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Differential Colon Cancer Cell Adhesion to E-, P-, and L-selectin: Role of Mucin-type Glycoproteins[1]

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ABSTRACT

E-, P-, and L-selectin support the adhesion of leukocytes to the vessel wall through the recognition of specific carbohydrate ligands, which often contain sialylated, fucosylated lactosamines such as sialyl Lewis x [sLe^x; Neu5Acα2–3Galβ1–4(Fucα1–3)GlcNAc−]. E-selectin expressed by activated endothelium has been shown to support the adhesion of sLe^x-bearing colon cancer cells. In the present study, we examine the interactions of multiple colon cancer cell lines with all three selectins. Three colon cancer cell lines (LS 180, T84, and COLO 205) bound to recombinant purified E-, P-, and L-selectin. The colon cancer line COLO 320 bound to P- and L-selectin but not E-selectin; conversely, HT-29 cells bound E-selectin but not P- and L-selectin. Caco-2 showed little or no interaction with any of the three selectins. Treatment of the cells with *O*-sialoglycoprotease from *Pasteurella haemolytica*, an enzyme that selectively cleaves mucin-type *O*-linked glycoproteins, reduced binding to purified P- and L-selectin in all cases. In addition, recombinant soluble P- and L-selectin bound to affinity-purified mucins from all adherent tumor cell lines. Of the four tumor cell lines that interacted with E-selectin, *O*-glycoprotease treatment substantially diminished adhesion of LS 180 and T84, had little effect on COLO 205, and failed to inhibit the binding of HT-29. As predicted by these data, E-selectin showed substantial binding only to mucins purified from LS 180 and T84. These findings suggest that L- and P-selectin interact primarily with mucin-type ligands on colon cancers, whereas E-selectin can recognize both mucin and nonmucin ligands. Binding of the colon cancer lines to purified selectins correlates with their adhesion to activated endothelial cells (E-selectin-dependent), platelets (P-selectin-dependent), and neutrophils (L-selectin-dependent). These differential tumor cell-selectin interactions may influence metastatic spread and may also contribute to the observed variability in host response to tumor progression.

INTRODUCTION

Cancer cells interact with a variety of host cells during growth and metastasis. Hematogenous dissemination brings cancer cells into contact with leukocytes, platelets, and endothelium. Interactions of blood-borne tumor cells with platelets and vascular endothelium may facilitate the arrest of metastatic cells in the microvasculature and organ colonization (1–5). Leukocyte binding to tumor cells can lead to a variety of outcomes, from tumor cell destruction to enhancement of metastatic spread (3, 6–9).

Certain inflammatory mediators induce vascular endothelium to express E- and P-selectin, which support the adhesion of leukocytes (reviewed in Refs. 10–13). P-Selectin is also expressed by activated platelets, where it contributes to platelet-leukocyte interactions. Neutrophils, monocytes, and most lymphocytes constitutively express L-selectin, a molecule that supports adhesive interactions with high endothelial venules of lymph nodes, as well as activated endothelium at sites of inflammation (reviewed in Refs. 10–13). Several recent studies have demonstrated that certain tumor cells interact with selectins. In particular, E-selectin has been shown to mediate adhesion of colon cancer cells to activated vascular endothelium (14–21). In addition, P-selectin can support the binding of activated platelets to lung cancer and neuroblastoma cells (22).

The tetrasaccharides sLe^x [5] and sLe^a can be recognized by endothelial E-selectin (reviewed in Refs. 10, 11). P- and L-selectin also recognize sLe^x and sLe^a, as well as several phosphate- and sulfate-containing molecules that do not bind E-selectin (10–13). Recent data suggest that the biologically relevant carbohydrate ligands for P- and L-selectin are more complex structures in which the cognate sugars are presented by cell surface mucin-type glycoproteins (reviewed in 10–13). Two sulfated mucin-type glycoproteins on lymph node endothelium were found to bind lymphocyte L-selectin (23–25), and a sialylated and fucosylated mucin-type glycoprotein binds P-selectin (26–28). In addition, MAdCAM-1, an L-selectin ligand present on the endothelium of mucosal lymphoid tissue, contains a mucin domain that must be correctly glycosylated for recognition (29). In contrast, some high affinity binding sites for E-selectin may be carried on *N*-linked oligosaccharides (30). These data suggest the possibility that tumor mucins could present selectin ligands that would facilitate their adhesion to normal cells in the vascular space. Supporting this thesis is the observation that certain colon cancers express sLe^x, sLe^a (31–36), and mucin-type glycoproteins (37–41). In the present study, we examined colon cancer cell interactions with selectins and the contribution of colon cancer mucins.

MATERIALS AND METHODS

Antibodies and Selectin-Immunoglobulin Fusion Proteins. 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) and H4/18 (IgG1, anti-E-selectin, nonblocker of adhesion); anti-VCAM-1 mAb E1/6 (IgG1); anti-ICAM-1 mAb E1/7 (IgG2a); and anti-p96 mAb H4/45 (IgG1; Refs. 14, 42–44). The following murine mAbs were provided as gifts: anti MHC class I mAb W6/32 (IgG2a; from D. Mendrick, Boston, MA; Ref. 45); anti-P-selectin mAbs G1 and S12 (IgG1; blocker and nonblocker of adhesion, respectively, from R. McEver, University of Oklahoma, Oklahoma City, OK; Ref. 46); anti-L-selectin mAb LAM 1–3 (IgG1, from T. Tedder, Dana-Farber Cancer Institute, Boston, MA; Ref. 47); and anti-CD18 mAb TS1/18 (IgG1, from T. Springer, Center for Blood Research, Boston, MA; Ref. 48). The anti-gpIIb/IIIa mAb P2 (IgG1) was from AMAC, Inc. (Westbrook, ME). Unless otherwise stated, ammonium sulfate-precipitated immunoglobulins or protein A-purified immunoglobulins were prepared as stock solutions in DPBS with calcium and magnesium (GIBCO-BRL, Grand Island, NY). In adhesion blocking studies, mAbs were diluted in RPMI 1640

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[5] The abbreviations used are: sLe^x, sialyl Lewis x [Neu5Acα2–3Galβ1–4(Fucα1–3)GlcNAc−]; sLe^a, sialyl Lewis a [Neu5Acα2–3Galβ1–3(Fucα1–4) GlcNAc−]; DPBS, Dulbecco's PBS; HSA, human serum albumin; TNF, tumor necrosis factor.

with 25 mM HEPES (GIBCO-BRL) and 1% FCS (RPMI-1% FCS; Hyclone Laboratories, Logan, UT).

Selectin-immunoglobulin fusion proteins are recombinant chimeric molecules containing extracellular regions of E-, P-, or L-selectin coupled to the hinge, CH2, and CH3 regions of human IgG1 (15, 49, 50). Each selectin-immunoglobulin contains the lectin domain, epidermal growth factor domain, and two (P-selectin-immunoglobulin, L-selectin-immunoglobulin) or six (E-selectin-immunoglobulin) complement regulatory repeats of the parent molecules. These fusion proteins were prepared by protein A affinity chromatography from culture media of COS cells transfected with cDNAs encoding E-, P-, or L-selectin-immunoglobulin in pCDM7 or pCDM8 vectors (49).

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 were subcultured (1:3 split ratio) using trypsin/versene (GIBCO). For use in adhesion experiments, cells (passages 2–4) were grown to confluence in Nunclon Terasaki MicroWell plates (Nunc, Naperville, IL) coated with 0.1% gelatin (Fischer Scientific, Pittsburgh, PA).

Platelets were isolated from human blood as described (51). Briefly, whole blood from normal donors was mixed with 0.11 volume of citrate buffer (70 mM citric acid-85 mM trisodium citrate) containing 110 mM glucose and 13 μg/ml prostaglandin E_1 (Sigma). Platelets were separated from blood cells by centrifugation at 160 × *g* for 10 min at room temperature and isolated by gel filtration on a Sepharose 2B column (Pharmacia, Piscataway, NJ). Platelets were suspended at 2×10^8/ml in 3.8 mM HEPES buffer (pH 7.3; Sigma) containing 137 mM NaCl, 3.8 mM NaH_2PO_4, 2.7 mM KCl, 1.0 mM $MgCl_2$, 5.6 mM glucose, and 0.35 mg/ml BSA (Pentex Fraction V, Miles, Inc., Kankakee, IL), and used for adhesion experiments within 30 min of isolation.

Centrifugation on a sodium metrizoate-dextran density gradient (Polymorphprep, Nycomed Pharma As, Oslo, Norway) was used to isolate neutrophils from blood anticoagulated with EDTA. Neutrophils were washed twice with DPBS; contaminating RBC were lysed with hypotonic buffer (ice-cold DPBS diluted 1:10 in distilled water). Neutrophils were suspended at 4×10^6/ml in ice-cold RPMI-1% HSA (Alpha Therapeutic Corp., Los Angeles, CA).

The following cell lines were obtained from American Type Culture Collection (Rockville, MD) and maintained in culture in the recommended medium: human colon carcinoma lines LS 180, T84, COLO 205, COLO 320, HT-29, and Caco-2 and human promyelocytic leukemia line HL60. In preparation for adhesion experiments, cells were detached from culture plates by mild trypsinization (0.25% trypsin/EDTA for 5 min at 37°C), a treatment that in preliminary experiments did not affect colon cancer cell ability to interact with E-, P-, or L-selectin. For tumor cell-endothelium, tumor cell-neutrophil, and tumor cell-selectin adhesion assays, cells were suspended at 1×10^6 cells/ml in RPMI-1% FCS; for tumor cell-platelet adhesion assays, cells were suspended at 3×10^6 cells/ml in RPMI-1% FCS.

Tumor Cell Binding to Endothelial Cells, Platelets, and Leukocytes. Endothelial monolayers grown to confluence in Nunclon Terasaki MicroWell plates were activated by incubation at 37°C for 4–6 h with Medium 199–20% FCS containing 200 units/ml of TNF-α (Biogen Corp., Cambridge, MA). Five μl of tumor cell suspensions were applied to microwells, and cells were allowed to adhere for 30 min at 37°C or, in certain studies, at 4°C. After removal of nonadherent cells by washing, adherent tumor cells were fixed with 2.5% glutaraldehyde in DPBS and counted. In certain studies, inhibition of adhesion by mAbs specific for the endothelial molecules E-selectin, VCAM-1, ICAM-1, p96, or MHC class I was evaluated. For this purpose, endothelial monolayers were preincubated at 4°C for 30 min with media containing mAbs at concentrations in excess of those required to obtain saturation in an immunobinding assay (RIA). For blocking mAbs, concentration curves were generated for blocking activity, and cell adhesion experiments were performed at concentrations that yielded maximal blocking activity.

Tumor cell interactions with platelets were assessed in a rosetting assay essentially as described (52). Freshly isolated platelets were activated by exposure to 0.15 unit/ml of thrombin (Sigma) at room temperature for 20 min. Nonactivated or activated platelets (4×10^6 in 20 μl) were mixed with 20 μl of RPMI-1% FCS (with or without 10 μg/ml anti-P-selectin or anti-gpIIb/IIIa mAbs) and incubated at room temperature for 20 min. Tumor cells (6×10^4 in 20 μl) were added and incubated for an additional 20 min at room temperature. Cells were fixed by adding glutaraldehyde in DPBS (1.25% final concentration) and gently resuspended. Phase contrast microscopy was used to determine the number of tumor cells with ≥2 bound platelets (rosettes)/100 tumor cells.

For tumor cell-leukocyte interactions, 2×10^5 neutrophils in RPMI-1% HSA (50 μl) were transferred into U-bottomed 96-well plates (Becton Dickinson, Oxnard, CA), and mixed with 2×10^4 tumor cells (20 μl). After centrifugation at 400 × *g* for 5 min, plates were incubated at 4°C for 15 min; supernatants were then discarded, and cell pellets were resuspended in 2.5% glutaraldehyde solution. Tumor cells with one or more bound neutrophils were counted using a phase contrast microscope. In certain studies, neutrophil suspensions were incubated at 4°C for 15 min with RPMI-1% HSA containing saturating concentrations of anti-L-selectin or anti-CD18 mAbs before the rosette assay.

Tumor Cell Adhesion to Purified, Immobilized Selectin-Immunoglobulin Fusion Proteins. Nunclon Terasaki Microwell plates (Nunc, Naperville, IL) were coated with protein A by overnight incubation at 4°C with a 10 μg/ml solution of recombinant protein A (Chemicon, Temecula, CA) in 50 mM carbonate/bicarbonate buffer, pH 9.5. Plates were washed with DPBS and then incubated at room temperature for 1 h with 5 μl of DPBS-1% BSA containing serially diluted E-, P-, or L-selectin-immunoglobulin fusion proteins. After washing, 5×10^3 tumor cells were added to each microwell and incubated at 4°C for 30 min. Nonadherent cells were removed by washing with DPBS; adherent cells were fixed with 2.5% glutaraldehyde in DPBS and counted microscopically. Different concentrations of selectin-immunoglobulin fusion proteins were tested before choosing the concentration that sustained maximal adhesion of tumor cells. Maximal tumor cell adhesion was reached using 5–10 μg/ml of E-selectin-immunoglobulin and 10–20 μg/ml of P- and L-selectin-immunoglobulin. In each experiment, a CD8-immunoglobulin fusion protein was used as a control.

Adhesion of *O*-Sialoglycoprotease-treated Tumor Cells to Selectin Immunoglobulin. To assess the participation of cell surface mucin-like glycoproteins in colon cancer cell adhesion to selectins, binding of tumor cells to selectin-immunoglobulins was assessed after treatment of the cells with *O*-sialoglycoprotease from *Pasteurella haemolytica* (Cedarlane Laboratories Ltd., Hornby, Ontario). In these studies, 2×10^6 tumor cells were suspended in 40 μl of RPMI-1% FCS and incubated at 37°C for 30 min with 10 μl (20 units) of *O*-sialoglycoprotease. After washing by centrifugation, cells were suspended at 1×10^6/ml in RPMI-1% FCS and assayed for adhesion to selectin-immunoglobulins immobilized in microtiter wells as described above. Control experiments showed that incubation of tumor cells with heat-inactivated *O*-sialoglycoprotease (100°C, 30 min) had no effect on cell adhesion to selectin-immunoglobulins.

Preparation of Mucin-enriched Glycoproteins from Colon Cancer Cells. Glycoproteins with *O*-linked oligosaccharides (including all mucins) were isolated from culture media of colon cancer lines by Jacalin column affinity chromatography (53, 54). For this purpose, confluent cultures of colon cancer cells were grown for 5–7 days in the absence of serum. Media were collected and loaded onto a 5-ml Jacalin-agarose column (4.0 mg of coupled lectin/ml; Vector Laboratories, Inc., Burlingame, CA) equilibrated with 175 mM Tris, ph 7.5. The column was washed with equilibration buffer and then eluted with 150 mM melibiose (Sigma) in the same buffer. Eluates were dialyzed against DPBS, and protein content was estimated by absorbance at 280 nm, assuming $E^{0.1\%}_{280nm} = 1.0$.

Binding of Selectin-Immunoglobulin Fusion Proteins to Immobilized Colon Cancer Cell Mucins. Binding of selectin-immunoglobulin to immobilized colon cancer mucins was assessed using an ELISA similar to that described previously (55). Polystyrene microwell plates (Corning Glass, Newark, CA) were incubated overnight at 4°C with 50 mM carbonate/bicarbonate buffer, pH 9.5, containing colon cancer mucins at the indicated concentrations. Plates were then incubated at room temperature for 2 h with a 1% BSA solution in assay buffer [20 mM HEPES (pH 7.4)-150 mM NaCl]. After two washes with assay buffer, microwells were exposed to a solution containing 50 nM E-selectin-immunoglobulin or 10 nM P-selectin-immunoglobulin in assay buffer supplemented with 1% BSA, 0.05% (v/v), Tween 20, and either 2 mM $CaCl_2$ or 5 mM EGTA. Solutions containing the selectins were incubated at room temperature for 2 h. After washing 3 times, 100 μl of assay buffer containing 1% BSA, 2 mM $CaCl_2$, and 1:3000 peroxidase-conjugated goat antihuman IgG (Jackson Immunoresearch Laboratories, Inc., West Grove, PA) was added to the wells. After 30-min incubation, plates were washed three

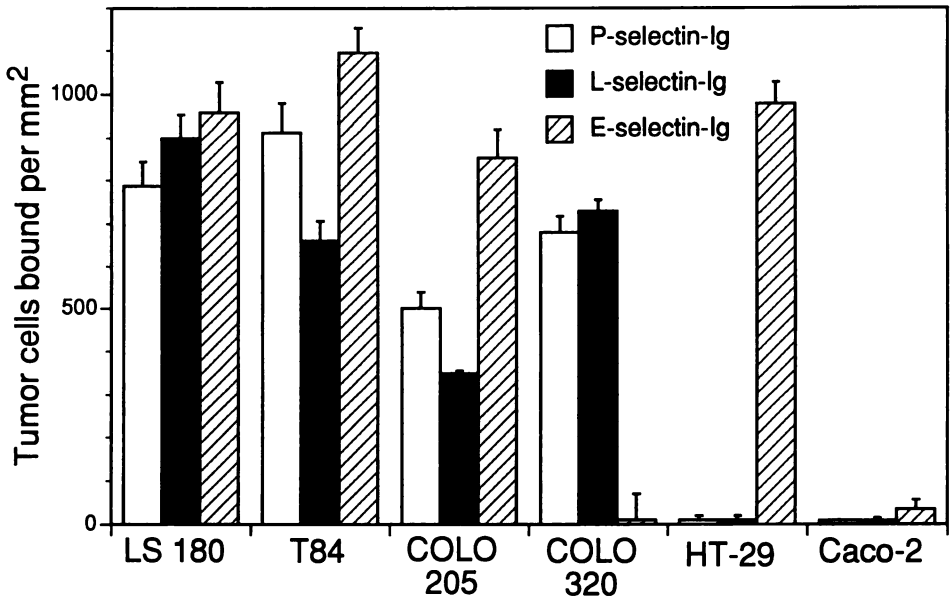

Fig. 1. Adhesion of colon cancer cells to purified E-, P-, and L-selectin-immunoglobulin (*Ig*). Protein A-coated microwells were incubated at room temperature for 1 h with DPBS-1% BSA containing E-selectin-immunoglobulin (5 μg/ml), P-selectin-immunoglobulin (20 μg/ml), or L-selectin-immunoglobulin (20 μg/ml) fusion proteins and then washed. Tumor cells were added and allowed to adhere at 4°C for 30 min. *Columns*, mean of quadruplicate wells in a representative experiment; *bars*, SE. Two additional experiments yielded similar results. In the same study, HL60 adhered at 913 ± 91 cells/mm^2 to P-selectin-immunoglobulin, 140 ± 20 cells/mm^2 to L-selectin-immunoglobulin, and 895 ± 78 cells/mm^2 to E-selectin-immunoglobulin. Adhesion of all tumor cell lines to the control CD8-immunoglobulin fusion protein was lower than 30 cells/mm^2.

times and incubated with 50 mM sodium citrate/sodium phosphate buffer, pH 5.0, containing 0.8 mg/ml *o*-phenylenediamine dihydrochloride/ml and 0.015% (v/v) hydrogen peroxide. Bound selectin-immunoglobulin was determined by measuring the absorbance at 450 nm at intervals of 12–30 s in a V_{max} microplate reader (Molecular Devices, Inc., Menlo Park, CA); an end point determination was made at 490 nm after stopping color development in the linear range with 4 N H_2SO_4. Specific binding was calculated by subtracting the signal generated in uncoated wells (nonspecific binding) from the signal obtained in wells coated with mucins. For studies of L-selectin-immunoglobulin binding, solutions containing 20 nM L-selectin-immunoglobulin were allowed to form multimeric aggregates with peroxidase-conjugated anti-immunoglobulin antibody (1:6000) for 30 min before incubation in mucin-coated plates. Subsequent steps were as described above.

RESULTS

Differential Binding of Colon Cancer Cell Lines to Selectins. We examined the adhesion of six well established colon cancer cell lines to purified recombinant E-, P-, and L-selectin. Three of the lines, LS 180, T84, and COLO 205, bound well to all three selectins (Fig. 1). The colon cancer cell line COLO 320 adhered to P- and L-selectin but not to E-selectin. Conversely, HT-29 bound to E-selectin but did not adhere to surfaces coated with P- or L-selectin. Of the six lines tested, only Caco-2 failed to demonstrate substantial binding to any of the selectins; low level binding to E-selectin was occasionally observed. The colon cancer lines that bound to P-, L-, or E-selectin-immunoglobulin also bound to COS cells transfected with corresponding cDNAs encoding full length transmembrane forms of the selectins (data not shown).

Binding to P- and L-Selectin Is Mediated Primarily by Mucin-type Glycoproteins. As noted in "Introduction," recent studies have suggested that P- and L-selectin can recognize mucin-type glycoproteins on leukocytes and endothelial cells, respectively. As shown in Fig. 2, adhesion of colon cancer cell lines to P- and L-selectin was substantially reduced or abolished by pretreatment of the cells with *O*-sialoglycoprotease, an enzyme that specifically cleaves mucin-type molecules [glycoproteins with multiple, closely spaced sialylated *O*-linked oligosaccharides (26, 56, 57)]. Adhesion of LS 180 and T84 cells to E-selectin-immunoglobulin was also inhibited by *O*-sialoglycoprotease, but this enzyme had little or no effect on binding of COLO 205 and HT-29 cells to E-selectin-immunoglobulin.

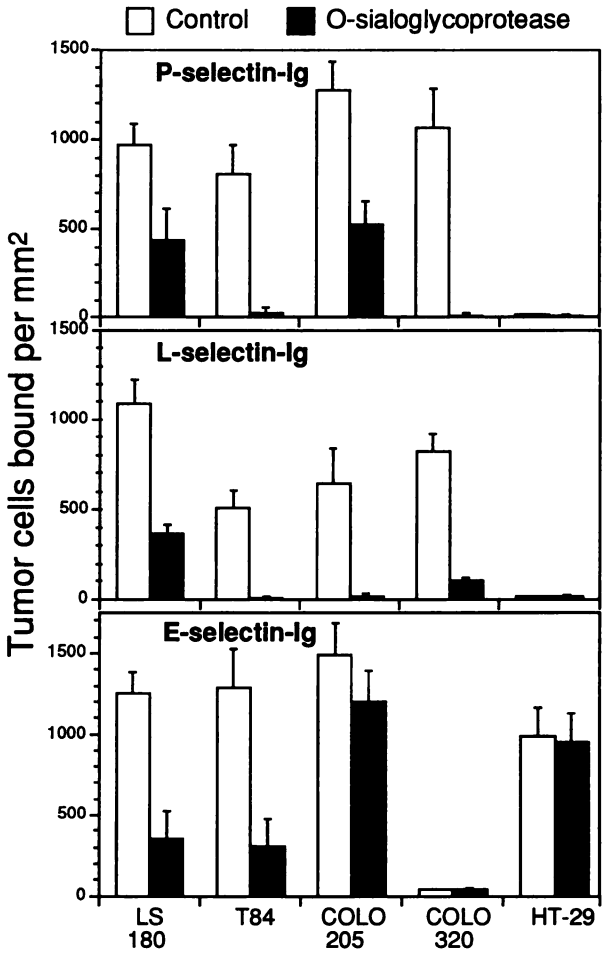

Fig. 2. Effect of *O*-sialoglycoprotease on colon cancer cell adhesion to selectins. Tumor cells were incubated at 37°C for 30 min in media containing *O*-sialoglycoprotease from *P. haemolytica*. Cells were washed, and their adhesion to immobilized selectin-immunoglobulin (*Ig*) fusion proteins was analyzed. *Columns*, mean of quadruplicate wells in a representative experiment; *bars*, SE. Two additional experiments yielded similar results. In the same study, HL60 adhesion was as follows: P-selectin-immunoglobulin, 226 ± 87 untreated cells/mm^2, 40 ± 23 treated cells/mm^2; L-selectin-immunoglobulin, 293 ± 86 untreated cells/mm^2, 7 ± 4 treated cells/mm^2; E-selectin-immunoglobulin, 432 ± 85 untreated cells/mm^2, 260 ± 62 treated cells/mm^2.

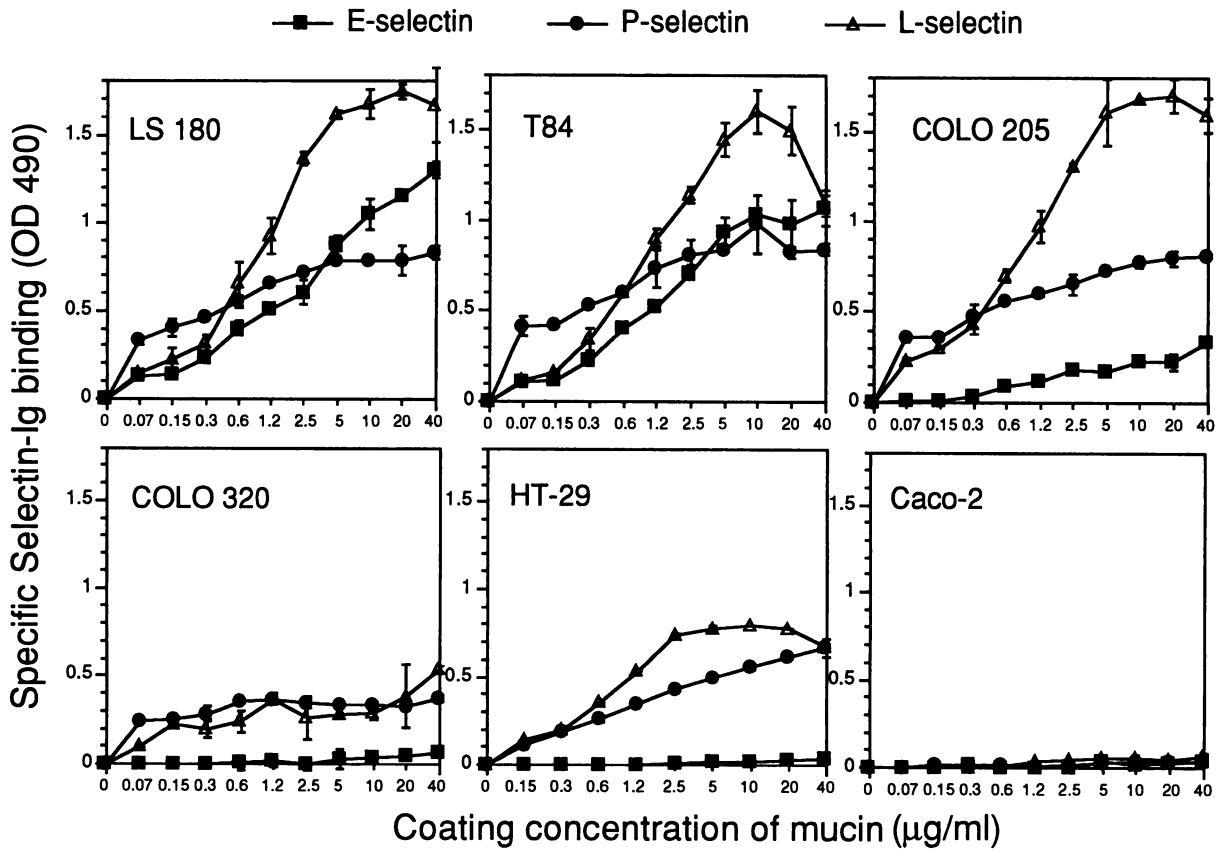

Fig. 3. Specific binding of P-, L-, and E-selectin-immunoglobulin to immobilized mucins from colon cancer cells. Microwell plates were incubated overnight at 4°C with solutions containing colon cancer cell mucins at the indicated concentrations. Binding of P-, L-, and E-selectin-immunoglobulin (*Ig*) to mucin-coated plates was quantitated in an ELISA assay by measuring absorbance (*OD*) at 490 nm. *Points*, mean absorbance in triplicate wells in a representative experiment; *bars*, SD. Two additional experiments performed using two independent mucin preparations yielded similar results.

Direct Demonstration of Interactions between Selectins and Mucin-type Glycoproteins from Colon Cancer Cells. Jacalin-purified glycoproteins from cultured cancer cell supernatants were used in direct binding interactions with recombinant selectin-immunoglobulin fusion proteins. As predicted by studies involving *O*-sialoglycoprotease treatment of whole tumor cells (see Fig. 2), shed mucins isolated from LS 180, T84, COLO 205, and COLO 320 cells supported a concentration-dependent binding of P- and L-selectin-immunoglobulin (Fig. 3). This binding was inhibited in the presence of 5 mM EGTA by 70–100% (L-selectin-immunoglobulin) and 40–60% (P-selectin-immunoglobulin), consistent with calcium-dependent binding interactions. E-selectin-immunoglobulin fusion protein showed substantial calcium-dependent binding to mucins isolated from LS 180 and T84 cells, as anticipated. Also in agreement with the *O*-sialoglycoprotease data are the observations that mucin-type glycoproteins derived from COLO 205 cells supported a low level binding of E-selectin, whereas those from COLO 320 and HT-29 cells showed no activity in this assay.

Selectins Mediate Binding of Colon Cancer Cells to Endothelial Cells, Platelets, and Neutrophils. Previous studies have demonstrated that colon cancers can bind to activated endothelial cells through an E-selectin-dependent mechanism (14–21). In the present study, we extend this observation to additional tumor cell lines (Fig. 4*a*) and demonstrate that interactions with purified recombinant E-selectin (see above) predict adhesion to activated endothelium. Colon cancer cell lines that bound to E-selectin-immunoglobulin also exhibited substantial adhesion to TNF-α-activated endothelium (Fig. 4*a*). As shown in Table 1, colon cancer cell adhesion was partially blocked by an anti-E-selectin antibody but not by antibodies against VCAM-1 and ICAM-1, adhesion molecules of the immunoglobulin superfamily known to be expressed by cytokine-activated endothelium (14, 44, 58–61).

The interaction of various colon cancer cells with recombinant P- and L-selectin suggested the possibility that adhesive interactions may also occur with platelets and neutrophils. As shown in Fig. 4*b*, thrombin-activated but not resting platelets bound to colon cancer cell lines LS 180, T84, COLO 205, and COLO 320. In contrast, HT-29 and Caco-2 cells supported little or no binding of either resting or activated platelets. Tumor cell-platelet adhesion was inhibited by an anti-P-selectin antibody but not by an antibody directed against gpIIb/IIIa (also designated αIIb/β3), a platelet integrin involved in other adhesive events (Table 2).

In studies parallel to those using platelets, LS 180, T84, COLO 205, and COLO 320 colon cancer cells formed aggregates with isolated human neutrophils (Fig. 4*c*). An anti-L-selectin mAb inhibited this tumor cell-leukocyte adhesion, wheras an anti-CD18 (β2 integrin) mAb had no effect (Table 3). In separate studies, tumor cell-neutrophil adhesion was inhibited by the polyphosphomonoester core of *O*-phosphonomannan (data not shown), a phosphorylated polysaccharide known to bind L-selectin (62).

DISCUSSION

In the last decade, substantial effort has been devoted to the elucidation of the molecules that mediate tumor-host cell interactions. In the present study, we demonstrate that P-selectin mediates adhesive interactions of some colon cancer cells with thrombin-activated platelets. During metastatic dissemination, tumor-platelet adhesion may result in the formation of neoplastic emboli that facilitate the arrest of

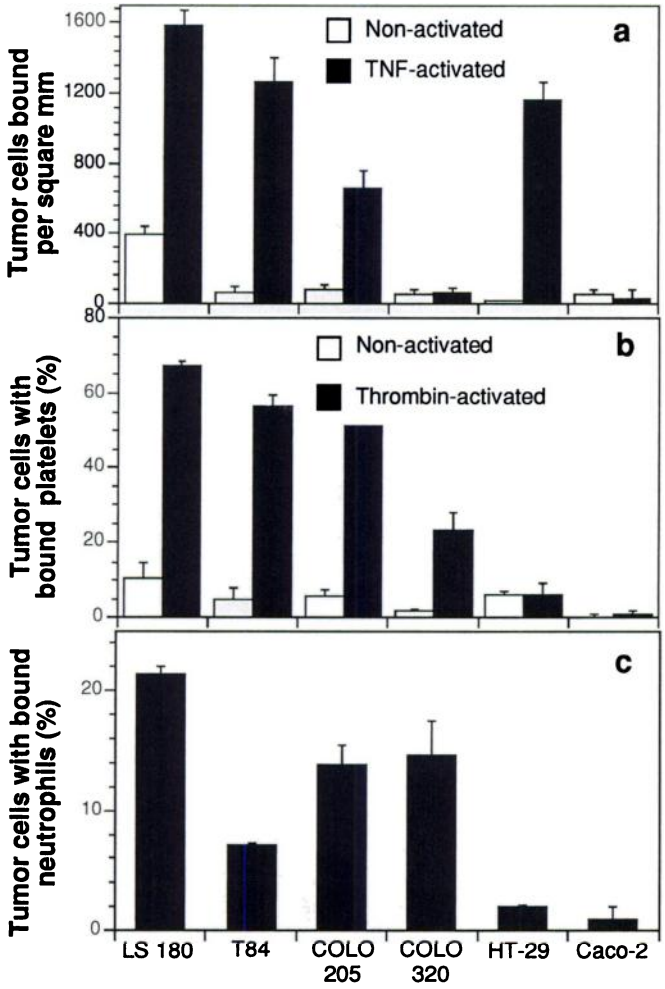

Fig. 4. Colon cancer cell adhesion to endothelial cells, platelets, and neutrophils. *a*, confluent endothelial monolayers were incubated at 37°C for 4–6 h in control media (RPMI-20% FCS) or in media containing 200 units/ml TNF-α.Tumor cell suspensions were applied and allowed to adhere at 4°C for 30 min. *Columns*, mean of quadruplicate samples in a representative experiment; *bars*, SE. Four additional experiments showed similar results. □, adhesion to control endothelium; ■, adhesion to TNF-α-stimulated endothelium. In the same study, HL60 cells adhered at 55 ± 25 cells/mm^2 to control endothelium and at 943 ± 70 cells/mm^2 to TNFα-stimulated endothelium. Similar tumor cell adhesion was obtained when the assay was performed at 37°C. *b*, human platelets were incubated at room temperature for 15 min with 0.15 unit/ml of thrombin. Tumor cells were added to stimulated platelets and coincubated at room temperature for 15 min. Tumor-platelet adhesion was visually quantitated as the percentage of tumor cells with two or more bound platelets (rosette). *Columns*, mean of triplicate samples in a representative experiment; *bars*, SE. □, rosettes with control platelets; ■, rosettes with thrombin-stimulated platelets. Two additional experiments yielded similar results. In the same study, HL60-platelet adhesion was 18 ± 4% rosettes with control platelets and 88 ± 3% rosettes with activated platelets. *c*, human neutrophils were coincubated with colon cancer cells at 4°C for 15 min. Tumor-neutrophil adhesion was visually quantitated as the percentage of tumor cells with bound neutrophils. *Columns*, mean of triplicate samples in a single experiment; *bars*, SE. Two additional experiments showed similar results.

tumor cells in the microvasculature of organs (1, 2, 63). In this process, cancer cells can activate the coagulation system (64, 65), resulting in the generation of thrombin, which in turn activates platelets and induces the expression of P-selectin. Previous reports have demonstrated a role for gpIIb/IIIa in the interactions of platelets with melanoma and colon cancer cells (66–68). In our studies, binding of thrombin-activated platelets to tumor cells was not affected by an antibody specific for gpIIb/IIIa capable of inhibiting platelet aggregation. It is possible that P-selectin and gpIIb/IIIa cooperate in the interactions of platelets with certain types of tumor cells. Because P-selectin is also expressed on activated vascular endothelium (10), our observations suggest that P-selectin may play a role in tumor cell-endothelium as well as tumor cell-platelet interactions.

L-selectin is expressed by a majority of leukocytes, including neutrophils, monocytes, natural killer cells, and most lymphocytes. Our studies indicate that L-selectin can support adhesive interactions of neutrophils with colon cancer cells; however, the overall effect of these interactions on cancer cells *in vivo* remains to be determined. Some observations suggest a tumoricidal effect of neutrophils (reviewed in Ref. 3); other studies have demonstrated a neutrophil-dependent enhancement of metastatic potential (3, 6, 7). Lymphocytes, natural killer cells, and lymphokine-activated killer cells exhibit cytotoxic activity toward tumor cells (8, 69). In this context, it has

Table 1 *Inhibition of tumor cell adhesion to cytokine-activated endothelium by anti-E-selectin, anti-VCAM-1, or anti-ICAM-1 mAbs*[a]

	% Inhibition of cytokine-dependent adhesion[b]		
Tumor cell line	Anti-E-selectin	Anti-VCAM-1	Anti-ICAM-1
LS 180	60 ± 6	3 ± 1	4 ± 1
T84	45 ± 5	−3 ± 1	−5 ± 1
COLO 205	41 ± 4	1 ± 1	−10 ± 3
HT-29	75 ± 4	7 ± 1	−5 ± 1

[a] TNF-stimulated endothelial monolayers were incubated at 4°C for 30 min with media containing mAb anti-E-selectin (H18/7), mAb anti-VCAM-1 (E1/6), or mAb anti-ICAM-1 (E1/7). Tumor cells were added and allowed to adhere. In the same study, antibody inhibition of HL60 cell adhesion was: anti-E-selectin, 55%; anti-VCAM-1, 10%; and anti-ICAM-1, 12%. Anti-p96, anti-MHC class I, and nonblocker anti-E-selectin (H4/18) mAbs had no effect on tumor cell adhesion to stimulated endothelium.

[b] Percent inhibition of tumor cell adhesion to stimulated endothelium exposed to indicated mAbs compared with adhesion to stimulated endothelium with no mAbs. Values of tumor adhesion to nonstimulated endothelium were subtracted from both control and mAb-treated samples, and are mean ± SE of 3–4 experiments, each performed in quadruplicate.

Table 2 *Inhibition of tumor cell interactions with thrombin-activated platelets by anti-P-selectin or anti-gpIIb/IIIa mAbs*[a]

	% Inhibition of thrombin-dependent adhesion[b]	
Tumor cell line	Anti-P-selectin mAb G1	Anti-gpIIb/IIIa mAb P2
LS 180	71 ± 8	−9 ± 3
T84	78 ± 8	4 ± 1
COLO 205	73 ± 10	−10 ± 2
COLO 320	87 ± 2	13 ± 3

[a] Thrombin-stimulated platelets were incubated at room temperature for 20 min with media containing anti-P-selectin or anti-gpIIb/IIIa mAbs, followed by addition of tumor cells. In the same study, antibody inhibition of platelet adhesion to HL60 cells was: anti-P-selectin (mAb G1), 98%; anti-gpIIb/IIIa (mAb P2), −5%. A nonblocking anti-P-selectin mAb (S12) had no significant effect on platelet adhesion to any tumor cell line (data not shown).

[b] Adhesion of activated platelets to tumor cells in the presence of indicated mAbs relative to adhesion in the absence of mAbs. Values of tumor interactions with nonstimulated platelets were subtracted from both control and mAb-treated samples and were typically less than 10% of adhesion to stimulated platelets. Values are mean ± SE of three experiments, each performed in triplicate.

Table 3 *Inhibition of tumor cell interactions with neutrophils by anti-L-selectin or anti-CD18 mAbs*[a]

	% Inhibition of adhesion[b]	
Tumor cell line	Anti-L-selectin mAb LAM 1–3	Anti-CD18 mAb TS1/18
LS 180	76 ± 7	−5 ± 1
T84	74 ± 9	6 ± 1
COLO 205	71 ± 6	5 ± 2
COLO 320	71 ± 8	4 ± 2

[a] Neutrophils were incubated at 4°C for 15 min in media containing anti-L-selectin or anti-CD18 mAbs, followed by addition of tumor cells.

[b] Tumor cell-neutrophil adhesion in the presence of indicated mAbs relative to adhesion in the absence of mAbs. Values are mean ± SE of three experiments, each performed in triplicate.

been reported that engagement of L-selectin on lymphocytes can stimulate their antitumor cell cytolytic activity (70).

Adhesion of colon cancer cells to P- and L-selectin followed congruent patterns, but adhesion to E-selectin was distinct. In particular, COLO 320 bound to P- and L-selectin but not E-selectin, whereas HT-29 bound to E-selectin but not P- and L-selectin. The interactions of P- and L-selectin with colon cancers appear to depend in large part on mucin-type glycoproteins. Adhesion of all colon cancer cells to recombinant P- and L-selectin was substantially inhibited after exposure of the cells to *O*-sialoglycoprotease. In addition, affinity purified *O*-linked glycoproteins enriched for mucin-type molecules from all adherent colon cancers supported binding of recombinant P- and L-selectin-immunoglobulin fusion proteins. P- and L-selectin are also known to interact with mucin-type glycoproteins present on leukocytes and endothelial cells (23, 24, 26–29, 71). In other studies, binding of activated platelets to myeloid tumor cells was reduced by inhibitors of *O*-linked glycosylation (18).

In more recent work, we have noted that E-selectin binding to this panel of colon cancer cells correlates well with their expression of sLe^x and sLe^a, whereas the binding of P- and L-selectin do not.[6] For example, the cell line COLO 320, which has very low levels of these sialylated antigens,[6] fails to bind to E-selectin but binds well to P- and L-selectin. It is possible that other polyanionic carbohydrate structures participate in these interactions. Previous studies have demonstrated that glycosaminoglycans (*e.g.*, heparin and heparan sulfate) bind P- and L-selectin but not E-selectin (55, 72–75). These and other studies suggest that selectin binding is determined by more than the expression of sialylated fucosylated lactosamines. One possibility is that P- and L-selectin binding involves recognition of anionic "clustered saccharide patches" that can be generated by more than one type of combination of oligosaccharide chains (11, 26, 74).

E-selectin appears to be able to support adhesion of colon cancer cells through interactions with both mucin-type and non-mucin-type glycoproteins. E-selectin interacts with several glycoproteins that bear *N*-linked carbohydrate chains (11, 30, 76), and isolated neutrophil glycolipids can sustain E-selectin-dependent cell adhesion (77). In addition, binding of neutrophils to E-selectin is resistant to several proteases (78), including *O*-sialoglycoprotease (79). Consistent with our observations that E-selectin-dependent HT-29 adhesion is unaffected by *O*-sialoglycoprotease treatment and that *O*-linked glycoproteins isolated from HT-29 cells do not support E-selectin-immunoglobulin binding, these colon cancer cells appear to express the E-selectin ligand sLe^x on glycolipids but not on glycoproteins (80).

Various glycoconjugates, including glycoproteins, glycolipids, and proteoglycans, have been proposed as natural ligands for the selectins (reviewed in Ref. 11). This study presents a panel of colon cancer cell lines that show differential binding to the selectins and provides a new approach to study the structural determinants of selectin binding. In particular, to our knowledge this is the first description of established cell lines that express mucin-type ligands for L-selectin: all other studies to date have been done using primary lymph node cultures or early passage cultures of high endothelial venular cells from this tissue (50, 81, 82). Mucin-type glycoproteins appear to play a key role, probably through the presentation of specific carbohydrate ligands to the selectins. Better knowledge of the structure of tumor cell ligands for selectins will enhance our understanding of tumor cell-host cell interactions.

[6] G. Mannori, D. Santoro, L. Carter, C. Corless, R. M. Nelson, and M. P. Bevilacqua, manuscript in preparation.

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REFERENCES

1. Gasic, G. J. Role of plasma, platelets, and endothelial cells in tumor metastasis. Cancer Metastasis Rev., *3:* 99–114, 1984.
2. Karpatkin, S., and Pearlstein, E. Role of platelets in tumor cell metastases. Ann. Intern. Med., *95:* 636–641, 1981.
3. Weiss, L., Orr, F. W., and Honn, K. V. Interactions between cancer cells and the microvasculature: a rate-regulator for metastasis. Clin. & Exp. Metastasis, *7:* 127–167, 1989.
4. Zetter, B. R. The cellular basis of site-specific tumor metastasis. N. Engl. J. Med., *322:* 605–612, 1990.
5. Pauli, B. U., Augustin-Voss, H. G., el Sabban, M. E., Johnson, R. C., and Hammer, D. A. Organ-preference of metastasis. The role of endothelial cell adhesion molecules. Cancer Metastasis Rev., *9:* 175–189, 1990.
6. Welch, D. R., Schissel, D. J., Howrey, R. P., and Aeed, P. A. Tumor-elicited polymorphonuclear cells, in contrast to "normal" circulating polymorphonuclear cells, stimulate invasive and metastatic potentials of rat mammary adenocarcinoma cells. Proc. Natl. Acad. Sci. USA, *86:* 5859–5863, 1989.
7. Orr, F. W., and Warner, D. J. Effects of systemic complement activation and neutrophil-mediated pulmonary injury on the retention and metastasis of circulating cancer cells in mouse lungs. Lab. Invest., *62:* 331–338, 1990.
8. Rosenberg, S. A. Immunotherapy and gene therapy of cancer. Cancer Res., *51:* 5074s–5079s, 1991.
9. Whitworth, P. W., Pak, C. C., Esgro, J., Kleinerman, E. S., and Fidler, I. J. Macrophages and cancer. Cancer Metastasis Rev., *4:* 319–351, 1990.
10. Bevilacqua, M. P., and Nelson, R. M. Selectins. J. Clin. Invest., *91:* 379–387, 1993.
11. Varki, A. Selectin ligands. Proc. Natl. Acad. Sci. USA, *91:* 7390–7397, 1994.
12. Rosen, S. D., and Bertozzi, C. R. The selectins and their ligands. Curr. Opin. Cell Biol., *6:* 663–673, 1994.
13. McEver, R. P. Selectins. Curr. Opin. Immunol., *6:* 75–84, 1994.
14. Rice, G. E., and Bevilacqua, M. P. An inducible endothelial cell surface glycoprotein mediates melanoma adhesion. Science (Washington DC), *246:* 1303–1306, 1989.
15. Walz, G., Aruffo, A., Kolanus, W., Bevilacqua, M., and Seed, B. Recognition by ELAM-1 of the sialyl-Le^x determinant on myeloid and tumor cells. Science (Washington DC), *250:* 1132–1135, 1990.
16. Lauri, D., Needham, L., Martin-Padura, I., and Dejana, E. Tumor cell adhesion to endothelial cells: endothelial leukocyte adhesion molecule-1 as an inducible adhesive receptor specific for colon carcinoma cells. J. Natl. Cancer Inst., *83:* 1321–1324, 1991.
17. Takada, A., Ohmori, K., Takahashi, N., Tsuyuoka, K., Yago, A., Zenita, K., Hasegawa, A., and Kannagi, R. Adhesion of human cancer cells to vascular endothelium mediated by a carbohydrate antigen, sialyl Lewis A. Biochem. Biophys. Res. Commun., *179:* 713–719, 1991.
18. Kojima, N., Handa, K., Newman, W., and Hakomori, S. Inhibition of selectin-dependent tumor cell adhesion to endothelial cells and platelets by blocking *O*-glycosylation of these cells. Biochem. Biophys. Res. Commun., *182:* 1288–1295, 1992.
19. Giavazzi, R., Foppolo, M., Dossi, R., and Remuzzi, A. Rolling and adhesion of human tumor cells on vascular endothelium under physiological flow conditions. J. Clin. Invest., *92:* 3038–3044, 1993.
20. Takada, A., Ohmori, K., Yoneda, T., Tsuyuoka, K., Hasegawa, A., Kiso, M., and Kannagi, R. Contribution of carbohydrate antigens sialyl Lewis A and sialyl Lewis X to adhesion of human cancer cells to vascular endothelium. Cancer Res., *53:* 354–361, 1993.
21. Sawada, R., Tsuboi, S., and Fukuda, M. Differential E-selectin-dependent adhesion efficiency in sublines of a human colon cancer exhibiting distinct metastatic potentials. J. Biol. Chem., *269:* 1425–1431, 1994.
22. Stone, J. P., and Wagner, D. D. P-selectin mediates adhesion of platelets to neuroblastoma and small cell lung cancer. J. Clin. Invest., *92:* 804–813, 1993.
23. Lasky, L. A., Singer, M. S., Dowbenko, D., Imai, Y., Henzel, W. J., Grimley, C., Fennie, C., Gillett, N., Watson, S. R., and Rosen, S. D. An endothelial ligand for L-selectin is a novel mucin-like molecule. Cell, *69:* 927–938, 1992.
24. Baumheter, S., Singer, M. S., Henzel, W., Hemmerich, S., Renz, M., Rosen, S. D., and Lasky, L. A. Binding of L-selectin to the vascular sialomucin CD34. Science (Washington DC), *262:* 436–438, 1993.
25. Hemmerich, S., and Rosen, S. D. 6′-sulfated sialyl $Lewis^x$ is a major capping group of GlyCAM-1. Biochemistry, *33:* 4830–4835, 1994.
26. Norgard, K. E., Moore, K. L., Diaz, S., Stults, N. L., Ushiyama, S., McEver, R. P., Cummings, R. D., and Varki, A. Characterization of a specific ligand for P-selection on myeloid cells. A minor glycoprotein with sialylated *O*-linked oligosaccharides. J. Biol. Chem., *268:* 12764–12774, 1993.
27. Sako, D., Chang, X. J., Barone, K. M., Vachino, G., White, H. M., Shaw, G., Veldman, G. M., Bean, K. M., Ahern, T. J., Furie, B., Cumming, D. A., and Larsen,

G. R. Expression cloning of a functional glycoprotein ligand for P-selectin. Cell, *75:* 1179–1186, 1993.
28. Moore, K., Eaton, S. F., Lyons, D. E., Lichenstein, H. S., Cummings, R. D., and Mcever, R. P. The P-selectin glycoprotein ligand from human neutrophils displays sialylated, fucosylated, *O*-linked poly-*N*-acetyllactosamine. J. Biol. Chem., *269:* 23318–23327, 1994.
29. Berg, E. L., McEvoy, L. M., Berlin, C., Bargatze, R. F., and Butcher, E. C. L-selectin-mediated lymphocyte rolling on MAdCAM-1. Nature (Lond.), *366:* 695–698, 1993.
30. Lenter, M., Levinovitz, A., Isenmann, S., and Vestweber, D. Monospecific and common glycoprotein ligands for E- and P-selectin on myeloid cells. J. Cell Biol., *125:* 471–481, 1994.
31. Fukushima, K., Hirota, M., Terasaki, P. I., Wakisaka, A., Togashi, H., Chia, D., Suyama, N., Fukushi, Y., Nudelman, E., and Hakomori, S. Characterization of sialosylated Lewis^x as a new tumor-associated antigen. Cancer Res., *44:* 5279–5285, 1984.
32. Itzkowitz, S. H., Yuan, M., Fukushi, Y., Palekar, A., Phelps, P. C., Shamsuddin, A. M., Trump, B. F., Hakomori, S., and Kim, Y. S. Lewis^x- and sialylated Lewis^x-related antigen expression in human malignant and nonmalignant colonic tissues. Cancer Res., *46:* 2627–2632, 1986.
33. Holmes, E. H., Hakomori, S., and Ostrander, G. K. Synthesis of type 1 and 2 lacto series glycolipid antigens in human colonic adenocarcinoma and derived cell lines is due to activation of a normally unexpressed β1–3N-acetylglucosaminyltransferase. J. Biol. Chem., *262:* 15649–15658, 1987.
34. Magnani, J. L., Nilsson, B., Brockhaus, M., Zopf, D., Steplewski, Z., Koprowski, H., and Ginsburg, V. A monoclonal antibody-defined antigen associated with gastrointestinal cancer is a ganglioside containing sialylated lacto-*N*-fucopentaose II. J. Biol. Chem., *257:* 14365–14369, 1982.
35. Saitoh, O., Wang, W. C., Lotan, R., and Fukuda, M. Differential glycosylation and cell surface expression of lysosomal membrane glycoproteins in sublines of a human colon cancer exhibiting distinct metastatic potentials. J. Biol. Chem., *267:* 5700–5711, 1992.
36. Hoff, S. D., Irimura, T., Matsushita, Y., Ota, D. M., Cleary, K. R., and Hakomori, S. Metastatic potential of colon carcinoma. Expression of ABO/Lewis-related antigens. Arch. Surg., *125:* 206–209, 1990.
37. Hirohashi, S., Clausen, H., Yamada, T., Shimosato, Y., and Hakomori, S. Blood group A cross-reacting epitope defined by monoclonal antibodies NCC-LU-35 and -81 expressed in cancer of blood group O or B individuals: its identification as Tn antigen. Proc. Natl. Acad. Sci. USA, *82:* 7039–7043, 1985.
38. Kjeldsen, T., Clausen, H., Hirohashi, S., Ogawa, T., Iijima, H., and Hakomori, S. Preparation and characterization of monoclonal antibodies directed to the tumor-associated *O*-linked sialosyl-2–6 α-*N*-acetylgalactosaminyl (sialosyl-Tn) epitope. Cancer Res., *48:* 2214–2220, 1988.
39. Itzkowitz, S. Carbohydrate changes in colon carcinoma. APMIS, *27* (Suppl.)*:* 173–180, 1992.
40. Niv, Y., Boland, C. R., and Kim, Y. S. Increased tumorigenicity after differentiation of colon cancer cell line: absence of association with mucin synthesis. Gastroenterology, *106:* 389–398, 1994.
41. Niv, Y., Byrd, J. C., Ho, S. B., Dahiya, R., and Kim, Y. S. Mucin synthesis and secretion in relation to spontaneous differentiation of colon cancer cells *in vitro*. Int. J. Cancer., *50:* 147–152, 1992.
42. Bevilacqua, M. P., Pober, J. S., Mendrick, D. L., Cotran, R. S., and Gimbrone, M. A. Identification of an inducible endothelial-leukocyte adhesion molecule. Proc. Natl. Acad. Sci. USA, *84:* 9238–9242, 1987.
43. Pober, J. S., Bevilacqua, M. P., Mendrick, D. L., Lapierre, L. A., and Fiers, W. G. M. Two distinct monokines, interleukin 1 and tumor necrosis factor, each independently induce biosynthesis and transient expression of the same antigen on the surface of cultured human vascular endothelial cells. J. Immunol., *136:* 1680–1687, 1986.
44. Rice, G. E., Munro, J. M., and Bevilacqua, M. P. Inducible cell adhesion molecule 110 (INCAM-110) is an endothelial receptor for lymphocytes. A CD11/CD18-independent adhesion mechanism. J. Exp. Med., *171:* 1369–1374, 1990.
45. Barnstable, C. J., Bodmer, W. F., Brown, G., Galfre, G., Milstein C., Williams, A. F., and Ziegler, A. Production of monoclonal antibodies to group A erythrocytes, HLA and other human cell surface antigens: new tools for genetic analysis. Cell, *14:* 9–20, 1978.
46. Geng, J. G., Bevilacqua, M. P., Moore, K. L., McIntyre, T. M., Prescott, S. M., Kim, J. M., Bliss, G. A., Zimmerman, G. A., and McEver, R. P. Rapid neutrophil adhesion to activated endothelium mediated by GMP-140. Nature (Lond.), *343:* 757–760, 1990.
47. Spertini, O., Kansas, G. S., Reimann, K. A., Mackay, C. R., and Tedder, T. F. Function and evolutionary conservation of distinct epitopes on the leukocyte adhesion molecule-1 (TQ-1, Leu-8) that regulate leukocyte migration. J. Immunol., *147:* 942–949, 1991.
48. Sanchez-Madrid, F., Krensky, A. M., Ware, C. F., Robbins, E., Strominger, J. L., Burakoff, S. J., and Springer, T. A. Three distinct antigens associated with human T-lymphocyte-mediated cytolysis: LFA-1, LFA-2, and LFA-3. Proc. Natl. Acad. Sci. USA, *79:* 7489–7493, 1982.
49. Aruffo, A., Kolanus, W., Walz, G., Fredman, P., and Seed, B. CD62/P-selection recognition of myeloid and tumor cell sulfatides. Cell, *67:* 35–44, 1991.
50. Aruffo, A., Dietsch, M. T., Wan, H., Hellstrom, K. E., and Hellstrom, I. Granule membrane protein 140 (GMP140) binds to carcinomas and carcinoma-derived cell lines. Proc. Natl. Acad. Sci. USA, *89:* 2292–2296, 1992.
51. Hanasaki, K., Nakano, T., and Arita, H. Two phasic generation of thromboxane A2 by the action of collagen on rat platelets. Thromb. Res., *46:* 425–436, 1987.
52. Larsen, E., Celi, A., Gilbert, G. E., Furie, B. C., Erban, J. K., Bonfanti, R., Wagner, D. D., and Furie, B. PADGEM protein: a receptor that mediates the interaction of activated platelets with neutrophils and monocytes. Cell, *59:* 305–312, 1989.
53. Hortin, G. L, and Trimpe, B. L. Lectin affinity chromatography of proteins bearing *O*-linked oligosaccharides: application of jacalin-agarose. Anal. Biochem., *188:* 271–277, 1990.
54. Hortin, G. L. Isolation of glycopeptides containing *O*-linked oligosaccharides by lectin affinity chromatography on jacalin-agarose. Anal. Biochem., *191:* 262–267, 1990.
55. Nelson, R. M., Dolich, S., Aruffo, A., Cecconi, O., and Bevilacqua, M. P. Higher-affinity oligosaccharide ligands for E-selectin. J. Clin. Invest., *91:* 1157–1166, 1993.
56. Abdullah, K. M., Udoh, E. A., Shewen, P. E., and Mellors, A. A neutral glycoprotease of Pasteurella haemolytica A1 specifically cleaves *O*-sialoglycoproteins. Infect. Immun., *60:* 56–62, 1992.
57. Sutherland, D. R., Abdullah, K. M., Cyopick, P., and Mellors, A. Cleavage of the cell-surface *O*-sialoglycoproteins CD34, CD43, CD44, and CD45 by a novel glycoprotease from Pasteurella haemolytica. J. Immunol., *148:* 1458–1464, 1992.
58. Rice, G. E., Munro, J. M., Corless, C., and Bevilacqua, M. P. Vascular and nonvascular expression of INCAM-110. A target for mononuclear leukocyte adhesion in normal and inflamed human tissues. Am. J. Pathol., *138:* 385–393, 1991.
59. Osborn, L., Hession, C., Tizard, R., Vassallo, C., Luhowskyj, S. C. R., and Lobb, R. Direct expression cloning of vascular cell adhesion molecule 1, a cytokine-induced endothelial protein that binds to lymphocytes. Cell, *59:* 1203–1211, 1989.
60. Dustin, M. L., Rothlein, R., Bhan, A. K., Dinarello, C. A., and Springer, T. A. Induction by IL-1 and interferon-γ: tissue distribution, biochemistry, and function of a natural adherence molecule (ICAM-1). J. Immunol., *137:* 245–254, 1986.
61. Pober, J. S., Gimbrone, M. A. J., Mendrick, D. L., Fiers, W. R. R., and Springer, T. A. Overlapping patterns of activation of human endothelial cells by interleukin 1, tumor necrosis factor, and immune interferon. J. Immunol., *137:* 1893–1896, 1986.
62. Stoolman, L. M., Tenforde, T. S., and Rosen, S. D. Phosphomannosyl receptors may participate in the adhesive interaction between lymphocytes and high endothelial venules. J. Cell Biol., *99:* 1535–1540, 1984.
63. Honn, K. V., Tang, D. G., and Crissman, J. D. Platelets and cancer metastasis: a causal relationship? Cancer Metastasis Rev., *11:* 325–351, 1992.
64. Rickles, F. R., Levine, M., and Edwards, R. L. Hemostatic alterations in cancer patients. Cancer Metastasis Rev., *11:* 237–248, 1992.
65. Gordon, S. G., and Chelladurai, M. Non-tissue factor procoagulants in cancer cells. Cancer Metastasis Rev., *11:* 267–282, 1992.
66. Boukerche, H., Berthier-Vergnes, O., Tabone, E., Dore, J. F., Leung, L. L., and McGregor, J. L. Platelet-melanoma cell interaction is mediated by the glycoprotein IIb-IIIa complex. Blood, *74:* 658–663, 1989.
67. Nierodzik, M. L., Plotkin, A., Kajumo, F., and Karpatkin, S. Thrombin stimulates tumor-platelet adhesion *in vitro* and metastasis *in vivo*. J. Clin. Invest., *87:* 229–236, 1991.
68. Karpatkin, S., Pearlstein, E., Ambrogio, C., and Coller, B. S. Role of adhesive proteins in platelet tumor interaction *in vitro* and metastasis formation *in vivo*. J. Clin. Invest., *81:* 1012–1019, 1988.
69. Trinchieri, G. Biology of natural killer cells. Adv. Immunol., *47:* 187–376, 1989.
70. Seth, A., Gote, L., Nagarkatti, M., and Nagarkatti, P. S. T-cell receptor-independent activation of cytolytic activity of cytotoxic T lymphocytes mediated through CD44 and gp90MEL-14. Proc. Natl. Acad. Sci. USA, *88:* 7877–7881, 1991.
71. Dowbenko, D., Watson, S. R., and Lasky, L. A. Cloning of a rat homologue of mouse GlyCAM 1 reveals conservation of structural domains. J. Biol. Chem., *268:* 14399–14403, 1993.
72. Skinner, M. P., Lucas, C. M., Burns, G. F., Chesterman, C. N., and Berndt, M. C. GMP-140 binding to neutrophils is inhibited by sulfated glycans. J. Biol. Chem., *266:* 5371–5374, 1991.
73. Imai, Y., Singer, M. S., Fennie, C., Lasky, L. A., and Rosen, S. D. Identification of a carbohydrate-based endothelial ligand for a lymphocyte homing receptor. J. Cell Biol., *113:* 1213–1221, 1991.
74. Norgard-Sumnicht, K. E., Varki, N. M., and Varki, A. Calcium-dependent heparin-like ligands for L-selectin in nonlymphoid endothelial cells. Science (Washington DC), *261:* 480–483, 1993.
75. Nelson, R. M., Cecconi, O., Roberts, W. G., Aruffo, A., Linhardt, R. J., and Bevilacqua, M. P. Heparin oligosaccharides bind L- and P-selectin and inhibit acute inflammation. Blood, *82:* 3253–3258, 1993.
76. Sawada, R., Lowe, J. B., and Fukuda, M. E-selectin-dependent adhesion efficiency of colonic carcinoma cells is increased by genetic manipulation of their cell surface lysosomal membrane glycoprotein-1 expression levels. J. Biol. Chem., *268:* 12675–12681, 1993.
77. Tiemeyer, M., Swiedler, S. J., Ishihara, M., Moreland, M., Schweingruber, H., Hirtzer, P., and Brandley, B. K. Carbohydrate ligands for endothelial-leukocyte adhesion molecule 1. Proc. Natl. Acad. Sci. USA, *88:* 1138–1142, 1991.
78. Larsen, G. R., Sako, D., Ahern, T. J., Shaffer, M., Erban, J., Sajer, S. A., Gibson, R. M., Wagner, D. D., Furie, B. C., and Furie, B. P-selectin and E-selectin. Distinct but overlapping leukocyte ligand specificities. J. Biol. Chem., *267:* 11104–11110, 1992.
79. Steininger, C. N., Eddy, C. A., Leimgrube, R. R. M., Mellors, A., and Welply, J. K. The glycoprotease of Pasteurella haemolytica A1 eliminates binding of myeloid cells to P-selectin but not to E-selectin. Biochem. Biophys. Res. Commun., *188:* 760–766, 1992.
80. Matsushita, Y., Hoff, S. D., Nudelman, E. D., Otaka, M., Hakomori, S., Ota, D. M., Cleary, K. R., and Irimura, T. Metastatic behavior and cell surface properties of HT-29 human colon carcinoma variant cells selected for their differential expression of sialyl-dimeric Le^x-antigen. Clin. & Exp. Metastasis, *9:* 283–299, 1991.
81. Aruffo, A., Stamenkovic, I., Melnick, M., Underhill, C. B., and Seed, B. CD44 is the principal cell surface receptor for hyaluronate. Cell, *61:* 1303–1313, 1990.
82. Bourin, M. C., and Lindahl, U. Glycosaminoglycans and the regulation of blood coagulation. Biochem. J., *289:* 313–330, 1993.