DIFFERENTIAL COLON CANCER CELL ADHESION TO E-, P-, AND L-SELECTIN: ROLE OF MUCIN-TYPE GLYCOPROTEINS

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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" and sLea can be recognized by endothelial E-selectin (reviewed in Refs. 10, 11). P- and L-selectin also recognize sLe" and sLea, 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 coge antigens 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", sLe" (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-96 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-gp120IIIa 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.
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 endotoxin, 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 at 37°C for 30 min. Nonadherent cells were removed by washing with DPBS; adherent cells were fixed with 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.

Adhesion of O-Sialoglycoprotease-treated Tumor Cells to Selectin Immunoglobulins. 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 before treatment of the cells with O-sialoglycoprotease from Pasteurella haemolytica (Cedarlane Laboratories Ltd., Hornby, Ontario). In these studies, 2 x 10^5 tumor cells were suspended at 5 x 10^5/ml in ice-cold RPMI-1% HSA (Alfa 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 x 10^6 cells/ml in RPMI-1% FCS; for tumor cell-platelet adhesion assays, cells were suspended at 3 x 10^6 cells/ml in RPMI-1% FCS.

Tumor Cell Binding to Endothelial Cells, Platelets, and Leukocytes. Endothelial monolayers grown to confluence in NuncTerasaki 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 endolectins 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 rosette 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 x 10^5 in 20 µl) were mixed with 20 µl of RPMI-1% FCS (with or without 10 µM anti-P-selectin or anti-CD18 mAbs) and incubated at room temperature for 20 min. Tumor cells (4 x 10^6 in 20 µl) were added and incubated for an additional 20 min at room temperature. Cells were fixed by adding glutaraldehyde (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 x 10^6 neutrophils in RPMI-1% HSA (50 µl) were transferred into U-bottomed 96-well plates (Becton Dickinson, Oxnard, CA), and mixed with 2 x 10^5 tumor cells (20 µl). After centrifugation at 400 x 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.

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 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 on to 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 E280nm = 1.0.

Binding of Selectin-Immunoglobulin Fusion Proteins to Immobilized Colon Cancer Cell Mucins. Binding of selectin-immunoglobulins 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 µM carboxyl/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 µM CaCl2 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 CaCl2, 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 times.
Fig. 1. Adhesion of colon cancer cells to purified E-, P-, and L-selectin-immunoglobulin (Ig). Protein A-coated microtiter wells 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/mm² to P-selectin-immunoglobulin, 140 ± 20 cells/mm² to L-selectin-immunoglobulin, and 895 ± 78 cells/mm² to E-selectin-immunoglobulin. Adhesion of all tumor cell lines to the control CD8-immunoglobulin fusion protein was lower than 30 cells/mm².

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 cells 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.
COLON CANCER INTERACTIONS WITH SELECTINS

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. 4a) 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. 4a). 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. 4b, 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 cIIb/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. 4c). An anti-L-selectin mAb inhibited this tumor cell-leukocyte adhesion, whereas 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...
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

<table>
<thead>
<tr>
<th>Tumor cell line</th>
<th>Anti-E-selectin</th>
<th>Anti-VCAM-1</th>
<th>Anti-ICAM-1</th>
</tr>
</thead>
<tbody>
<tr>
<td>LS 180</td>
<td>60 ± 6</td>
<td>3 ± 1</td>
<td>4 ± 1</td>
</tr>
<tr>
<td>T84</td>
<td>45 ± 5</td>
<td>1 ± 5</td>
<td>5 ± 1</td>
</tr>
<tr>
<td>COLO 205</td>
<td>41 ± 4</td>
<td>1 ± 1</td>
<td>10 ± 3</td>
</tr>
<tr>
<td>HT-29</td>
<td>75 ± 4</td>
<td>7 ± 1</td>
<td>5 ± 1</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Tumor cell line</th>
<th>Anti-P-selectin</th>
<th>Anti-gpIIb/IIIa</th>
</tr>
</thead>
<tbody>
<tr>
<td>LS 180</td>
<td>71 ± 8</td>
<td>9 ± 3</td>
</tr>
<tr>
<td>T84</td>
<td>78 ± 8</td>
<td>4 ± 1</td>
</tr>
<tr>
<td>COLO 205</td>
<td>73 ± 10</td>
<td>4 ± 2</td>
</tr>
<tr>
<td>COLO 320</td>
<td>87 ± 2</td>
<td>13 ± 3</td>
</tr>
</tbody>
</table>

### Table 3 Inhibition of tumor cell interactions with neutrophils by anti-L-selectin or anti-CD18 mAbs

<table>
<thead>
<tr>
<th>Tumor cell line</th>
<th>Anti-L-selectin</th>
<th>Anti-CD18</th>
</tr>
</thead>
<tbody>
<tr>
<td>LS 180</td>
<td>76 ± 7</td>
<td>5 ± 1</td>
</tr>
<tr>
<td>T84</td>
<td>74 ± 9</td>
<td>6 ± 1</td>
</tr>
<tr>
<td>COLO 205</td>
<td>71 ± 6</td>
<td>5 ± 2</td>
</tr>
<tr>
<td>COLO 320</td>
<td>71 ± 8</td>
<td>4 ± 2</td>
</tr>
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</table>

a Thrombin-stimulated endothelial monolayers were incubated at 4°C for 20 min in 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-p56, anti-MHC class I, and nonblocker anti-E-selectin (H4/18) mAbs had no effect on tumor cell adhesion to stimulated endothelium.

b Adhesion of activated platelets to tumor cells in the presence of indicated mAbs was determined by subtracting the percentage of tumor cell adhesion to nonstimulated endothelium from the percentage of tumor cell adhesion to stimulated endothelium with no mAbs. Values of tumor cell adhesion to nonstimulated endothelium were subtracted from both control and mAb-treated samples, and are mean ± SE of 3–4 experiments, each performed in triplicate.

c 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. In the same study, antibody inhibition of platelet adhesion to HL60 cells was: anti-P-selectin (mAb G1), 96%; anti-gpIIb/IIIa (mAb P2), 56%. A nonblocking anti-P-selectin mAb (S12) had no significant effect on platelet adhesion to any tumor cell line (data not shown).

d 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.
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 sLeA and sLeX, whereas the binding of P- and L-selecton do not. For example, the cell line COLO 320, which has very low levels of these sialylated antigens, 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-sialoglycopeptase (79). Consistent with our observations that E-selectin-dependent HT-29 adhesion is unaffected by O-sialoglycopeptase 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 sLeX 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.

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