Influence of Tumor-derived Interleukin 1 on Melanoma-Endothelial Cell Interactions in Vitro


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ABSTRACT

Human melanoma cell lines that express high constitutive levels of the metastasis-associated marker intercellular adhesion molecule 1 (ICAM-1) were found to secrete interleukin 1 (IL-1) in vitro. Experiments with neutralizing antibodies showed that this cytokine was responsible for their expression of ICAM-1 but not that of two other progression/metastasis markers, Muc-18 and Gp IIb/IIIa. The IL-1 present in melanoma-conditioned medium induced the expression of vascular cell adhesion molecule 1, endothelial-leukocyte adhesion molecule 1, and ICAM-1 on human umbilical vein endothelial cells (ECs) in culture and increased the rate at which melanoma cells and ECs adhered to each other. IL-1-producing melanoma lines adhered significantly more rapidly to ECs than did non-IL-1-producing lines, and this enhancement was reduced by prior incubation of the melanoma cells with neutralizing anti-IL-1 antibodies. Similarly, endothelial cells treated with conditioned medium from IL-1-producing melanoma lines adhered significantly more rapidly to melanoma cells than did ECs treated with medium from non-IL-1-producing melanoma lines, and this enhancement was abolished by addition of anti-IL-1 antibodies to EC cultures in conditioned medium. Blocking antibodies to endothelial vascular cell adhesion molecule 1, endothelial-leukocyte adhesion molecule 1, and ICAM-1 failed to inhibit melanoma-EC adhesion, but an antibody to tumor cell GpIIb/IIIa did block adhesion by up to 44%. The ability to secrete IL-1 could increase the metastatic potential of melanoma cells by stimulating tumor cell-EC adhesion.

INTRODUCTION

The majority of deaths in cancer patients are caused not by the primary tumor but by their metastases. Understanding the process by which malignant tumor cells spread throughout the body has therefore become a major goal of cancer research. Metastasis is in part a nonrandom event, resulting from the selective growth of subpopulations of tumor cells endowed with specific prometastatic properties (1), the acquisition of which should be reflected in the expression of new phenotypic markers. Mabs2 have now identified three such markers, ICAM-1 (2, 3), GpIIb/IIIa (4, 5), and Muc-18 (6) in human malignant melanoma.

ICAM-1 (7), together with its ligand, the lymphocyte function-associated-1 antigen, mediates a wide range of adhesive interactions within the immune system (8). GpIIb/IIIa, a member of the integrin family of membrane receptors, plays a vital role in platelet aggregation (9). GpIIb/IIIa on activated platelets binds a variety of adhesive proteins including fibrogenin, fibronectin, von Willebrand factor, and vitronectin via a common peptide sequence, Arg-Gly-Asp (RGD) (9). A precise function of the Muc-18 molecule has yet to be identified, but it is a member of the immunoglobulin superfamily, suggesting a possible role in intercellular adhesion (10).

Permanent cell lines established from late primary and metastatic human melanomas manufacture IL-1 (11, 12), a pleiotropic cytokine with a broad spectrum of activities in immune, inflammatory, and acute phase responses (reviewed in Refs. 13 and 14). IL-1 induces ICAM-1 on vascular ECs and a variety of other cell types, including melanoma cells (15, 16, 39). IL-1 also induces ECs to express ELAM-1 (17) and VCAM-1 (18, 19) which normally mediate EC adhesion to neutrophils and lymphocytes, respectively, but can also serve as receptors for the binding of tumor cells (20, 21). Furthermore, recent in vitro and in vivo data demonstrate that IL-1 promotes the metastatic spread of melanoma cells (22), probably by increasing melanoma-EC adhesion (20, 23, 24). Taken together these observations suggest that the ability to synthesize IL-1 or another cytokine with a similar spectrum of activities (e.g., tumor necrosis factor α) could increase the metastatic potential of melanoma cells by inducing adhesion molecules on ECs (i.e., paracrine effects) or on the melanoma cells themselves (autocrine effects) which enhance melanoma-EC adhesion.

In this study we sought a causal connection between constitutive IL-1 synthesis and expression of ICAM-1, GpIIb/IIIa, and Muc-18 by a panel of human melanoma cell lines and investigated the influence of melanoma IL-1 and tumor and EC CAMs on melanoma-EC adhesion.

MATERIALS AND METHODS

Unless otherwise specified, all reagents were purchased from Sigma Chemical Co., Ltd. (Poole, England).

Cells and Culture Conditions. Seven human melanoma cell lines were used in this study: A375 (25); DX.3 (26); MJM (27); SK MEL23 (28); HMB.2; T8; and VUP (29). Cell lines were cultured in Eagle’s minimum essential medium supplemented with 10% fetal calf serum, 2.4 mm l-glutamine, 200 units/ml penicillin, and 100 μg/ml streptomycin. Cultures were maintained at 37°C in a humidified atmosphere of 90% air/10% CO2. For some experiments rIL-1α (20 IU/ml) or polyclonal anti-cytokine antisera [sufficient to neutralize 200 units/ml (200 NU/ml)] were added to cultures 24–96 h and 96 h before use, respectively (see "Antibodies and Cytokines").

HUVEC were isolated from umbilical cords as described previously (30). Cultures were maintained in Medium 199 (Gibco-Biocolt, Ltd., Paisley, Scotland) supplemented with Earle’s salts, 20% fetal calf serum, endothelial cell growth supplement (0.12 mg/ml), 0.09 mg/ml heparin, glutamine, and antibiotics at 37°C in 5% CO2 in air. The cells were passaged in tissue culture flasks precoated with 1% (w/v) gelatin (BDH, Poole, England) and were used at the third or fourth passage. Cultures were judged to be >95% EC by morphological criteria and by

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2 The abbreviations used are: Mab, monoclonal antibody; BSA, bovine serum albumin; CAM, cell adhesion molecule; CM, conditioned medium; CFSE, colony-stimulating factor; EC, endothelial cell; ELAM-1, endothelial-leukocyte adhesion molecule 1; ELISA, enzyme-linked immunosorbent assay; FACS, fluorescence-activated cell sorter; GAG, S-galactosidase-α-S-galactosidase; GM-CSF, granulocyte-macrophage colony-stimulating factor; HUVEC, human umbilical vein endothelial cells; ICAM-1, intercellular adhesion molecule 1; PBS, phosphate-buffered saline; rIL-1α, recombinant human interleukin 1; VCAM-1, vascular cell adhesion molecule 1; VWF, von Willebrand factor; NU, neutralizing unit(s).
positive staining of monolayers with anti-vWF Mab, F8/86 (31).

For some FACS analyses, IL-α (20 units/ml), melanoma CM (1:3, v/v), or anti-cytokine antisera (200 NU/ml) was added for 24 h before harvesting. For ELISAs and adhesion assays, similar pretreatment was carried out on HUVEC in the wells of microtiter plates.

Antibodies and Cytokines. The Mabs used in this study have all been described previously (5, 17, 31–37) and are listed in Table 1. Mabs H4/18 and Muc18-1 were generously provided by Dr. M. Bevilacqua (Department of Pathology, Harvard Medical School, Boston, MA) and Dr. J. P. Johnson (Institute for Immunology, Munich, Germany), respectively. P256 and DCI 4C7 were kind gifts from the Director’s laboratory, Imperial Cancer Research Fund (London). Concentrated culture supernatant F8/86 was purchased from DAKO Ltd. (High Wycombe, England) and used at 1:50 dilution. Mabs MRC-OX-7 (IgG1) and HSR-1 (IgG2a) were from Dr. A. Williams (MRC Cellular Immunology Unit, Oxford, England) and Dr. M. Pfreundschuh (Department of Medicine, Cologne, Germany); they were used at 20 μg/ml as nonbinding control antibodies in all assays. GAG immune complexes for ELISAs were prepared by mixing 150 μg purified DCI4C7 IgG with 0.5 mg (equivalent to 500 units) β-galactosidase (3.2.1.23) from Escherichia coli in 1 ml 0.05 M Tris buffer, pH 7.4, for 24 h at 4°C and remained stable at 4°C for up to 6 months (35).

Sheep anti-rat IL-1α antisera are described in the following section. Neutralizing sheep antisera of high titre against human IL-4 (564) and GM-CSF (567) were the generous gift of Dr. R. Thorpe (Division of Immunology, National Institute of Biological Standards and Control, South Mimms, Hertfordshire, England). rIL-1α (International Standard, preparation 86/632) was diluted to 10^6 units/ml in PBS, sterile filtered, and stored at −70°C until used.

Anti-IL-1 Antiserum and IL-1 Binding Assays. Polyclonal antiserum to rIL-1α and rIL-1β were raised in sheep by repeated i.m. injections of 200–500 μg rIL-1 as described previously. The anti-IL-1α antisem reacted with rIL-1α and peptide analogues of IL-1α but not with IL-1β, tumor necrosis factor α, granulocyte-CSF, GM-CSF, IL-2, or IL-6. The anti-IL-1α antisem reacted with rIL-1β and peptide analogues but not with IL-1α or any of the cytokines listed above. Sheep anti-IL-1α antisem neutralized the activity of 100 pg/ml (10 IU/ml) IL-1α in the EL-4 NOB-1/cytotoxic T-lymphocyte line bioassay when diluted 1/12,500. Anti-IL-1β antisem displayed similar neutralizing activity against IL-1β (100 pg/ml = 10 IU/ml) in the same assay when diluted 1/2500. For use in IL-1 neutralization experiments, anti-IL-1α antisem was diluted to a final concentration calculated to neutralize 2 ng/ml IL-1α in melanoma-conditioned medium was quantified using a sensitive immunodiagnostic assay as described previously (38). The lower limit of detection of the assay was 10 pg/ml (1 unit/ml). Melanoma CM samples were screened for immunoreactive IL-1β by immunoradiometric assay as described previously. The lower limit of detection of the assay was 40 pg/ml (4 units/ml). In each case the specific sheep antisem to IL-1α and IL-1β were used to detect cytokine in conditioned medium.

ELISA. Melanoma cells (5–10 × 10^5/ml) or HUVEC (2.5–5 × 10^5/ml) in their respective growth media were distributed in 200-μl volumes into the wells of 96-well flat-bottomed microtiter plates (Falcon 3072; precoated in 1% w/v gelatin for HUVEC cultures). Test reagents (100 μl/well) were added in triplicate, and the plates were incubated at 37°C for 2 h before washing and development with 0.1% w/v gelatin (350 μl/well). After two washes in PBS containing 0.2% (w/v) BSA and 0.2% (w/v) NaN3 (PBS-BSA-N3) were added to the wells of round-bottomed 96-well microtiter plates (Falcon 3910). Optimal dilutions of Mabs were distributed in 50-μl volumes, and the plates sealed. After 15 min, the cells were washed four times by centrifuging the plates at 800 x g for 30 s, removing the supernatants, and resuspending the cells in 150 μl/well PBS-BSA-N3. Rabbit anti-mouse IgG-fluorescein isothiocyanate (ICN, High Wycombe, England), diluted 1:10 in PBS-BSA-N3, was distributed in 50-μl volumes. The cells were incubated a further 15 min and washed as before, and cell-associated fluorescence was measured on a FACScan (Becton Dickinson, Fullerton, CA). Data were analyzed with the CONSORT 30 program.

Adhesion Assay. Melanoma cells were pretreated with rIL-1α (24 h) or a mixture of sheep anti-IL-1 and anti-IL-18 antisem (96 h) as described in "Cells and Culture Conditions." HUVEC were introduced into the wells of flat-bottomed 96-well plates (Falcon 3072) at confluent

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As reported by Paleolog et al. (40) and independently confirmed by us, the F8/86 signal was dependent on cell number alone and was not altered by any of the experimental treatments.

Crystal Violet Assay. Duplicate wells of dried melanoma cells as used in ELISAs were fixed with methanol for 10 min. After the plates were air-dried, 100 μl of crystal violet (0.1% w/v in distilled water) were added to each well for 10 min. The stained cells were washed in tap water until the washings were colorless. The stain was eluted with 33% (v/v) acetic acid (100 μl/well) and cellular material in the wells was estimated by absorbance at 620 nm on a Titertek Multiskan reader (Flow). These data were used to adjust test Mab readings from melanoma cell ELISAs to correct for variations in cell density according to the formula

\[
\text{Corrected fluorescence intensity} = \frac{\text{Test Mab signal} - \text{negative control Mab signal}}{\frac{F8/86 \text{ spinal} - \text{negative control Mab signal}}{100}}
\]

Indirect Immunofluorescence. Melanoma cells and HUVEC were prepared for FACS analyses as previously described (41). All manipulations were carried out at room temperature. Briefly, 50 μl of cell suspension at 2–3 × 10^6 cells/ml in PBS containing 0.2% (w/v) BSA and 0.2% (w/v) NaN3 (PBS-BSA-N3) were added to the wells of round-bottomed 96-well microtiter plates (Falcon 3910). Optimal dilutions of Mabs were distributed in 50-μl volumes, and the plates sealed. After 15 min, the cells were washed four times by centrifuging the plates at 800 x g for 30 s, removing the supernatants, and resuspending the cells in 150 μl/well PBS-BSA-N3. Rabbit anti-mouse IgG-fluorescein isothiocyanate (ICN, High Wycombe, England), diluted 1:10 in PBS-BSA-N3, was distributed in 50-μl volumes. The cells were incubated a further 15 min and washed as before, and cell-associated fluorescence was measured on a FACScan (Becton Dickinson, Fullerton, CA). Data were analyzed with the CONSORT 30 program.
RESULTS

Detection and Characterization of Melanoma-derived IL-1. CM from seven melanoma cell lines were analyzed for content of human IL-1α and IL-1β. The results are shown in Fig. 1. Only the CM from two lines, DX.3 and T8, contained significant amounts of IL-1α. DX.3 cells produced 170–220 pg/ml, and T8 cells produced 75 pg/ml. The levels of IL-1α in CM from the A375, HMB.2, MJM, SK23, and VUP lines were below the level of detection by radioimmunoassay (10 pg/ml).

DX.3 CM was concentrated 20-fold on an Amicon YM-2 ultrafiltration membrane and fractionated on a Sephacryl S-200 column. Each fraction was analyzed for induction of ICAM-1 on HUVECs by ELISA. All ICAM-1-inducing activity eluted in fractions 122–125, corresponding to an estimated molecular weight of 15,000–20,000 (Fig. 1, inset). All ICAM-1-inducing activity both in active fractions and unfractionated DX.3 CM could be abolished by prolonged exposure to trypsin, heat treatment (95°C, 10 min), and harvested onto glass fiber filters using a Titerkt cell harvester, and the radioactivity on the filters was measured using a liquid scintillation spectrometer (LKB 1205 Betaplate).

Expression and Regulation of ICAM-1 by Melanoma Cell Lines. As shown in Fig. 2, high constitutive ICAM-1 expression was restricted to the two lines DX.3 and T8, which secrete IL-1α (Fig. 1). Treatment with exogenous IL-1 did not increase ICAM-1 levels in these lines, possibly indicating that the amounts of IL-1 produced by the cells were sufficient for maximal ICAM-1 induction. Indeed, exogenous IL-1 actually decreased ICAM-1 expression by DX.3 and T8 cells, perhaps through a negative feedback mechanism. Four other lines (A375, MJM, SK23, and VUP), which had low levels of ICAM-1, showed 2–5-fold increases in ICAM-1 expression in the presence of exogenous IL-1. One line, HMB.2, did not express ICAM-1 either constitutively or when stimulated with IL-1, suggesting that it lacks the machinery to respond to this cytokine or that it cannot process ICAM-1. Sheep anti-IL-1 antiserum did not affect ICAM-1 levels in low-ICAM-1-expressing cell lines but, by contrast, significantly inhibited ICAM-1 expression by the IL-1-producing high-ICAM-1-expressing DX.3 and T8 lines. The neutralizing effect of the anti-IL-1 antiserum was specific since it did not neutralize other cytokines (see “Materials and Methods”). Furthermore, sheep antisera of irrelevant specificity (to IL-4 or GM-CSF) did not affect ICAM-1 levels in any lines (data not shown).

Since the ELISA will detect intracellular antigens, FACS analyses of ICAM-1 expression by the seven lines (with and without IL-1 or anti-IL-1) were carried out to determine whether ICAM-1 expression and regulation occurred at the cell surface. The results obtained were qualitatively the same as the ELISA data in all cases. For example, in the T8 line, pretreatment with neutralizing anti-IL-1 for 4 days caused a 4-fold decrease in mean fluorescence intensity when the cells were stained with anti-ICAM-1 Mab 6.5B5 (Fig. 2, inset).

Expression and regulation of two other melanoma progression/metastasis markers, Muc18 and Gpllb/IIIa (CD41/CD61) were assessed alongside ICAM-1. Although the levels of both markers varied among the lines in the panel, no evidence of an influence of exogenous or endogenous IL-1 was found (data not shown).

These findings indicate that apparent constitutive expression of the metastasis-associated marker ICAM-1, but not Muc-18 or GpIb/IIia, in human melanoma cell lines might well be dependent upon autologous IL-1 secretion in vitro.

Induction of Endothelial Cell CAMs by Melanoma-derived IL-1. CM from the melanoma panel were assayed by ELISA for their ability to induce the cytokine-regulated CAMs, ICAM-1, ELAM-1, and VCAM-1, in HUVECs. As shown in Fig. 3, IL-1α-containing DX.3 and T8 CM upregulated all three CAMs in HUVEC in a manner analogous to that of rIL-1α. In each case, induction was blocked by addition of neutralizing sheep anti-IL-1 to the HUVEC/CAM cultures but not by the control antibodies anti-IL-4, anti-IL-6, or anti-GM-CSF (results not shown). By contrast, HUVEC incubated with CM from the five non-IL-1-producing lines exhibited ICAM-1, ELAM-1, and VCAM-1 levels that did not differ significantly from those of control HUVEC incubated with unconditioned medium (Fig. 3). T8 and DX.3 CM (Fig. 3, inset) also induced significant up-regulation of the three CAMs on the HUVEC cell surface, and this induction was blocked by anti-IL-1 antiserum. Furthermore, the kinetics of induction of HUVEC ICAM-1, ELAM-
MELANOMA-DERIVED IL-1 AND ENDOTHELIAL CELLS

Fig. 2. Expression of ICAM-1 on melanoma cells and its regulation by IL-1. Specific binding of anti-ICAM-1 Mab 6.5B5 to melanoma cell lines after culture in regular medium (■), medium containing rIL-1α for 24 h (■), or neutralizing sheep anti-human IL-1 antiserum for 96 h (■). Binding was determined by GAG ELISA. Measurements were made in triplicate. Columns, arithmetic mean; bars, 2 x SEM. Inset, cell surface ICAM-1 expression by T8 cells measured by indirect immunofluorescence using the FACS. Cells were stained with anti-ICAM-1 MAb 6.5B5 after being left untreated (——) or being treated with rIL-1α (— — — —) or anti-IL-1 antiserum (——) as above. ·······, T8 cells stained with an irrelevant isotype-matched Mab DX-7.

Fig. 3. Induction of HUVEC adhesion molecules by CM from melanoma lines. Specific binding of Mabs anti-ICAM-1, 6.5B5 (■); anti-VCAM-1, 1.4C3 (■); and anti-ELAM-1, 1.2B6 (■) to HUVEC after treatment for 24 h (ICAM-1, VCAM-1) or 6 h (ELAM-1) with melanoma CM. Control cultures were treated with rIL-1α or fresh medium alone for the same time periods. Binding levels were measured by GAG ELISA. Columns, mean percentage of binding of reference Mab, anti-vWF F8/86; bars, 2 x SEM. Inset, cell surface binding of anti-ICAM-1 (——), anti-VCAM-1 (— — — —), anti-ELAM-1 (········), or negative control antibody OX-7 (——) measured by indirect immunofluorescence using the FACS. HUVEC were treated with DX.3 CM for 6 or 24 h before assay.

1, and VCAM-1 by DX.3 CM (Fig. 4, top) was the same as that of rIL-1α (Fig. 4, bottom).

Thus IL-1-producing melanoma cell lines are able to up-regulate cell surface levels of endothelial cell-CAMs which can serve as receptors for tumor cells (20, 21).

Effect of Autologous IL-1 on Melanoma-EC Adhesion. To assess the influence, if any, of tumor-derived IL-1 on melanoma-EC adhesiveness, a restricted panel of four lines, DX.3 and T8 (IL-1-producing) and A375 and HMB.2 (nonproducing) was selected for further study. Adhesion of untreated, IL-1-treated, and anti-IL-1-treated melanoma cells to HUVEC preincubated with regular medium, rIL-1α, or autologous melanoma CM was measured. The results are shown in Figs. 5 and 6.

The HUVEC cultures were confirmed as fully confluent immediately before each experiment to exclude the possibility that the melanoma cells were attaching to the subendothelial matrix rather than to the HUVEC themselves. Preincubation of HUVEC with DX.3 or T8 CM increased the subsequent rate of adhesion of DX.3 and T8 cells by approximately 2-fold and 3-fold, respectively. The time taken for 50% of DX.3 tumor cells to adhere (50% adhesion time) was reduced from 14 to 8 min by preincubating the HUVEC with DX.3 CM, and the 50% adhesion time for T8 cells was reduced from 20 to 7 min by preincubating the HUVEC with T8 CM (Fig. 5a). By contrast, A375 and HMB.2 cells adhered more slowly to unstimulated HUVEC with 50% adhesion times of 28 min or more, and pretreatment of HUVEC with A375 or HMB.2 CM had no effect on the rate of attachment of either line (Fig. 5b).

The enhancing effect of tumor-derived IL-1 on the adhesion of melanoma cells and HUVEC was mediated partly through an effect on the HUVEC and partly through an effect on the tumor cells themselves. With DX.3 cells, blocking of the autocrine IL-1 effects by pretreatment of DX.3 cells for 96 h with neutralizing anti-IL-1 antiserum increased the 50% adhesion time to unstimulated HUVEC from 15 to 26 min and to CM-treated HUVEC from 8 to 21 min (Fig. 6a). Blocking of the influence of IL-1 in DX.3 CM on the HUVEC (paracrine effects) increased 50% adhesion times to a somewhat lesser extent, from 8 to 15 min for untreated DX.3 cells and from 21
cells to unstimulated HUVEC by 33.0%, 34.4%, and 45.4%, respectively (Fig. 7a), and to rIL-1-stimulated HUVEC by 19.6%, 11.0%, and 34.9%, respectively (results not shown). Thus, a GpIIb/IIIa-related structure on melanoma cells appears to contribute to their ability to adhere to both unstimulated and IL-1-stimulated HUVEC in this system.

When blocking Mabs to VCAM-1, ELAM-1, and ICAM-1 were added to HUVEC pretreated with rIL-1α for 6 h, no inhibition of adhesion of any melanoma line was seen (Fig. 7b). The same results were found with unstimulated, 24-h IL-1-treated and DX.3 CM-treated HUVEC and with blocking Mabs added in pairs, or with the combination of all three (results not shown). Thus it appears probable that none of the previously characterized cytokine-inducible EC CAMs plays a significant role in melanoma adhesion to IL-1- and DX.3/T8 CM-stimulated HUVEC in this system, indicating that additional IL-1-regulated mechanisms contribute to tumor cell adhesion to activated EC.

DISCUSSION

The major findings to emerge from the present study are that IL-1 produced by melanoma cells induces ICAM-1 expression in an autocrine fashion on the melanoma cells themselves, induces ICAM-1, ELAM-1, and VCAM-1 expression on ECs,
calculated as for Fig. 5. The SD of triplicate measurements were always < 15% of
(b) to 111All . Melanoma cells were cultured in regular medium (•, •) or
and enhances melanoma-EC adhesion by a mechanism which is
and enhances melanoma-EC adhesion by a mechanism which is
apparently independent of the aforementioned CAMs. Since
adhesion of melanoma cells and ECs may be a critical step in
the process of metastasis, these findings may help to explain
the observed enhancement by IL-1 of metastasis formation in
animal melanoma models (22, 24).

The causal link between ICAM-1 expression and IL-1 pro-
duction established in this study could explain why ICAM-1 is not preferentially distributed in metastatic lesions of tumors
other than melanomas (3). Since IL-1 is rarely produced by
nonmelanoma tumor cells (11, 12, 42), it is probable that
ICAM-1 expression in most carcinomas is a passive event
resulting from induction by infiltrating leukocytes and is un-
related to the tumor cells themselves. In melanoma, it is possible
that ICAM-1 plays no direct part in metastasis but that both
ICAM-1 expression and increased metastatic potential are con-
sequences of melanoma cell IL-1 synthesis.

IL-1α produced by DX.3 and T8 melanoma cells was re-
ponsible for the observed melanoma and EC CAM induction be-
cause all of the inductive effects described and the kinetics of
EC CAM induction were precisely emulated by recombinant
IL-1α and abrogated by an anti-IL-1 antisemur which does not
neutralize the activity of any other cytokine. Biochemical analy-
sis revealed DX.3-derived ICAM-1-inducing activity to be me-
liated by a heat- and trypsin-sensitive polypeptide with molec-
ular weight of 15,000–20,000, in agreement with previous
reports of tumour-derived IL-1 (11, 12, 42).

Manufacture of IL-1 by DX.3 and T8 cells is probably con-
stitutive. It was thought possible that low levels of lipopolysac-
charide in culture media stimulated release of the cytokine (13),
but this is unlikely because only very low levels of lipopolysac-
charide (1.2–2.4 IU/ml were detected in a Limulus amoebocyte
lysate assay (43) (data not shown). Furthermore, if lipopolysac-
charide was present, it was at levels below that giving induction
of ICAM-1 on HUVEC or non-IL-1-secreting melanoma lines.

Although a link between IL-1 production and high ICAM-1 expression by melanoma cells was established, neutralizing anti-
IL-1 antibodies did not completely inhibit ICAM-1 expression
by DX.3 and T8 cells. This result indicates that synthesis of
basal levels of ICAM-1 is independent of endogenous IL-1 or
that the antibodies were unable to neutralize all IL-1 bioactivity
produced by the cells. The latter explanation is supported by
observations that, with other cytokines, autocrine growth stim-
ulation of tumor cells (44), their antiviral status (45), and
antigen expression (46) are incompletely blocked by specific
antibodies. It is possible that melanoma IL-1 triggers autocrine
ICAM-1 expression by interacting with its receptor in an intra-
cellular compartment inaccessible to the antibody or immedi-
ately after its appearance at the cell surface (47). Pretreatment
of DX.3 and T8 cells with IL-1 inhibitors having an intracellular
mode of action could resolve this issue (48).

Melanoma-derived IL-1α up-regulated ICAM-1, ELAM-1,
and VCAM-1 on the endothelial cell surface, as anticipated
from the known effects of IL-1 on ECs (7, 15, 17, 18, 33).
However, from adhesion blocking experiments it appeared that
these three molecules contribute little or nothing to the observed
increase in melanoma-EC adhesion induced by IL-1. The failure
of antibodies to ICAM-1 and ELAM-1 to block adhesion
accords with the findings of previous studies (21, 23, 24) and is
probably explained by the absence on melanoma cells of LFA-
1 and the ligand recognized by ELAM-1 on IL-1-activated
HUVEC. However, the failure of the anti-VCAM-1 antibody
4B9 to block melanoma-EC adhesion was unexpected since the
DX.3 line used in the present study expresses the VCAM-1
ligand VLA-4α4 and because another anti-VCAM-1 antibody,
E1/6, has been reported to block melanoma-EC adhesion (21).
It is possible that the two Mabs recognize independent func-
tional determinants of VCAM-1 and that only one of these
determinants (that bound by E1/6) is recognized by VLA-4α
on melanoma cells.

Tumor cell GpIIb/IIIa appears to contribute to adhesion
between HUVEC and the melanoma lines used in this study,
but its role is probably independent of IL-1 since melanoma
GpIIb/IIIa expression is not IL-1 regulated and anti-GpIIb/IIIa
Mab P256 did not abrogate the increase in melanoma-EC
adhesion attributable to IL-1. In tumor cell lines, GpIIb/IIIa in
part mediates adhesion to subendothelial matrix, platelets,
and ECs (51). Platelet adhesion results in the formation of a throm-
botic “cocoon” which is thought to protect tumor cells arrested
in the circulation during the early stages of extravasation (52).
The third melanoma progression/metastasis marker, Muc-18
was not regulated by IL-1, and anti-Muc-18 antibodies did not
block melanoma-EC adhesion, suggesting that Muc-18 is not
involved in this process. However, we do not at present know
whether the Muc-18–1 antibody blocks the binding of the Muc-
18 molecule on melanoma cells and its putative receptor on
other cells, and so a role for Muc-18 in melanoma-EC adhesion
cannot be formally excluded (10).

It should be stressed that IL-1 synthesis could affect tumorig-
genicity and metastatic spread of melanoma cells by mecha-

Fig. 6. Influence of tumor-derived IL-1 on adhesion of DX.3 (a) and T8 cells
(b) to HUVEC. Melanoma cells were cultured in regular medium (■, □) or
preincubated with sheep anti-IL-1 antiserum for 96 h (△, ○) and applied to
HUVEC monolayers which had been incubated with melanoma CM for 24 h in
the absence (■, □) or presence (△, ○) of anti-IL-1. Percentage adhesion
was calculated as for Fig. 5. The SD of triplicate measurements were always <15% of
the arithmetic mean value and are not shown.
nisms other than promotion of tumor cell-EC adhesion. Melanoma cell ICAM-1 as induced by autologous IL-1 may promote metastasis by mechanisms not involving ECs, such as inhibition of complement activation at the melanoma cell surface or the formation of large intravascular clusters of tumor cells and circulating leukocytes which become readily lodged in small capillary beds at secondary sites (49). IL-1-induced CAMs on ECs support inflammatory processes by regulating leukocyte attachment (8) and augment secondary localization of tumors at inflammatory sites (50). IL-1α, such as is produced by the tumor cells in the present study and in previous reports (11, 12), blocks IL-β-induced immunostimulation in vivo by competition for mutual receptor sites (53) and may be responsible for the local immunosuppression noted in patients with metastasizing melanoma (54). Finally, the catabolic activity of IL-1 contributes to local tumor invasion by induction of tissue-degrading enzymes (13), and its angiogenic (13) and melanoma mitogenic (12) activity suggest that IL-1 could stimulate tumor growth at secondary sites. Thus IL-1 production potentially contributes to malignant behavior at all stages of the metastatic process (1).

In conclusion, melanoma-derived IL-1 induces cell-cell adhesion molecules and increase reciprocal adhesiveness of melanoma and endothelial cells, suggesting that IL-1 confers upon melanoma cells the ability to create a prometastatic microenvironment for themselves. These findings support recent evidence from animal models (22, 24) and clinical trials (55) that therapy with inflammatory cytokines such as IL-1 in melanoma may be counterproductive and further indicate that inhibitors of IL-1 (48, 56) could have therapeutic value in this disease.

REFERENCES


29. Siracky, J., Blasko, M., Borovansky, J., Kovarik, K., Svec, J., and Vrba, M.


Influence of Tumor-derived Interleukin 1 on Melanoma-Endothelial Cell Interactions *in Vitro*


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