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Heparan Sulfate-Like Proteoglycans Mediate Adhesion of Human Malignant Melanoma A375 Cells to P-Selectin Under Flow

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Selectins, a family of cell adhesion molecules, bind to sialylated and fucosylated carbohydrates, such as sialyl Lewis\(^a\) (SL\(\text{Le}^a\)) and its derivatives, as their minimal recognition motif. Here we report that P-selectin bound to human malignant melanoma A375 cells and mediated their adhesion under flow. However, probing with a specific Ab failed to detect any apparent expression of SL\(\text{Le}^a\). This finding was bolstered by reduced expression of \(\alpha\)-1,3-fucosyltransferase VII mRNA and by absence of the cell surface expression of P-selectin glycoprotein ligand-1. Instead, they expressed heparan sulfate-like proteoglycans on their cell surfaces. Treatment with \(\beta\)-D-xyloside (a proteoglycan biosynthesis inhibitor) or heparinases could reduce the binding of these cells to P-selectin. In the competition assays, heparin, but not other proteoglycans, could abolish the P-selectin recognition. Further, we found that P-selectin could bind specifically to human tongue squamous cancer Tca-8113 cells, which had negative staining of SL\(\text{Le}^a\) but positive staining of heparan sulfates. Both \(\beta\)-D-xyloside and heparinases could reduce the binding of P-selectin to Tca-8113 cells. Our results thus indicate that heparan sulfate-like proteoglycans can mediate adhesion of certain types of non-blood borne, “epithelial-like” human cancer cells to P-selectin. The Journal of Immunology, 2000, 165: 558–565.

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euocyte-endothelial cell interactions are mediated by at least four families of cell adhesion molecules. They are selectins (CD62), selectin ligands, integrins, and the IgG superfamily of cell adhesion molecules. The selectin family includes L-selectin (CD62L), a constitutively expressed receptor on most leukocytes; E-selectin (CD62E), a cytokine-inducible molecule exclusively expressed on stimulated endothelial cells; and P-selectin (CD62P), a presynthesized protein stored in the Weibel-Palade bodies of endothelial cells and the \(\alpha\) granules of platelets (1–4).

After inflammatory and thrombogenic challenges, P-selectin translocates, by exocytosis, from the cellular granules to the cell surfaces of endothelial cells and platelets in seconds. Further, P-selectin can be up-regulated by de novo synthesis in the stimulated endothelial cells in hours. P-selectin interacts with P-selectin glycoprotein ligand-1 (PSGL-1\(^{-1}\); CD162), a principal ligand expressed on a majority of leukocytes, to mediate tethering (initial attachment), rolling, and weak adhesion of leukocytes on the activated endothelial cells. P-selectin also mediates heterotypic aggregation of the activated platelets to leukocytes (2, 3).

Structurally, the extracellular portion of P-selectin has an NH\(_2\)-terminal domain of \(\sim\)120 residues homologous to the Ca\(^{2+}\)-dependent, carbohydrate-recognition domain (C-type animal lectin).

Functionally, the binding of P-selectin to leukocytes requires sialylated and fucosylated carbohydrate structures (a lectin/carbohydrate pair); their prototype is \(\text{S}i\alpha\text{ar}2–3\text{Gal}\beta1–4(\text{Fuc}\alpha1–3)\text{GlcNAc}\), called sialyl Lewis\(^a\) (SL\(\text{Le}^a\)) (1–4). The existence of SL\(\text{Le}^a\)-like moieties on O-linked glycans of PSGL-1 further supports this notion (5, 6).

The observation that all three selectins interact with SL\(\text{Le}^a\)-like oligosaccharides has also been demonstrated in vivo. A congenital defect of endogenous fucose metabolism leading to an inability to synthesize fucosylated carbohydrates, including SL\(\text{Le}^a\) and related structures, is clinically identified as leukocyte adhesion deficiency type II. These children suffered from severe recurrent bacterial infection, which characteristically demonstrated no pus formation despite the marked elevation of blood leukocyte counts (7). Their neutrophils rolled poorly on the postcapillary venules under shear stress (8). Further, mice lacking \(\alpha\)-1,3-fucosyltransferase VII (FucT-VII) exhibited a leukocyte adhesion deficiency demonstrated by absence of P- and E-selectin ligand activity on leukocytes and deficiency in L-selectin ligand activity on endothelial venules. These mice had leukocytosis, impaired leukocyte extravasation in inflammation, and faulty lymphocyte homing (9).

However, the above conclusion is mainly drawn from the studies of high endothelial venules of lymphatic tissues and circulating leukocytes. Our understanding of the carbohydrate ligand structures for P-selectin on non-blood-borne, “epithelial-like” cancer cells is still limited. For example, probing the P-selectin-binding small cell lung cancer cells and neuroblastoma cells with specific Abs failed to detect any apparent expression of SL\(\text{Le}^a\) (10). A SL\(\text{Le}^a\)-negative cell line of human malignant melanoma, NKI-4, bound to E-selectin (11) and P-selectin (12). Further, an SL\(\text{Le}^a\)-deficient variant of HL-60 cells exhibited high levels of adhesion to P- and E-selectin (13). These findings raised the question as to whether, in addition to SL\(\text{Le}^a\) and its derivatives, P-selectin might react with other oligosaccharide structures, especially on somatic cancer cells. In this study, we investigated the potential carbohydrate structures for P-selectin on A375 cells, a cell line of a human...
malignant melanoma, and, to a lesser extent, on Tca-8113 cells, a cell line of a human tongue squamous cancer.

Materials and Methods

Proteins and Abs

P-selectin receptor-globulin (P-selectin Rg; constructed by fusing the lectin domain, the epidermal growth factor-like domain, and the two complement CUB repeats of P-selectin with the Fc portion of human IgG1) was prepared as before (12, 14). G1, a leukocyte adhesion blocking Ab against P-selectin, was characterized as previously described (15). PS1, a leukocyte adhesion nonblocking IgM Ab against P-selectin, was prepared according to the previously described methods (16). Fab\(\text{\textsubscript{2}}\) fragments of G1 and PS1 Abs were prepared using an ImmunoPure Fab\(\text{\textsubscript{2}}\) Preparation Kit (Pierce, Rockford, IL).

MAA, an IgM mAb to Lewis\(\text{b}^{\text{\textsubscript{\textit{a}}}}\) (Le\(\text{\textsubscript{a}}\)), was purchased from Becton Dickinson Immunocytometry Systems (San Jose, CA). CSLEX, an IgM mAb to SL\(\text{e}^{\text{\textsubscript{\textit{a}}}}\), was prepared as before (16). 10E4, an IgM mAb to the native heparan sulfate chains of proteoglycans, was a gift from Dr. Guido David (17).

Rabbit preimmune IgG and an Ab raised against a synthetic peptide corresponding to residues 41–55 of the PSGL-1 amino acid sequence were prepared as described (12, 18). The PSGL-1 peptide Ab was initially isolated by protein A chromatography and further affinity purified on the immobilized synthetic peptide.

Cell lines

Human cell lines of promyeloid cells (HL-60; CCL 240) and malignant melanoma (A375; CRL 1619) were purchased from American Tissue Culture Collection (Manassas, VA). Tca-8113 was purchased from the Cell Bank of Type Culture Collection of Chinese Academy of Sciences (Shanghai, China). They were cultured in RPMI 1640 (Life Technologies, Gaithersburg, MD) supplemented with 10% heat-inactivated newborn bovine calf serum (BCS), 4 mM L-glutamine, 100 U/ml penicillin, and 100 µg/ml streptomycin at 37°C in the presence of 5% CO\(\text{\textsubscript{2}}\).

Flow cytometric assays

Adherent cells were detached by PBS (PBS, pH 7.4) containing 0.02% EDTA (Versene, Life Technologies). All cells were washed once and re-suspended in PBS/BCS (PBS supplemented with 1 mM CaCl\(\text{\textsubscript{2}}\), 1 mM MgCl\(\text{\textsubscript{2}}\), and 1% heat-inactivated BCS). 1 × 10\(\text{\textsuperscript{6}}\) cells/ml. Each aliquot (0.5 ml) of cells was incubated with 1–3 µg mouse IgM (Calbiochem-Novobiochm, La Jolla, CA), rabbit preimmune IgG, MAA mAb, CSLEX mAb, 10E4 mAb, or a PSGL-1 peptide Ab followed by 3 µg of an FITC-conjugated Ab against mouse IgM or rabbit IgG (Sigma, St. Louis, MO) at 22°C for 1 h with end-to-end rotation.

For the cell surface P-selectin binding assay, each aliquot (0.5 ml) of cells was incubated with 3 µg human IgG (Sigma) or P-selectin Rg followed by 3 µg of an FITC-conjugated Ab against human IgG (Pierce) at 22°C for 1 h with end-to-end rotation. Cells were sedimented at 1500 rpm for 5 min on a tabletop centrifuge, and supernatants were discarded. For Ab inhibition experiments, 3 µg P-selectin Rg were preincubated with 10 µg G1 (Fab\(\text{\textsubscript{2}}\)), or PS1 (Fab\(\text{\textsubscript{2}}\)) in 50 µl PBS/BCS at 22°C for 30 min. For proteoglycan inhibition experiments, 1 µg P-selectin Rg was preincubated with 0.3 mg heparin, chondroitin sulfate, keratan sulfate, or hyaluronic acid (all from Sigma) in 100 µl PBS/BCS at 22°C for 30 min. Each aliquot was then resuspended in 0.5 ml PBS/BCS for immediate flow cytometric analysis (FACScan, Becton Dickinson, Mountain View, CA).

Semi-quantitative RT-PCR for FucT-VII and core 2 transferase (C2GnT) mRNA

Total cellular RNA was isolated from ~10\(\text{\textsuperscript{6}}\) cells using TRIzol (Life Technologies), and ~1 µg total RNA was used as template in a 20-µl reverse transcription reaction. PCR amplification of cDNA was conducted with 35 cycles of 30 s at 94°C, 45 s at 56°C, and 1 min at 72°C. Preliminary experiments demonstrated that this cycle number was well below the plateau phase for PCR products. Hence, the amounts of the PCR products could reflect the amounts of mRNA initially present (data not shown). Primers were as follows: for detection of FucT-VII mRNA, sense 5’-CCC ACC GTG GCC CAG TAC CGC TTC T-T3’ and antisense 5’-CTG ACC TCT GTG CCC AGC CTC CGC TTC T-T3’, for detection of C2GnT mRNA, sense 5’-TCT GGG AGT GCC GAT CTC GCC TAC TCT GTC TTC-3’ and antisense 5’-ATG CTC ATC CAA ACA CGT GAT GCC AAA-3’ (13). RT-PCR (reverse transcriptase-PCR) products were assayed by 2% agarose gel electrophoresis.

Proteoglycan biosynthesis inhibition and heparinase digestion

For experiments of proteoglycan biosynthesis inhibition, cells were cultured in RPMI 1640 (Life Technologies) supplemented with 10% BCS with or without 1 mM β-T-xylene (4-methylumbelliferyl-β-T-xylene, Sigma) for 1 wk (19–21). For heparinase digestion experiments, cells were washed twice with an equal volume mixture of DME medium (high glucose) and Ham’s F-12 medium (both from Life Technologies) supplemented with 1 mM CaCl\(\text{\textsubscript{2}}\) and MgCl\(\text{\textsubscript{2}}\). The washed cells were resuspended at 1 × 10\(\text{\textsuperscript{6}}\) cells/ml in the same media. Each aliquot of 0.5 ml cells was digested with 1 U/ml heparinasises I, II, and III (Sigma), in the presence of a cocktail of protease inhibitors (10 µg/ml leupeptin, 10 µg/ml pepstatin, 20 µg/ml aprotinin, and 10 mM benzamidine, all from Sigma), at 37°C for 1 h with end-to-end rotation. The cells were then washed twice and resuspended in PBS/BCS for further experimentation.

Laminar flow assay

Polystyrene slides were cut from bacteriological petri dishes (Falcon 1058, Becton Dickinson Labware, Lincoln Park, NJ) and fitted into a parallel plate laminar flow chamber (22, 23). Slides were coated with 2 ml of 10 µg/ml human IgG or P-selectin Rg in 20 mM Tris-HCl (pH 9.5), 140 mM NaCl, 0.02% NaN\(\text{\textsubscript{3}}\) at 4°C overnight and blocked with 3% human serum albumin at 22°C for 2 h. Slides were mounted on the stage of an inverted phase contrast Olympus microscope (Olympus Optical, Tokyo, Japan) connected to a time lapse video cassette recorder STLV-24P (Samsung Electronics, Suwon, Korea) using a Panasonic color CCTV camera wv-GP410/G (Matsushita Communication Industrial, Osaka, Japan). A375 cells were resuspended at 0.5 × 10\(\text{\textsuperscript{6}}\) cells/ml in PBS supplemented with 10 mM HEPES, pH 7.4, and 2 mM CaCl\(\text{\textsubscript{2}}\). The cells were injected into the flow chamber at 22°C using a syringe pump. The wall shear stress was 0.5 dyne/cm\(\text{\textsuperscript{2}}\) unless specifically indicated. The numbers of bound cells were quantified from videotape recordings of 10–20 fields of view obtained (3–4 min after flowing cells through the chamber) while scanning the lower plate of the flow chamber using a 10X objective lens. Adhesive interactions between cellular Fe receptors and the Fn domain of P-selectin Rg were eliminated by preincubation of the cells with 10 µg/ml human IgG at 22°C for 20 min. For inhibition experiments, the immobilized P-selectin Rg was preincubated with 10 µg/ml G1 (Fab\(\text{\textsubscript{2}}\)) or PS1 (Fab\(\text{\textsubscript{2}}\)) at 22°C for 20 min. Treatment of β-T-xylene and heparinasises were the same as above.

Results

P-selectin binding to A375 cells

Using a cell surface-binding assay, we examined the interaction of P-selectin Rg with A375 cells. In this assay, a FITC-conjugated Ab to human IgG was used to report the binding of P-selectin Rg to these cells by flow cytometry. As shown in Fig. 1, P-selectin Rg, but not human IgG, bound to HL-60 cells (a cell line of human promyeloid cells) and A375 cells (a cell line of a human malignant melanoma). Preincubation of P-selectin Rg with G1 Fab\(\text{\textsubscript{2}}\) (a leukocyte adhesion blocking mAb to P-selectin), inhibited this binding, indicating the binding specificity of P-selectin to A375 cells.

Cell surface expression of heparan sulfate-like proteoglycans

We then investigated the carbohydrate structures expressed on A375 cells using oligosaccharide-specific mAbs. Fig. 2 shows that compared with mouse IgM, MAA (a mAb to Le\(\text{\textsubscript{a}}\)) bound to both HL-60 cells and A375 cells. However, CSLEX (a mAb to SLe\(\text{\textsuperscript{a}}\)) reacted to HL-60 cells but did not react with A375 cells. In contrast, 10E4 (a mAb to heparan sulfates) recognized A375 cells but did not recognize HL-60 cells. These results suggested that although HL-60 cells expressed SLe\(\text{\textsuperscript{a}}\), they had no apparent expression of heparan sulfate-like proteoglycans detectable to 10E4 mAb. In contrast, although A375 cells did not express SLe\(\text{\textsuperscript{a}}\) detectable to CSLEX mAb, they expressed heparan sulfate-like proteoglycans on their cell surfaces.

Studies in transfected cell lines and in normal T lymphocytes have demonstrated that the functional binding of P-selectin requires certain glycosyltransferases, such as FucT-VII and C2GnT (24,
25). To support the above findings, we measured mRNA for FucT-VII and C2GnT by semiquantitative RT-PCR. Fig. 3 shows that HL-60 cells and A375 cells expressed indistinguishable high levels of mRNA for C2GnT, indicating that both cell lines were equally capable of generation of the branched O-linked glycans. However, A375 cells apparently had less FucT-VII mRNA than HL-60 cells, suggesting that there might have been less fucosylated glycans synthesized in A375 cells. This finding is consistent with the finding of the apparent negative expression of SLex on A375 cells (Fig. 2).

To further support our findings, we examined whether A375 cells expressed PSGL-1, a principal leukocyte ligand for P-selectin known to carry the SLex-like structures (5, 6). Fig. 4 shows that a PSGL-1 peptide Ab, but not rabbit preimmune IgG, bound avidly to HL-60 cells. By contrast, when compared with rabbit preimmune IgG, this Ab apparently did not recognize A375 cells, suggesting that there might have been less fucosylated glycans synthesized in A375 cells. This finding is consistent with the finding of the apparent negative expression of SLex on A375 cells (Fig. 2).

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Reduction of P-selectin binding by a proteoglycan inhibitor

To investigate whether the cell surface heparan sulfate-like proteoglycans might play a role in P-selectin binding to these cancer cells, we conducted the following experiments. In these experiments, the biosynthesis of proteoglycans was inhibited by β-D-xyloside (19–21), before P-selectin was bound to them. Fig. 5 shows that compared with human IgG, P-selectin Rg bound to HL-60 cells and A375 cells. β-D-Xyloside inhibited the binding of P-selectin to A375 cells. It also marginally reduced the binding of 10E4 (a mAb to heparan sulfates) to A375 cells. In contrast, the same β-D-xyloside treatment had no inhibitory effects on the binding of P-selectin to HL-60 cells. These results indicated that some proteoglycans on A375 cells, but not on HL-60 cells, might participate in the binding of A375 cells to P-selectin.

Abolishment of P-selectin binding by heparin

To corroborate the above findings, we examined which proteoglycan could inhibit the P-selectin binding. Fig. 6 shows that compared with human IgG, P-selectin Rg bound to A375 cells. Heparin, but not keratan sulfate or hyaluronic acid, could abolish the binding of P-selectin to A375 cells. Interestingly, chondroitin sulfate could also partially inhibit the P-selectin binding. However, higher concentrations of chondroitin sulfates (up to 3 mg) could not completely inhibit the P-selectin binding (data not shown). These data provided the convergent evidence indicating that the cell surface heparan sulfate-like proteoglycans facilitated the P-selectin binding to A375 cells.
Inhibition of P-selectin binding by heparinase digestion

To further strengthen the above conclusion, we digested A375 cells with a combination of three heparinases (heparinase I, II, and III). As shown in Fig. 7, compared with human IgG, P-selectin Rg bound to HL-60 cells and A375 cells. Treatment with heparinases clearly reduced the binding of P-selectin to A375 cells but did not reduce the binding of P-selectin to HL-60 cells. They also reduced the binding of 10E4 (a mAb to heparan sulfates) to A375 cells. These data were consistent with the results of the proteoglycan biosynthesis inhibition experiments (Fig. 5) and further suggested that among various kinds of proteoglycans, it was heparan sulfate-like proteoglycans that appeared to be involved in the binding of P-selectin to A375 cells.

Neutralization of adhesion of A375 cells to P-selectin under flow by β-D-xyloside and heparinases

In an attempt to correlate the above findings from the cell surface binding assay with an assay in a more physiological setting, we performed experiments for measurements of adhesion of A375 cells to P-selectin under flow conditions by using a flow cytometry-based assay.
cells to P-selectin under physiological flow conditions. We first measured the adhesive properties of HL-60 cells and A375 cells. As shown in Fig. 8A, both cell lines adhered avidly to immobilized P-selectin Rg at 0.5 dyne/cm². However, at 1–1.5 dyne/cm², significantly less amounts of A375 cells could adhere to P-selectin when compared with those of HL-60 cells, indicating a sharp difference in the binding kinetics between these two cell lines.

Using this assay, we investigated the effects of β-D-xylolyside and heparinases on the adhesion of A375 cells to P-selectin. Fig. 8B shows that A375 cells adhered to immobilized P-selectin Rg, but not to immobilized human IgG, under shear forces similar to those of capillary venules (0.5 dyne/cm²). G1 F(ab)_2 (a leukocyte adhesion blocking mAb to P-selectin), but not PS1 F(ab)_2 (a leukocyte adhesion nonblocking mAb to P-selectin), neutralized adhesion of A375 cells to P-selectin, indicating the specificity of this adhesion. Treatment with β-D-xylolyside (an inhibitor of proteoglycan biosynthesis) and digestion with heparinas both markedly reduced the adhesion of A375 cells to P-selectin, further supporting our conclusion that the cell surface heparan sulfate-like proteoglycans could mediate adhesion of A375 cells to P-selectin. In contrast, treatment with β-D-xylolyside or heparinases had no inhibitory effects on the adhesion of HL-60 cells to P-selectin (data not shown). This finding is fully consistent with the P-selectin binding data (Figs. 5 and 7).

**Requirement of cell surface heparan sulfate-like proteoglycans for P-selectin binding to Tca-8113 cells**

We finally searched several cell lines of human cancers to explore whether our findings that heparan sulfate-like proteoglycans could mediate adhesion of P-selectin to A375 cells were applicable to other human cancer cells. Among these cell lines, we found that heparan sulfate-like proteoglycans indeed could mediate adhesion of P-selectin to Tca-8113 cells, a cell line of a human tongue squamous cancer. As summarized in Table I, P-selectin Rg, but not human IgG, bound to Tca-8113 cells. G1 F(ab)_2, but not PS1 F(ab)_2, could inhibit this binding. These cells had no apparent expressions of Le^a, SLe^a, and PSGL-1 detectable to MMA (a mAb to Le^a), CSLEX (a mAb to SLe^a), and the PSGL-1 peptide Ab. However, 10E4 (a mAb to heparan sulfates) bound avidly to them, indicating the presence of heparan sulfate-like proteoglycans on Tca-8113 cells. Further, treatment of these cells with β-D-xylolyside and heparinases both reduced the binding of P-selectin to these cells, attesting to the functional roles of heparan sulfates on Tca-8113 cells for P-selectin recognition. Taking together these observations, we conclude that P-selectin can react with the cell surface heparan sulfate-like proteoglycans to mediate adhesion of certain kinds of cancer cells, such as A375 cells and Tca-8113 cells.

**Discussion**

Prior work has shown that heparin and heparan sulfates bound to P- and L-selectin but did not bind to E-selectin (26–32). In addition, heparin and heparan sulfates were shown to inhibit leukocyte adhesion mediated by P- and L-selectin but not leukocyte adhesion mediated by E-selectin (27–31). However, given a large number of the “heparin-binding” proteins (33) and a broad range of naturally and artificially sulfated molecules that inhibit leukocyte adhesion mediated by P- and L-selectin (27–29, 31, 32), it is generally assumed that there is relatively little specificity to these inhibitory effects. As a result, their biological relevance has not been well defined.

In the present study, we showed that P-selectin bound to A375 cells, a cell line of a human malignant melanoma. Interestingly, A375 cells had no apparent expression of SLe^a detectable to CSLEX (a mAb to SLe^a) and had a reduced amount of FucT-VII mRNA. Instead, they expressed heparan sulfate-like proteoglycans on their cell surfaces. Both β-D-xylolyside (a proteoglycan biosynthesis inhibitor) and heparinases appeared to reduce adhesion of A375 cells to P-selectin. Heparin, but not other proteoglycans, abolished the P-selectin binding to these cells. Further, we found the similar results for Tca-8113 cells, a cell line of a human tongue squamous cancer.
squamous cancer. On the basis of these findings, we conclude that the cell surface heparan sulfate-like proteoglycans can mediate adhesion of A375 cells and Tca-8113 cells to P-selectin. We believe that our results may have important impacts on the current understanding of the molecular mechanisms for the interactions of P-selectin with its cognate ligands on human somatic cancers.

It is well known that under flow conditions, human leukocytes and cell lines derived from human leukocytes, such as HL-60 cells and U937 cells, can roll on and adhere to immobilized P-selectin (23). However, we observed that A375 cells had no apparent rolling on the immobilized P-selectin. In addition, unlike HL-60 cells, they could specifically adhere to the immobilized P-selectin mainly at 0.5 dyne/cm² (Fig. 8). These findings are consistent with the previous reports using human melanoma A375 M cells (34) and 397 cells (35), indicating that the binding properties for P-selectin recognition of human leukocytes and human melanoma cells are quite distinct.

P-selectin has been shown to bind to several human cancers and human cancer-derived cell lines in vitro, such as colon cancer, lung cancer including small cell lung cancer, breast cancer, malignant melanoma, gastric cancer, and neuroblastoma (10–12, 22, 34–40). Recently, using P-selectin deficiency mice, it has been demonstrated that P-selectin can significantly promote the growth and metastasis of human colon carcinoma (41). This finding indicates that adhesion of human colon carcinoma cells to P-selectin must play critical roles in the in vivo pathological processes of this cancer. By extrapolation, we suspect that this molecular mechanism may participate in the growth and the metastasis of other P-selectin-binding cancer cells, such as those described above.

FIGURE 7. Effects of heparinases on P-selectin binding. HL-60 cells and A375 cells were incubated with human IgG, P-selectin Rg, mouse IgM, or 10E4 (a mAb to heparan sulfates) followed by an FITC-conjugated Ab against human IgG or mouse IgM after digestion with heparinases I, II, and III. The binding events were analyzed by flow cytometry, as described above. Results are representative of three separate experiments.

FIGURE 8. Effects of β-α-xylolide (β-DX) and heparinases on adhesion of A375 cells to P-selectin under flow. Adhesion of A375 cells and HL-60 cells to immobilized human IgG or immobilized P-selectin Rg (designated as +), from 0.5 to 1.5 dyne/cm² (A) or at 0.5 dyne/cm² (B), was measured (square millimeters after perfusion of the flow chamber with 1 × 10⁶ cells). For Ab inhibition experiments, P-selectin Rg was preincubated with G1 F(ab)₂ (a leukocyte adhesion blocking mAb to P-selectin) or PS1 F(ab)₂ (a leukocyte adhesion nonblocking mAb to P-selectin). A375 cells were also pretreated with β-α-xylolide and heparinases. All results were expressed as the mean number (± SD) of adherent A375 cells in 10–20 fields of view using a 10× objective lens from five to six separate experiments.
In literature, heparin and heparan sulfate-like proteoglycans have been shown to attenuate adhesion of several cancer cells in in vivo metastasis models (reviewed in Refs. 42–46). They included, for example, a melanoma (B16-BL6) model (47), a human pancreatic adenocarcinoma (Ca-Pan-2) model, a melanoma (B16-F10) lung metastasis model (48), a murine cancer cell (3LL-HH) liver metastasis model (49), and a rat model of tumor implantation after laparoscopy (50). However, the molecular mechanism(s) for these therapeutic effects remained to be determined. It is our supposition that their therapeutic effects on cancer metastasis could be due partly to the abrogation of the P-selectin-mediated adhesion of cancer cells, as illustrated in this study.

In conclusion, our findings indicate that P-selectin can interact with both SLEx and its derivatives on leukocytes and heparan sulfate-like proteoglycans responsible for the recognition of P-selectin. We believe that an extension of these lines of investigation should facilitate our understanding of the molecular mechanisms for P-selectin-mediated adhesion of leukocytes and cancer cells.

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