American Journal of Pathology, Vol. 151, No. 1, July 1997

Inhibition of Colon Carcinoma Cell Lung Colony Formation by a Soluble Form of E-Selectin

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During metastasis, tumor cells adhere to vascular endothelia. E-selectin is an adhesive protein expressed by cytokine-activated endothelium that can support adhesion of colon cancer cells through the recognition of specific carbohydrate ligands. Using a series of colon carcinoma cell lines that displayed E-selectin adhesiveness and an increased metastatic capacity in cytokine-treated mice, we examined possible inhibition of cytokine-dependent experimental lung metastasis by a soluble form of E-selectin, the recombinant fusion protein E-selectin-immunoglobulin. We found that E-selectin-immunoglobulin bound to the surfaces of HT-29 colon carcinoma cells and blocked the formation of cytokine-inducible experimental lung metastases; control L-selectin-immunoglobulin also bound to HT-29 cells but had no effect on tumor cell lung colonization. E-selectin-immunoglobulin was found to interfere with E-selectin-dependent adhesion of HT-29 cells to activated vascular endothelium and to block the retention of these cells in the lung, a process that implies tumor cell adhesive interactions with the host vasculature. Our results demonstrate that E-selectin-immunoglobulin inhibits adhesion and formation of lung metastases by colon carcinoma cells and suggest that impairment of tumor cell-endothelium adhesion might represent a therapeutic approach to the metastatic diffusion of tumors. (Am J Pathol 1997, 151:233–243)

Adhesion of blood-borne tumor cells to the endothelial lining of vessel walls is thought to facilitate the arrest of metastatic cells in the microvasculature and colonization of organs.[1–3] A growing body of evidence indicates that cancer cells may adhere to vascular endothelium through the same molecules that sustain adhesion of leukocytes at sites of inflammation. In particular, recent reports indicated that certain types of tumor cells can interact with E-, P-, and L-selectin, a superfamily of adhesion molecules that support binding of leukocytes to the endothelium through the recognition of specific carbohydrate ligands.[4–8] E- and P-selectin can be expressed by activated vascular endothelium, whereas L-selectin is present on most leukocytes; P-selectin is also found on activated platelets.[4–8] Several observations showed that colon carcinoma cells could adhere to activated endothelium through E-selectin.[9–17] Subsequently, colon carcinoma cells were found to bind to P- and L-selectin[15]; in addition, P-selectin could support adhesion of lung cancer and neuroblastoma cells.[18] It was also demonstrated that colon cancers expressed certain carbohydrate structures, such as sialyl Lewis^x (sLe^x; NeuAcα2–3Galβ1–4[Fucα1–3]GlcNac—)[19,20] and sialyl Lewis^a (sLe^a; NeuAcα2–3Galβ1–3[Fucα1–4]GlcNac—),[21] which were known to act as ligands for the selectins.[22–30] In addition, sLe^x was found to be expressed in greater amounts on highly metastatic colon carcinoma lines than on their less metastatic counterparts.[31,32] Finally, a monoclonal antibody against sLe^a-related structures inhibited lung retention of colon carcinoma cells injected intravenously

Supported by funds from the Pew Charitable Trusts and the Howard Hughes Medical Institute. During the course of the majority of these studies, M. P. Bevilacqua was an investigator at the Howard Hughes Medical Institute and a Pew Scholar in Biomedical Science. G. M. worked in Dr. Bevilacqua's laboratory at the Howard Hughes Medical Institute as part of her Ph.D. Program in Experimental Pathology at the University of Florence.

Accepted for publication April 11, 1997.

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into nude mice.[33] Taken all together, these observations suggested that the selectins may play a role in the metastatic spread of tumor cells.

Several studies reported that soluble forms of the selectins could inhibit adhesion of leukocytes[34] and their extravasation at sites of inflammation.[35–39] In particular, an E-selectin-immunoglobulin fusion protein (E-selectin-Ig) was found to impair the accumulation of leukocytes in inflamed lung tissue.[36] Anti-inflammatory effects have also been reported with recombinant P-selectin-Ig[36,37] and L-selectin-Ig.[36,38,39] In this study, we used the soluble fusion protein E-selectin-Ig to inhibit adhesion and formation of experimental lung metastases by colon carcinoma cells. Experiments were performed using a series of human colon carcinoma cell lines that adhered to E-selectin and produced experimental lung metastases in mice stimulated with interleukin (IL)-1, a treatment that is known to induce the expression of E-selectin in tissues.[40] The data demonstrate that E-selectin-Ig can impair adhesion and organ colonization by colon carcinoma cells and expand our understanding of the participation of E-selectin in tumor metastasis.

Materials and Methods

Antibodies and Selectin-Ig Fusion Proteins

Monoclonal antibody (MAb) anti-sLe^a was purchased from Signet (Dedham, MA); anti-sLe^x MAb was produced in ascites fluid using CSLEX1 hybridoma cells (American Type Culture Collection, Rockville, MD). The following MAbs were generated by immunization of mice with cytokine-activated human endothelial cells: anti-E-selectin MAbs H18/7 (IgG2a, blocker of adhesion)[41] and H4/18 (IgG1, nonblocker of adhesion),[42] anti-VCAM-1 MAb E1/6 (IgG1),[9] and anti-ICAM-1 MAb E1/7 (IgG2a).[43] Unless otherwise stated, MAbs were used as affinity-purified or ammonium-sulfate-precipitated immunoglobulins. For adhesion blocking studies and immunobinding studies, MAbs were diluted in Dulbecco's modified Eagle's medium (DMEM; Gibco BRL, Grand Island, NY) containing 1% fetal calf serum (FCS; Hyclone, Logan, UT).

E- and L-selectin-Ig fusion proteins are recombinant chimeric molecules containing extracellular regions of E-selectin and L-selectin, respectively, coupled with the Fc portion of human IgG1.[44,45] Each selectin-Ig contains the lectin domain, epidermal growth factor domain, and two (L-selectin-Ig) or six (E-selectin-Ig) complement regulatory repeats of the parent molecules. These fusion proteins were prepared by transfecting BHK cells with cDNAs encoding E-selectin-Ig or L-selectin-Ig in pNUT vector (kindly donated by Dr. R. MacGillivray, University of British Columbia, Vancouver, British Columbia, Canada).[46] Transfected cells were seeded in plastic roller bottles (Corning, Corning, NY) and grown in DMEM/F12 medium (Gibco) added to ITS culture supplement (Becton Dickinson, Bedford, MA). Culture media of BHK cells were collected and replaced every 2 days. E- and L-selectin-Ig secreted into growth media were purified by affinity column chromatography using protein A Sepharose (Pharmacia Biotech, Piscataway, NJ) and prepared as stock solutions in Dulbecco's phosphate-buffered saline containing calcium and magnesium (DPBS). Protein purity was assessed by SDS-polyacrylamide gel electrophoresis followed by silver staining; protein concentration was determined using the bicinchoninic acid reagent (Pierce, Rockford, IL). Stock solutions were tested for the presence of endotoxin using a quantitative chromogenic *Limulus* amebocyte lysate assay (Whittaker Bioproducts, Walkersville, MD).

Cells and Culture Conditions

Primary cultures of human umbilical vein endothelial cells were obtained from Clonetics Corp. (San Diego, CA). Cells were grown in Medium 199 (Gibco) containing 20% FCS, 50 μg/ml endothelial cell growth supplement, and 100 μg/ml heparin (Sigma Chemical Co., St. Louis, MO) and subcultured (1:3 split ratio) using trypsin/versene (Gibco). For use in adhesion experiments, cells (passages 2 to 4) were grown to confluence in Nunclon Terasaki MicroWell plates (Nunc, Naperville, IL) coated with 0.1% gelatin (Fisher, Pittsburgh, PA).

The human colon carcinoma cell lines HT-29, LS 180, LS 174T, T84, COLO 320, Caco-2, and the human melanoma cell line Hs 294T were obtained from American Type Culture Collection and maintained in culture as recommended. BHK cells were grown by using DMEM/F12 medium containing 10% FCS and subcultured using versene (Gibco). In preparation for adhesion and immunostaining experiments, human tumor cells were harvested using trypsin-versene and suspended at 1×10^6 cells/ml in DMEM/1% FCS. For lung colony formation and lung retention experiments, tumor cells were suspended in DPBS at 5×10^6 and 2×10^6 cells/ml, respectively.

Isolation of a Colon Carcinoma Variant, Caco-2(E+), by Panning on Immobilized E-Selectin

Tissue culture dishes (10 cm diameter; Corning) were coated with recombinant protein A (10 μg/ml, 4°C, 12 hours) (Chemicon, Temecula, CA) using a solution of 50 mmol/L carbonate/bicarbonate buffer, pH 9.5. After three washes with DPBS, plates were incubated at room temperature for 1 hour with a solution of 1% bovine serum albumin (BSA; Boehringer Mannheim Corp., Indianapolis, IN) in DPBS (DPBS/1% BSA) containing 5 μg/ml recombinant E-selectin-Ig and then washed again. Caco-2 cells suspended at 1×10^6 cells/ml in DPBS/1% BSA were added to these plates and incubated for 45 minutes at 4°C. After removal of nonadherent cells by washing, fresh culture medium was added and adherent cells were allowed to grow for several days. When cultures were approximately 70% confluent, cells were harvested by treatment with versene (37°C, 5 minutes), washed, and applied at 4°C for 45 minutes to a fresh plate coated with E-selectin-Ig. E-selectin-adherent Caco-2 cells (Caco-2(E+)) were the result of six consecutive rounds of panning and growth.

Tumor Cell Surface Staining with Anti-Carbohydrate MAbs and Selectin-Ig Fusion Proteins

Tumor cells (1×10^6 cells) were incubated at 4°C for 45 minutes with DMEM/1% FCS containing MAbs anti-sLe^a or anti-sLe^x, or fusion proteins E-selectin-Ig or L-selectin-Ig. All antibodies and fusion proteins were used at concentrations in excess of those required to obtain binding saturation (1:100 dilution ascites fluid containing anti-sLe^x MAb, 10 μg/ml anti-sLe^a MAb, and 1 mg/ml selectin-Ig fusion proteins). Cell surface binding of anti-carbohydrate MAbs or selectin-Ig fusion proteins was revealed using fluorescein-conjugated anti-immunoglobulin antibodies and quantitated by flow cytometry. Specific staining of tumor cells was expressed as mean fluorescence intensity and compared with the staining of tumor cell samples incubated with an irrelevant primary MAb or with the nonbinding CD8-Ig fusion protein followed by the appropriate fluorescein-conjugated anti-immunoglobulin antibody.

Tumor Cell Adhesion to Immobilized E-Selectin

Nunclon Terasaki Microwell plates were coated with protein A and then incubated at room temperature for 1 hour with 5 μl of DPBS/1% BSA containing 5 μg/ml recombinant E-selectin-Ig. After washing, 5×10^3 tumor cells were added to each microwell and incubated at 37°C for 30 minutes. Nonadherent cells were removed by washing with DPBS, and the adherent cells per unit area of microwells were enumerated with the aid of an ocular reticle. In each experiment, a CD8-Ig fusion protein was used as a negative control. The specificity of tumor cell-selectin interactions was assessed by inhibition studies using anti-E-selectin MAbs.

Experimental Lung Colony Formation in IL-1-Stimulated Nude Mice

Athymic BALB/c mice (male, 3 to 4 weeks old; Simonsen Labs, Gilroy, CA) were injected via the lateral tail vein with DPBS containing 1 μg of recombinant human IL-1 (specific activity, 3×10^8 U/mg of protein, D10 assay; kindly donated by Dr. P. Lomedico, Hoffman-La Roche, Nutley, NJ). Control animals received DPBS only. After 5 hours, single-cell suspensions of tumor cells (containing 5×10^6 cells/ml in DPBS) were injected intravenously into all animals (0.2 ml per mouse). Mice were sacrificed 8 weeks later, or earlier if moribund. Metastatic nodules on lung surfaces were visualized by intratracheal injection of India ink[47] and counted with the aid of a dissecting microscope. Extrapulmonary organs with suspect metastatic lesions were removed, fixed in 10% buffered formalin, and processed for histological examination.

To evaluate the effect of E-selectin-Ig fusion protein on experimental lung colony formation, suspensions of HT-29 tumor cells were incubated at 4°C for 45 minutes with DPBS containing E-selectin-Ig or the control fusion protein L-selectin-Ig and then injected into mice. Before injection, tumor cells were assessed for viability by using the trypan blue exclusion assay.

Effect of E-Selectin-Ig Fusion Protein on HT-29 Tumor Cell Clonogenicity in Agar

HT-29 colon carcinoma cells were incubated at 4°C for 45 minutes with control medium or with medium containing 1 mg/ml E-selectin-Ig. Tumor cells were then suspended at 1×10^4/ml in DMEM/21% FCS containing 0.3% agar (Sigma). Single-cell suspen-

Table 1. *Carbohydrate Expression and E-Selectin Adhesiveness of Human Colon Carcinoma Cells*

Cell line	Carbohydrate expression*		Adhesiveness†
	sLe^a	sLe^x	
HT-29	52	62	978 ± 50
LS 180	67	85	958 ± 70
LS 174T	38	96	645 ± 32
T84	64	87	1095 ± 58
COLO 320	3	3	10 ± 30
Caco-2	9	16	55 ± 21
Caco-2(E+)	3	68	1050 ± 110

* Tumor cell suspensions were incubated at 4°C for 30 minutes with MAbs anti-sLe^a or anti-sLe^x. Binding of anti-carbohydrate antibodies was revealed by reaction with fluorescein-conjugated anti-immunoglobulin antibodies and quantitated using flow cytometry analysis. Values are percentages of tumor cells that reacted specifically with anti-sLe^a or anti-sLe^x MAbs. Similar results were obtained in two additional experiments.

† Protein A-coated microwells were incubated at room temperature for 1 hour with DPBS/1% BSA containing 5 μg/ml E-selectin-Ig. Tumor cells were added and allowed to adhere at 37°C for 30 minutes. After removing nonadherent cells by washing with DPBS, the adherent cells per mm^2 were enumerated microscopically. Values are mean ± SE of quadruplicate determinations in a representative experiment. Two additional experiments yielded similar results. In the same study, anti-E-selectin MAb H18/7 abolished adhesion of all colon carcinoma cell lines. Tumor cell adhesion to the control CD8-Ig fusion protein was lower than 30 cells/mm^2.

sions were layered on dishes coated with a 1% agar solution and maintained in regular growth conditions for 7 days. Colonies of tumor cells grown in semisolid agar were enumerated and used to calculate cloning efficiency.

Effect of E-Selectin-Ig Fusion Protein on HT-29 Tumor Cell Adhesion to Endothelial Monolayers

Endothelial monolayers grown to confluence in Nunclon Terasaki Microwell plates were activated by incubation at 37°C for 4 hours with growth medium containing 200 U/ml recombinant human IL-1. Suspensions of HT-29 colon carcinoma cells were incubated at 4°C for 45 minutes with control medium or with medium containing 1 mg/ml E-selectin-Ig or L-selectin-Ig fusion proteins. Five microliters of tumor cell suspensions were then applied to microwells and incubated at 37°C for 30 minutes. After removal of nonadherent cells by washing, adherent tumor cells were fixed with 2.5% glutaraldehyde in DPBS and counted. To evaluate possible inhibition of tumor cell binding by MAbs specific for endothelial adhesion molecules, endothelial monolayers were preincubated at 4°C for 30 minutes with media containing anti-E-selectin, anti-VCAM-1, or anti-ICAM-1 MAbs, using concentrations of immunoglobulins in excess of those required to obtain saturation in an immunobinding assay.

Effect of E-Selectin-Ig Fusion Protein on Lung Retention of ^{125}I-Labeled HT-29 Colon Carcinoma Cells

Subconfluent cultures of HT-29 colon carcinoma cells were metabolically labeled by growth for 24 hours in medium containing 0.5 μCi/ml 5-[^{125}I]iodo-2′-deoxyuridine (specific activity, 2000 Ci/mmol; Amersham Corp., Arlington Heights, IL) and then harvested. Cells were washed twice by centrifugation and suspended in medium with or without 0.5 mg/ml E-selectin-Ig. After a 45-minute incubation at 4°C, 0.2 ml of tumor cell suspensions were injected intravenously into control or IL-1-treated mice. Animals were sacrificed 24 hours after tumor cell inoculation. Lungs were removed, washed extensively with 70% ethanol, and processed for count of radioactivity. Triplicate samples of tumor cell suspensions were retained and counted for input radioactivity.

Results

Colon Carcinoma Cells Adhere to E-Selectin and Produce Cytokine-Dependent Experimental Lung Metastases

As shown in Table 1, colon carcinoma cell lines HT-29, LS 180, LS 174T, and T84 expressed the E-selectin carbohydrate ligands sLe^a and sLe^x on cell surfaces. These two oligosaccharide antigens were barely detectable on surfaces of COLO 320 cells; Caco-2 cells also failed to react with antibodies directed against these carbohydrate epitopes. By contrast, a high expression of sLe^x was observed in Caco-2(E+) cells, a variant that we isolated by panning of Caco-2 tumor cells in culture dishes coated with purified recombinant E-selectin. All colon carcinoma cell lines that showed a high cell surface expression of E-selectin ligands (HT-29, LS 180, LS 174T, and T84 lines) adhered in high numbers to recombinant human E-selectin immobilized on culture dishes. This group included Caco-2(E+) cells;

Table 2. *Effect of IL-1 Injection on Experimental Lung Colony Formation by Human Colon Carcinoma Cells*

Cell line	Control mice*		IL-1-treated mice	
	Number of nodules†	Incidence‡	Number of nodules	Incidence
HT-29	4 (1–40)	11/11	86 (20–190)§	9/9
LS 180	8 (1–37)	8/8	39 (10–139)§	8/8
LS 174T	4 (1–15)	5/5	45 (11–190)§	8/8
T84	5 (1–63)	9/9	65 (5–320)§	9/9
COLO 320	0 (0–4)	3/7	0 (0–2)	1/6
Caco-2	1 (0–4)	3/8	0 (0–1)	1/10
Caco-2(E+)	0 (0–1)	1/9	12 (5–20)§	9/9

* Animals were given DPBS or IL-1 5 hours before injection of tumor cells.
† Median number of lung colonies per animal and range in parenthesis.
‡ Number of mice with lung colonies/number of mice injected.
§ Significantly different from number of nodules in control mice ($P < 0.01$; Mann-Whitney U-test).

notably, these cells were found to be specifically adhesive for E-selectin, as they were found to bind to E- but not to P- or L-selectin (data not shown). As reported in Table 1, COLO 320 and parental Caco-2 cell lines, which did not express either sLe^a or sLe^x on cell surfaces, did not bind to E-selectin. In parallel studies, all colon carcinoma cell lines that adhered to purified human E-selectin also bound to COS cells transfected with a cDNA encoding the murine homologue of human E-selectin[40] (data not shown).

As shown in Table 2, the E-selectin-adherent colon carcinoma cell lines (HT-29, LS 180, LS 174T, T84, and Caco-2(E+) variant) produced higher numbers of lung colonies in cytokine-treated than in unstimulated mice. By contrast, COLO 320 and parental Caco-2 cells, which did not bind to E-selectin, did not display an increased metastatic capacity in cytokine-treated compared with unstimulated mice.

E-Selectin-Ig Fusion Protein Inhibits Formation of Experimental Lung Metastases and Adhesion of Colon Carcinoma Cells

Immunofluorescence studies showed that E-selectin-Ig fusion protein present in the solution phase of the assay stained specifically HT-29 colon carcinoma cells (Figure 1). Solution-phase L-selectin-Ig showed a comparable or even higher level of binding to HT-29 cell surfaces and therefore was considered as a suitable control for E-selectin-Ig in inhibition studies.

As shown in Table 3, HT-29 tumor cells suspended in medium that contained E-selectin-Ig displayed a reduced lung-colonizing capacity upon injection into cytokine-treated mice. This inhibitory effect was dependent on the concentration of E-selectin-Ig in suspension medium, being maximal by using this fusion protein at 0.5 mg/ml; by contrast, L-selectin-Ig used at the same concentration had no effect. E-selectin-Ig did not affect HT-29 cell coloni-

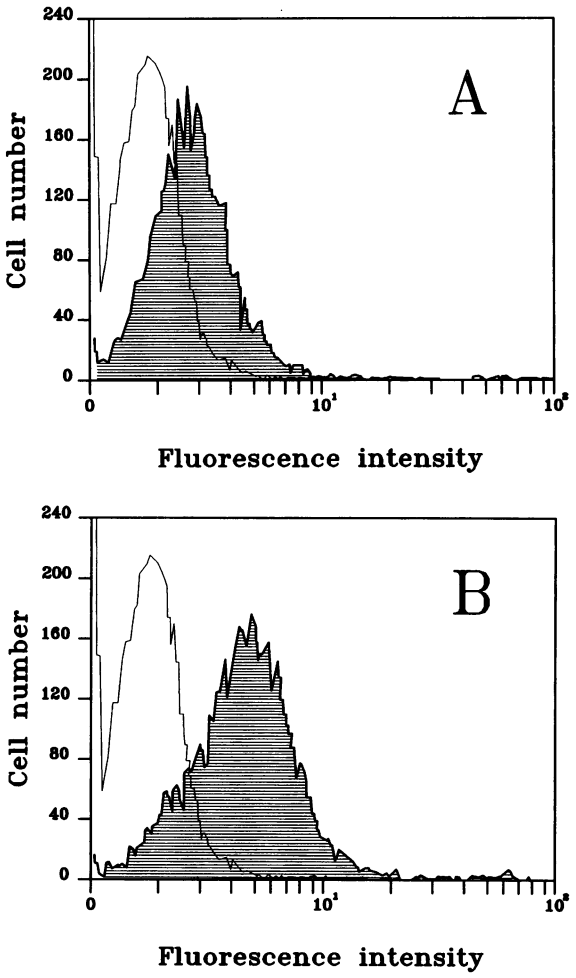

Figure 1. *Flow cytometric analysis of HT-29 tumor cell staining by fusion proteins E-selectin-Ig* (**A**) *or L-selectin-Ig* (**B**). *HT-29 tumor cells were incubated at 4°C for 45 minutes with medium containing indicated fusion proteins at 1 mg/ml. Binding of selectin-Igs was revealed by reaction with fluorescein-conjugated anti-immunoglobulin antibodies and quantitated using flow cytometric analysis. Specific staining of tumor cells was expressed as mean fluorescence intensity and compared with the staining of tumor cell samples incubated with the nonbinding CD8-Ig fusion protein followed by fluorescein-conjugated anti-immunoglobulin antibody. E- and L-selectin-Ig bound specifically to 36 and 75%, respectively, of HT-29 cells. Two additional experiments gave similar results.*

Table 3. *Inhibition of Experimental Lung Colony Formation by E-Selectin-Ig Fusion Protein*

Cells and fusion proteins	Control mice*		IL-1-treated mice	
	Number of nodules†	Incidence‡	Number of nodules	Incidence
HT-29	1 (0–11)	4/7	30 (10–90)	9/9
Plus E-selectin-Ig				
0.25 mg/ml	ND	ND	12 (4–43)	7/7
0.5 mg/ml	ND	ND	4 (1–15)§	7/7
1.0 mg/ml	3 (0–21)	5/6	3 (0–27)§	6/7
Plus L-selectin-Ig				
0.5 mg/ml	ND	ND	25 (5–80)	7/7
Hs 294T	15 (3–90)	8/8	70 (9–160)	9/9
Plus E-selectin-Ig				
0.5 mg/ml	ND	ND	60 (14–150)	6/6

Tumor cells were suspended in control media or in media containing E- or L-selectin-Ig fusion proteins at indicated concentrations. Cell suspensions were incubated at 4°C for 1 hour and then injected into mice. Incubation with selectin-Ig fusion proteins did not affect tumor cell viability, as assessed by trypan blue exclusion assay. ND, not determined.

* As in Table 2.

† As in Table 2.

‡ As in Table 2.

§ Significantly different from number of nodules produced by untreated HT-29 cells in IL-1-stimulated mice ($P < 0.01$; Mann-Whitney U-test).

zation of the lung in unstimulated mice. In addition, this fusion protein did not impair the lung-colonizing capacity of Hs 294T cells, a melanoma cell line that binds to VCAM-1 but not to E-selectin.[9]

To evaluate possible effects of E-selectin-Ig on tumor cell growth, we tested the clonogenic capacity of E-selectin-Ig-treated HT-29 cells. These cells displayed a 34.3 ± 5% cloning efficiency in control medium and a 33.1 ± 6% cloning efficiency in medium containing E-selectin-Ig (two experiments), showing that this fusion protein does not affect the proliferative capacity of HT-29 cells. By contrast, we found that E-selectin-Ig interfered significantly with HT-29 cell adhesiveness to vascular endothelia. As shown in Figure 2, HT-29 cells displayed increased adhesion to cytokine-activated endothelial monolayers by comparison with unstimulated monolayers. Cell adhesion to activated but not to unstimulated endothelia was significantly inhibited by the presence of E-selectin-Ig; control L-selectin-Ig had no effect. Antibody inhibition studies showed that HT-29 cell adhesion to activated endothelial monolayers was largely mediated by E-selectin but not by VCAM-1 or ICAM-1, adhesion molecules of the immunoglobulin superfamily known to be expressed by cytokine-activated endothelium.[9,43,48,49] In parallel studies, E-selectin-Ig was found to inhibit adhesion of HT-29 cells to tissue culture dishes coated with immobilized E-selectin (data not shown).

Arrest and survival of tumor cells within the lung microvasculature represent a prerequisite for establishing experimental lung metastases. As tumor cell adhesive interactions with vascular endothelia are thought to play an important role in these processes,[2,3,50] we examined the effect of E-selectin-Ig fusion protein on the arrest of HT-29 tumor cells in the lung vasculature. As shown in Figure 3, HT-29 cells showed an increased lung arrest in cytokine-treated compared with unstimulated mice; lung retention in cytokine-treated animals was found to be substantially impaired by the presence of E-selectin-Ig in tumor cell suspension media.

Discussion

During metastatic dissemination, tumor cells interact with several normal cells of the host, such as

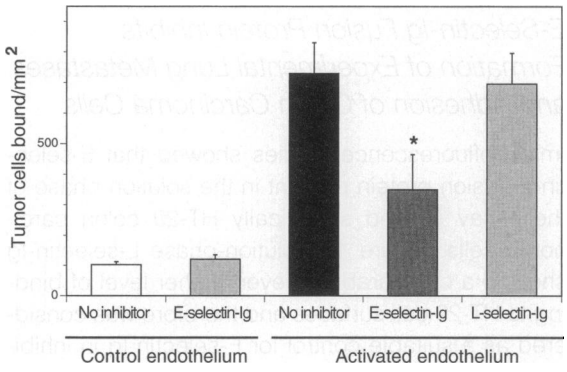

Figure 2. *Effect of E-selectin-Ig fusion protein on HT-29 colon carcinoma cell adhesion to IL-1-activated endothelium. Confluent endothelial monolayers were activated by incubation at 37°C for 4 hours with growth medium containing 200 U/ml IL-1 (D10 units). Suspensions of colon carcinoma cells were incubated at 4°C for 45 minutes with control medium or with medium containing E- or L-selectin-Ig at 1 mg/ml and then allowed to adhere at 37°C for 30 minutes onto endothelial monolayers. In the same study, MAbs specific for the endothelial molecules E-selectin, VCAM-1, or ICAM-1 inhibited cytokine-dependent HT-29 tumor cell adhesion by 97, 4, and − 5%, respectively. Values are means of quadruplicate samples ± SE in a representative experiment. Two additional experiments gave similar results. *Significantly different from the adhesion of untreated cells to activated endothelium (P < 0.01; Student's t-test).*

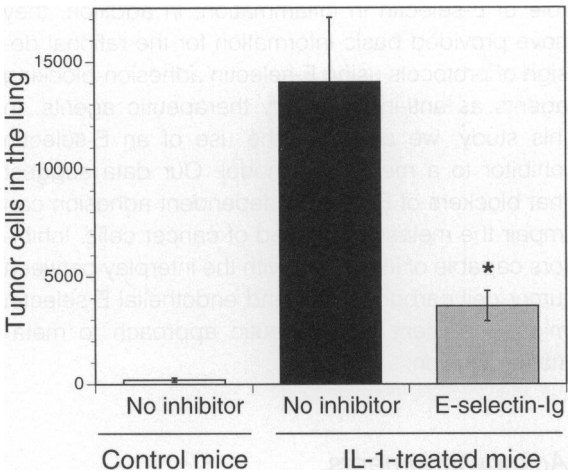

Figure 3. *Effect of E-selectin-Ig fusion protein on the arrest of HT-29 colon carcinoma cells in the lungs. HT-29 colon carcinoma cell cultures were metabolically labeled by growth for 24 hours in medium containing 0.5 μCi/ml 5-[^{125}I]iodo-2′-deoxyuridine. Suspensions of radiolabeled tumor cells were incubated at 4°C for 45 minutes with control medium or with medium containing 0.5 mg/ml E-selectin-Ig and then injected intravenously into control or IL-1-treated mice. Radioactivity present in the lungs was counted 24 hours after injection of labeled tumor cells. Results are expressed as number of tumor cells present in the lungs. Values are means of quadruplicate samples ± SE in a representative experiment. Two additional experiments gave similar results. *Significantly different from the lung arrest of untreated cells in IL-1-stimulated mice* ($P < 0.01$; *Student's* t-*test*).

platelets, leukocytes, and endothelial cells.[1,50–52] These interactions may result in immunological destruction of blood-borne tumor cells, thus reducing the efficiency of the metastatic process. However, contact with certain host tissue components may also offer biological advantages to metastatic cells. For example, adhesion to vascular endothelium is thought to promote tumor cell arrest in the microvasculature, therefore facilitating colonization of organs.[2,3,50] Several observations have implicated a role of the endothelial adhesion molecule E-selectin in metastatic dissemination. For example, it was demonstrated that colon carcinoma cells can bind to vascular endothelium through E-selectin.[9–17,53] Separate studies found that the E-selectin ligand sLe^x was expressed in abundance on colon cancers but not on most normal colonic epithelial cells.[19,20] The observations that the carbohydrate sLe^a is also highly expressed on surfaces of colon carcinoma cells[21] and can serve as a ligand for E-selectin[22–27] strengthened the emerging concept that E-selectin might participate in the metastatic spread of cancers. However, direct evidence of a role of E-selectin in metastasis has not been provided. In addition, very few studies have sought to impair the metastatic spread of cancer cells by using inhibitors capable of interfering with tumor cell adhesion to E-selectin.[33,54]

In this study, we showed that E-selectin-Ig, a recombinant fusion protein that was found to bind to the surfaces of colon carcinoma cells, blocked cancer cell formation of experimental lung metastases. We suggest that the blocking effect of E-selectin-Ig can be due to impairment of adhesive interactions established by tumor cells with E-selectin present on host vasculature. Indeed, we found that E-selectin-Ig was able to inhibit E-selectin-dependent adhesion of colon carcinoma cells to activated endothelial monolayers; in addition, E-selectin-Ig blocked colon cancer cell retention in the lung, a process that is thought to involve attachment of tumor cells to the host vasculature.[2,3,50] Taken together, our findings indicate that inhibition of tumor-cell/E-selectin adhesive interactions can affect the colonization of organs by colon carcinoma cells. Previous observations suggested that P- and L-selectin may play a role in the metastatic spread of certain types of tumor cells, including colon cancers, by mediating tumor cell adhesive interactions with the host's platelets and leukocytes.[15,18] In the present study, we demonstrate that a soluble form of L-selectin did not impair lung colonization by HT-29 colon carcinoma cells (although it bound to the surfaces of these tumor cells). This result is consistent with our previous observation showing that HT-29 tumor cells were not able to adhere to L-selectin and did not display L-selectin-dependent aggregation with leukocytes.[15] The possibility that different members of the selectin family act cooperatively in the metastatic diffusion of colon carcinoma cells deserves further attention.

Previous observations showed that colon carcinoma[55] and other types of tumor cells[56–59] produced higher numbers of metastases in cytokine-stimulated mice than in untreated animals. By using a series of colon carcinoma cell lines showing a different adhesiveness to E-selectin, we demonstrate here that formation of IL-1-inducible lung colonies by colon carcinoma cells is correlated with their adhesion to E-selectin. In cancer patients, endothelial activation and expression of adhesion molecules, including E-selectin, might occur for several reasons. For example, both malignant cells and macrophages within a solid tumor may produce tumor necrosis factor and other cytokines,[60–65] which could activate vascular endothelium in downstream vasculature. In addition, tumor cells lodged in microvessels may be able to activate adjacent endothelium. Finally, vascular endothelium might be activated by various pathophys-

iological insults, including infections, that occur in cancer patients as well as in healthy individuals.[66]

It is known that colon cancers frequently metastasize to extrapulmonary sites (mostly the liver) and only to a lesser extent to the lungs.[67] Although our studies are focused on colon carcinoma cell colonization of the lung, it should be noted that most of the colon carcinoma cell lines studied here colonized also lymph nodes, skeletal muscles, and subcutaneous tissues; however, their extrapulmonary dissemination was not increased by stimulation of mice with IL-1 (data not shown). This noninducible pattern of extrapulmonary dissemination might be due to a differential expression of E-selectin in tissues as a consequence of the intravenous route of cytokine administration. In this regard, a previous observation showed that IL-1 injected intravenously into mice induced a high expression of E-selectin in heart and lungs but not in extrapulmonary organs, such as spleen.[40] In cancer patients, however, induction of E-selectin expression may well occur in vascular endothelia of extrapulmonary organs and, therefore, mediate tumor cell extravasation at these sites. This possibility is supported by a recent study showing that transgenic mice constitutively expressing E-selectin in liver developed massively infiltrating hepatic metastases after injection with an E-selectin-binding tumor cell line.[68]

In different models of metastatic cells, specific changes of glycoconjugates have been associated with the expression of a high metastatic capacity.[47,69–71] Particularly relevant to the present study are the E-selectin carbohydrate ligands sLe^x and sLe^a, components of cell surface glycolipids and glycoproteins that are highly expressed by colon cancers.[19–21] In this study, all the colon carcinoma lines that expressed sLe^a and sLe^x were found to adhere to E-selectin and to produce high numbers of lung colonies in IL-1-stimulated mice; the E-selectin-adhesive Caco-2(E+) variant, which also produced experimental lung metastases in cytokine-treated mice, failed to display sLe^a but expressed sLe^x. Previous studies suggested a role of sLe^x [31,32] and sLe^a-related structures[33] in the metastatic diffusion of colon cancers. These carbohydrates may participate in the metastatic process by mediating tumor cell adhesion to E-selectin expressed by activated vascular endothelia.

Over the past few years, several molecules have been used to block the adhesive function of E-selectin *in vivo*, eg, anti-E-selectin MAbs,[35,72–74] peptides,[75] soluble E-selectin,[35] and E-selectin-Ig fusion protein.[36] Taken all together, these studies have contributed substantially to the demonstration of a role of E-selectin in inflammation; in addition, they have provided basic information for the rational design of protocols using E-selectin adhesion-blocking agents as anti-inflammatory therapeutic agents. In this study, we extended the use of an E-selectin inhibitor to a metastasis model. Our data suggest that blockers of E-selectin-dependent adhesion can impair the metastatic spread of cancer cells. Inhibitors capable of interfering with the interplay between tumor cell carbohydrates and endothelial E-selectin might represent a therapeutic approach to metastatic diffusion.

Acknowledgments

G. M. is grateful to Drs. G. Mugnai and S. Ruggieri, Institute of General Pathology, University of Florence, for helpful discussion in the preparation of the manuscript, and to Dr. O. Cecconi, Howard Hughes Medical Institute, University of California, San Diego, for critical review and suggestions.

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