the SK-Mel-28 cell line.

Adhesion of melanoma cells to HUVEC

To determine the adhesion capacity of melanoma

Fig. 6. Modulation of ICAM-1 (A), HLA-DR (B), and LFA-1 (C) expression on HUVEC by $\text{TNF-}\alpha$ and $\text{IFN-}\gamma$. HUVEC was treated with 200u/mL of each cytokine for 20 hours and evaluated by cytofluorimetric analysis. Control indicates the expression of ICAM-1, HLA-DR and LFA-1 on HUVEC without cytokine treatment

cells to cultured HUVEC monolayer, HUVEC was treated with various doses of cytokines ($\text{TNF-}\alpha$ or IL-1), which showed the modulating activity of ICAM-1 on both HUVEC and melanoma cells. Under basal conditions (i.e., no cytokine treatment), Malme-3M (61.1% adhesion) adhered to endothelial cells more than SK-Mel-28 (34.8% adhesion) did. Adhesion of Malme-3M cells increased to 70.6% adhesion after $\text{TNF-}\alpha$ treatment of HUVEC for 4 hours compared to basal condition 62% adhesion (Fig. 10). However, the % increment of adhesion was very low and this increment was not dose dependent. Malme-3M adhesion to HUVEC treated with IL-1 for 4 hours started to show minimal increase from baseline 60.2% adhesion to 75% adhesion and this was dose dependent (Fig. 11). There was no increase in adhesion of SK-Mel-28 cells to HUVEC treated with either $\text{TNF-}\alpha$ or IL-1 for 4 hours (Fig. 10 & 11).

DISCUSSION

Cancer cell metastasis is a multistep process involving the release of cancer cells from a primary

tumor and their entry into blood circulation, and their dissemination to distant organ sites. Subsequently, these cancer cells cross the vessel wall to enter the extravascular tissue and proliferate and form the secondary tumors (Nicolson, 1988; Zetter, 1990). It has been generally accepted that metastatic tumor cells adhere preferentially to the extracellular matrix underlying the vascular endothelial cells, and specific receptors for subendothelial components have been identified (Kramer et al., 1980; Liotta, 1984). However, during the metastatic spread, the restraint and attachment of tumor cells to the vascular endothelium precedes their extravasation and, adhesion of cancer cells to the endothelium appears to be a prerequisite for their escape from blood circulation (Liotta, 1984). The presence of a specific recognition mechanism between the endothelium and tumor cells has been hypothesized (Nicolson, 1988), and recent *in vitro* studies suggest that endothelial cells can be activated to express surface factors that mediate their adhesive interaction with circulating cells (Gamble et al., 1985). However, the molecules involved in adhesion

Fig. 7. Modulation of ICAM-1 expression on melanoma cell lines (A; Malme-3M, B; SK-Mel-28) by TNF- α , IL-1 and IFN- γ . Melanoma cells were treated with 200u/mL of each cytokine for 20 hours and evaluated by cytofluorimetric analysis. Control indicates the expression of ICAM-1 on the melanoma cell lines without cytokine treatment.

Fig. 8. Modulation of HLA-DR expression on melanoma cell lines (A; Malme-3M, B; SK-Mel-28) by TNF- α , IL-1 and IFN- γ . Melanoma cells were treated with 200u/mL of each cytokine for 20 hours and evaluated by cytofluorimetric analysis. Control indicates the expression of HLA-DR on the melanoma cell lines without cytokine treatment.

Fig. 9. Cytokine effect on CAM expression of melanoma cells. A; Malme-3M, B; SK-Mel-28. Values are mean $\pm$ SEM of 2 or 4 independent experiments.

of tumor cells to endothelial cells remains unclear. Cytokines are important regulators of endothelial cells and tumor cells. TNF- α or IFN- γ induces the loss of components in the basement membrane and in-

duces the overlapping of cells (Alan et al., 1986). The endothelial cells stimulated by TNF- α or IL-1 show increased adhesion with neutrophils (Moser et al., 1989), and T lymphocytes (Jordan et al., 1987). TNF- α also

Fig. 10. Effect of TNF- α on melanoma cell binding to HUVEC. Values are mean $\pm$ SEM of 3 independent experiments.

Fig. 11. Effect of IL-1 on melanoma cell binding to HUVEC. Values are mean $\pm$ SEM of 2 independent experiments.

increases the expression of MHC antigens and various adhesion molecules on the surface of endothelial cells (Collins et al., 1986; Jordan et al., 1987). Two main effects of cytokines on tumor cells are direct anti-proliferative activity and modulation of molecules which play a role in the interaction of tumor cells with the immune system. Among the most important cell-surface molecules involved in the generation and regulation of immune responses, HLA class-I, -II, and ICAM-1 are reactive to cytokine-mediated modulation (Anichini et al., 1986; Rothlein et al., 1988; Roberta et al., 1990). The release of cytokines at the sites of inflammation and immune response cause cell activation and result in augmented expression of adhesion molecules on the surfaces of host cells and tumor cells (Rothlein et al., 1988). Cytokines also affect malignant progression by modulation of CAMs on tumor cells, and it is likely that the tumor cell's repertoire of surface molecules may influence not only the immune response but also metastatic characteristics of tumor cells.

In our study, TNF- α or IL-1 induced proliferation of HUVEC in a dose-dependent manner only under the condition of no gelatin coated on plastic surface. It was likely that rapid proliferation of cells from gelatin-coated culture plates might mask the proliferative effects of cytokines, and actually there was no difference in proliferation between the unstimulated and the cytokine stimulated group in gelatin-coated plates. However, in the absence of gelatin, the proliferative effects of cytokines directly resulted in increased numbers of HUVEC. TNF- α or IL-1 also induced morphological change to spindle shape in endothelial cells, but it is not certain what made the cells changed in terms of morphology, and further studies should be performed to investigate the mechanisms of the morphological change.

The effects of cytokines such as TNF- α , IL-1, and IL-6 on expression of CAM on HUVEC was examined. In our study, TNF- α and IL-1 treatment for 2 hours increased the expression of ICAM-1 on the surface of HUVEC in a dose-dependent manner, measured by

ELISA. In contrast, IL-6 did not affect ICAM-1 expression on HUVEC. In FACS analysis, TNF- α significantly increased the expression of ICAM-1 of HUVEC, which was correlated with that of ELISA, while IL-1 treatment for 20 hours at 200u/ml induced little increases of ICAM-1. IFN- γ treatment for 20 hours induced minimal increase of ICAM-1 on HUVEC. TNF- α appeared to be a more potent inducer of ICAM-1 than IFN- γ . This is consistent with the notion that the induction of ICAM-1 expression after IFN- γ treatment was much slower than that after TNF- α treatment (Dustin and Springer, 1988). Even though IL-1 was a strong inducer of ICAM-1 expression on HUVEC when HUVEC was treated for 2 hours with IL-1, the expression of ICAM-1 returned to basal level after 20 hour treatment with IL-1. HUVEC did not express either LFA-1 or HLA-DR in basal condition (i.e., unstimulated condition). The expression of HLA-DR and LFA-1 on HUVEC was induced by treatment with TNF- α , IL-1 or IFN- γ . Although HLA-DR on HUVEC was not induced by treatment with 200 u/ml of IFN- γ for 20 hours in our study, the increased expression of HLA-DR on human dermal microvascular endothelial cells (HDMEC) was reported at higher concentration (500u/ml) and longer duration (72 hours) of IFN- γ treatment by other investigators (Swierlick et al., 1991).

The surface molecules of two melanoma cell lines, Malme-3M and SK-Mel-28 showing different metastatic potentials, were also measured. Flow cytometric analysis of both of the melanoma cell lines revealed negligible difference in basal level ICAM-1 expression. After stimulation with TNF- α , IL-1, or IFN- γ , the levels of ICAM-1 in both melanoma cell lines were increased. LFA-1, which reacts with ICAM-1, was not expressed on melanoma cell surfaces either constitutively or when stimulated with cytokines. TNF- α and IL-1 did not influence the expression of HLA-DR in either melanoma cell line. HLA-DR expression was induced by IFN- γ only in SK-Mel-28, not in Malme-3M, suggesting that Malme-3M lacks the machinery to respond to this cytokine and that the reactivity to cytokines varies from cell line to cell line. TNF- α and IFN- γ showed different modulating activities of ICAM-1 expression on HUVEC and melanoma cells. While TNF- α acts as the most active cytokine for enhancing the ICAM-1 expression in HUVEC, IFN- γ appeared to be the strongest enhancer of ICAM-1 on melanoma cells.

It has been demonstrated that various cancer cells can attach to endothelial cells *in vitro* and *in vivo* (Auerbach et al., 1987; Dejana et al., 1988; Rice et al., 1988), and the ability of cultured tumor cell lines to form metastases correlates with their ability of adhering to vascular endothelial cells *in vitro* (Auerbach et al.,

1987). This adhesion is significantly increased by treatment of endothelial cells with inflammatory cytokines such as IL-1 and TNF- α (Dejana et al., 1988; Rice et al., 1988). This phenomenon might be related to the increased leukocyte adhesion to cytokine activated endothelial cells (Carlos and Harlan, 1990) and could be related in part to the expression and *de novo* synthesis of the same endothelial cell-derived adhesion molecules such as ELAM-1 (Bevilacqua et al., 1989), VCAM-1 (Osborn et al., 1989), inducible cell adhesion molecule 110 (ICAM-110) (Rice and Bevilacqua, 1989), and ICAM-1 (Springer, 1990).

ICAM-1 is an adhesion molecule which is expressed mainly on the surface of endothelial cells and is detected on the surface of other fibroblasts, thymic epithelium, and mucosal epithelium (Dustin et al., 1986). Its molecular weight is about 90 kd, and it interacts with LFA-1 (Rothlein et al., 1988). ICAM-1 plays a role in lymphocyte trafficking by allowing ICAM-1 bearing endothelial cells to interact with LFA-1 on lymphocytes in inflammatory diseases (Barton et al., 1989) and also in malignant diseases (Johnson et al., 1989). Recently, it was reported that the incidence of positivity for ICAM-1 was higher in patients with malignancy than in patients with benign diseases and both the expression and shedding of ICAM-1 of malignant tumor cells were increased by IFN- γ treatment in a dose- and time-dependent manner (Tsujiaki et al., 1991). Also, elevated serum levels of ICAM-1 were demonstrated in patients with malignant melanoma (Ronald et al., 1991).

Increased expression of ICAM-1 on endothelial cells by TNF- α or IL-1 can increase the adhesion between endothelial cells and lymphocytes (Gamble et al., 1985; Moser et al., 1989). ICAM-110 expression is associated with melanoma cell adhesion to cultured endothelial cells and appeared to be involved in the adhesion of lymphocytes to endothelial cells or follicular dendritic cells (Freedman et al., 1990; Rice and Bevilacqua, 1989). ELAM-1 not only facilitates leukocyte adhesion to cytokine activated endothelial cells but also promotes the adhesion of some colon carcinoma cells to cytokine-treated endothelial cells (Bevilacqua et al., 1987). Thus the role of CAMs in malignant tumor cells can be explained by the adhesion mechanism between tumor cells and endothelial cells during the metastatic process. In other words, it is possible to suggest that the more expressed CAMs on endothelial cells or tumor cells, the more adhesion has occurred between tumor cells and endothelial cells. However, the exact role of these adhesion molecules in malignant metastatic process and the effects of inflammatory cytokines on these molecules, especially

in tumor cell-endothelial cell adhesion are not fully characterized, because little is known about the surface antigens of tumor cells and their heterogeneous expression and distribution in each cell.

In our study, the increase of Malme-3M adhesion to TNF- α or IL-1 treated HUVEC was low and the increase of SK-Mel-28 adhesion to TNF- α or IL-1 treated HUVEC was negligible. The increase of melanoma cell-HUVEC adhesion after treatment with TNF- α or IL-1 was relatively lower than those of melanoma cell-HUVEC or of melanoma cell-HDMEC after treatment with TNF- α or IL-1 by reports of other investigators (Lee et al., 1992). This discrepant result from the other report might depend on how vigorously the non-binding melanoma cells were washed out from cytokine treated endothelial cells. The increase of melanoma cell-HUVEC adhesion did not coincide with the result of increased ICAM-1 expression on both HUVEC and melanoma cells by TNF- α or IL-1. In addition to this result, the FACS analysis which showed the absence of LFA-1 on both HUVEC and melanoma cells, either constitutively or cytokine treated, could lead us to conclude that the adhesion between melanoma cells and HUVEC was probably mediated by molecular interactions other than ICAM-1/LFA-1. Our results indicate that the increased expression of ICAM-1 in malignant disease does not play a crucial role in the adhesion mechanism between endothelial cells and melanoma cells. The exact role of ICAM-1 and the cytokine effects to their expression in malignant progression remain to be evaluated. For the further understanding of metastatic processes, the advance in the study of various other molecules expressed on the surface of tumor cells and endothelial cells and also study about the effects of cytokines on those molecules should be done.

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