Heterogeneity in Human Melanoma Cell Adhesion to Cytokine Activated Endothelial Cells Correlates with VLA-4 Expression

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Abstract

Tumor cell attachment to endothelial cells (EC) is one of the critical steps of the metastatic process. It was previously reported that interleukin 1 treatment of EC induces expression of membrane molecules that promote tumor cell adhesion. In this paper we report that a panel of six clones isolated from a human metastatic melanoma presented a marked heterogeneity in their ability to adhere to interleukin 1 activated EC. This was correlated with integrin VLA-4 expression by the clones. Antibodies directed to VLA-4 and to its endothelial ligand INCAM110/VCAM-1 abolished interleukin 1 induced increase in melanoma cell adhesion to EC. These data demonstrate intratumor heterogeneity in the expression of VLA-4 and that this can represent a crucial determinant of tumor cell interaction with EC during secondary spread.

Materials and Methods

Cells. EC were isolated and cultured from human umbilical vein as described previously (13). Melanoma cell clones from a human s.c. metastasis (Mel 665/2) were obtained and cultured as reported (14). Antibodies. mAb HP2/1 directed to VLA-4 subunit has been characterized previously (15); mAb B1E5 (16) directed to VLA-5 subunit was kindly donated by Dr. C. Damsky (University of California, San Francisco, CA); both of them were incubated with TC suspension at a 1:5 dilution.

mAb E1/6 (6) and mAb 4B5 (17) to INCAM110/VCAM-1 were obtained through the courtesy of Dr. M. P. Bevilacqua (Harvard Medical School, Boston, MA) and Dr. J. M. Harlan (University of Washington, Seattle, WA), respectively; mAb 6.5B5 (18) directed to ICAM-1 was generously donated by Dr. D. Haskard (Guy's Hospital, London, United Kingdom) and mAb H18/7 (19) to ELAM-1 was from Dr. M. P. Bevilacqua. mAbs were used to a final concentration of 1:10, except mAb 4B5 and mAb 6.5B5 at concentrations of 1:100 and 1:20, respectively.

Rabbit polyclonal antiserum to purified fibronectin (20) was added during the adhesion assay at 1:50 dilution.

Antibody concentration was selected to obtain maximal biological activity as described in the pertinent references.

Fluorescence flow cytometric analysis was performed by an EPICS C cytometer as described previously (12).

Adhesion Assay. EC were activated with 20 units/ml of human recombinant IL-1β (specific activity, 106 units/µg; Sclavo, Siena, Italy) for 6 h (3). mAbs directed to EC adhesive receptors were preincubated with EC for 30 min/37°C before addition of TC and then allowed to stay during the adhesion assay. 125I]-iododeoxyuridine (1 µCi/ml for 18 h; Amersham International, Amersham, United Kingdom) labeled melanoma cells were detached, washed twice with phosphate buffered saline, and finally resuspended in M199—20% newborn calf serum at 6 × 106 cells/ml as described (3). mAbs directed to integrins were added to TC 30 min before the adhesion test. Then an aliquot (50 µl) of radiolabeled TC suspension containing the mAbs was added to each well and incubated for 30 min at 37°C. Nonadherent cells were then removed by washing the plates three times with phosphate buffered saline plus 2% newborn calf serum. The content of each well was solubilized with 100 µl of NaOH-1% sodium dodecyl sulfate and the lysate was counted in a 125I gamma counter (Beckman, Fullerton, CA) as described (3).
Fig. 1. (A) Fluorescence flow cytometric analysis of VLA-4 integrin expression on melanoma clones from Mel65/2. Columns, percentage of positive cells for VLA-4 after subtracting the nonspecific background staining with an irrelevant antibody. Numbers inside columns, mean intensity of fluorescence (arbitrary units on a linear scale). The analysis was performed at least three times for each clone with comparable results. (B) Adhesion of human melanoma clones to IL-1 (20 units/ml, 6 h) stimulated EC for 30 min at 37°C. Results are expressed as percentage of control adhesion. Percent increment was calculated as (TC bound to stimulated EC − TC bound to untreated EC)/(TC bound to untreated EC × 100). Mean ± SD (bars) of 3 experiments, 6 replicates/experiment. *, P < 0.01 versus control adhesion; O, P < 0.01 versus adhesion to IL-1 activated EC. Statistical analysis by Dunnet’s test.

Results

Fig. 1 compares different clones of melanoma cells for the percentage and intensity of fluorescence of VLA-4 expression (Fig. 1A) and for IL-1 induced increment in adhesion to EC (Fig. 1B). The melanoma clones were quite heterogeneous for these parameters. However, a correlation was observed between VLA-4 expression and cell adhesion to IL-1 treated EC.

We then directly investigated the role of VLA-4 and of its EC ligand INCAM110/VCAM-1 by antibody inhibition studies. A VLA-4 mAb (Fig. 2) and an INCAM110/VCAM-1 mAb (Fig. 3) abolished IL-1 induced increment in adhesion of melanoma clones, except clones 2/14, 2/4, and 2/61 which were only partially inhibited by an INCAM110/VCAM-1 mAb. The data reported in Fig. 3 have been obtained with mAb H1/6 but comparable results were found with mAb 4B5.

Both mAbs slightly but consistently inhibited melanoma cell adhesion to untreated EC. This inhibition reached statistical significance for clones 2/4, 2/62, 2/61, and 2/60 with both antibodies. ELAM-1, ICAM-1, VLA-5, and fibronectin antibodies did not affect adhesion of melanoma clones to either untreated or IL-1 treated EC (data not shown).

Discussion

The characterization of the molecules involved in TC adhesion to the endothelium might help one to understand how TC localize and eventually metastasize in different organs. The data reported indicate that VLA-4 expression by clones of human melanoma cells correlates with the increase in adhesion induced by IL-1 treatment of EC and that a VLA-4 mAb can block this increment.

The recently identified EC ligand for VLA-4 was named INCAM110/VCAM-1 (6) and belongs to the immunoglobulin superfamily (7). INCAM110/VCAM-1 mAbs strongly inhibited cell adhesion to IL-1 activated EC. However, while the VLA-4 antibody was fully inhibitory with all the clones used, INCAM110/VCAM-1 antibodies in three cases (clones 2/14, 2/4, and 2/61) were only partially inhibitory. We do not have, at present, a direct explanation for this discrepancy but it is
conceivable that these clones recognize an additional VLA-4 ligand on activated EC. A possible candidate could be fibronectin since VLA-4 can also bind this protein and fibronectin is present in EC extracellular matrix and possibly on EC surface. However, anti-fibronectin antibodies did not affect adhesion of melanoma clones to either untreated or IL-1 activated EC. The possibility that the cells bind to other known IL-1 dependent EC adhesive antigens is also unlikely since ELAM-1 and ICAM-1 mAbs were ineffective. The existence of a second ligand for VLA-4 therefore remains unclarified and requires further and more direct studies.

VLA-4 and INCAM110/VCAM-1 antibodies slightly inhibited adhesion of melanoma clones to untreated EC. This observation is in agreement with previous reports indicating the presence of low but biologically significant amounts of INCAM110/VCAM-1 on the surface of resting EC (21, 22).

The biological relevance of VLA-4/VCAM-1 type of recognition in melanoma cell dissemination and metastasis remains to be elucidated.

IL-1 and TNF can be released in tissues and reach appreciable circulating concentrations during inflammatory reactions (23). Many cell types including vascular cells, macrophages, and TC of hematopoietic and nonhematopoietic origin have the potential to release these cytokines (23).

Circulating or locally released TNF and IL-1 can activate EC to express adhesive structures for TC and this in turn facilitates the metastatic process. We reported (5) that IL-1 treatment of cultured human endothelial cell. J. Clin. Invest. 92: 1466-1470, 1988.

References
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