The Binding of T Cell-expressed P-selectin Glycoprotein Ligand-1 to E- and P-selectin Is Differentially Regulated*

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The HECA452 carbohydrate epitope, also termed cutaneous lymphocyte antigen, is known to bind to E-selectin and defines a human T cell subset preferentially found in inflamed skin. Activated T cells can express a functional form of the P-selectin glycoprotein ligand-1 (PSGL-1), the major ligand known for P-selectin. Here we show that PSGL-1 can exist in two forms, of which only one carries the HECA452 epitope and binds to E-selectin, while the other only binds to P-selectin. We have analyzed the glycoprotein ligands for E- and P-selectin on the mouse CD8⁺ T cell clone 4G3 at 4, 8, and 12 days after antigen-specific activation. Only at day 4 did the cells bind to E-selectin, whereas cells at all three activation stages bound to P-selectin. Expression of the HECA452 epitope correlated with E-selectin binding. In affinity isolation experiments, PSGL-1 was isolated as the major ligand by E-selectin-IgG and by P-selectin-IgG; however, PSGL-1 only bound to E-selectin at day 4, whereas it bound to P-selectin at all three activation stages. Immunoprecipitated PSGL-1 from cells at day 4, but not from cells at days 8 and 12, was recognized in immunoblots by monoclonal antibody HECA452. In immunoblots of total extracts of cells at day 4, HECA452 recognized a 240/140-kDa pair of protein bands as the major antigen. These bands could be completely removed by depletion of cell extracts with anti-PSGL-1 antibodies. Our data suggest that the carbohydrate requirements for binding of PSGL-1 to P-selectin differ from those necessary for binding to E-selectin. Furthermore, we conclude that the major glycoprotein carrier for the HECA452 epitope on activated 4G3 cells is PSGL-1.

The selectins are a family of three carbohydrate-binding cell adhesion molecules that mediate initial interactions between leukocytes and endothelial cells leading to leukocyte extravasation (1). Among other leukocytes, the two endothelial selectins E- and P-selectin bind previously activated “memory” T cells (2–4). This interaction is indeed relevant for the migration of T cells into inflamed tissue because of P-selectin (5). In addition, sulfation of tyrosine residues within the first 10 N-terminal amino acids of the mature protein was found to be essential for P-selectin binding (5–7). P-selectin was shown to be the major P-selectin ligand on human neutrophils and on activated lymphocytes (13, 14). Cloning of mouse PSGL-1 allowed to generate antibodies against an N-terminal peptide, which blocked binding of mouse neutrophils to P-selectin in vitro (15). With the help of similar polyclonal and monoclonal antibodies, the importance of mouse PSGL-1 for the entry of neutrophils into inflamed peritoneum and of T helper 1 lymphocytes into inflamed skin could recently be demonstrated in vivo (16, 17).

In addition to P-selectin, E-selectin can also bind to PSGL-1. We showed recently (16, 17) that mouse PSGL-1 is identical to a 130-kDa glycoprotein (dimeric form, 230 kDa) which had been found by affinity isolation as a common ligand for mouse E- and P-selectin on mouse neutrophils (18). Human PSGL-1 was also demonstrated to bind to E-selectin (8), although binding was reported to be weaker than binding to human P-selectin (9). The structural requirements for binding of PSGL-1 to P-selectin differ from those for the binding to E-selectin, since tyrosine sulfation of PSGL-1 is only necessary for P-selectin binding (19). It has not yet been analyzed whether the carbohydrate modifications that mediate the binding of PSGL-1 to P-selectin differ from those that are involved in the binding to E-selectin.

A subpopulation of human T lymphocytes that avidly binds to E-selectin and is enriched in sites of chronic inflammation in the skin was reported to express a carbohydrate antigen defined by the monoclonal antibody (mAb) HECA452 (2, 3). Based on the enrichment of this antigen on skin-located T cells, it was termed cutaneous lymphocyte antigen. The mAb HECA452 was shown to block lymphocyte binding to E-selectin (20, 21), suggesting a role of this epitope as a ligand for E-selectin. The tetrasaccharide moieties sialyl Lewis x (sLex) and its stereoisomer sialyl Lewis a (sLeα) are recognized by HECA452. However, the exact nature of the carbohydrate epitope that is recognized by this antibody on skin-located T cells is still unknown, since these cells are largely negative for sLeα and sLeα (21, 22). It is also still unknown which glycoproteins are the carriers of the interesting HECA452 carbohydrate epitope.

In this study, we have analyzed the glycoprotein ligands of E- and P-selectin on the mouse T cell clone 4G3 after antigen-
PSGL-1 Binding to E-selectin Requires the HECA452 Epitope

**EXPERIMENTAL PROCEDURES**

**Cell Culture**—The mouse T lymphoma cell line EG7-OVA, derived by transfection of EL4 cells with the gene for ovalbumin (23), was cultured in Dulbecco's modified Eagle's medium (Life Technologies, Inc., Eggenstein, Germany) supplemented with 10% fetal calf serum (Life Technologies, Inc.). The CD8+ cytotoxic T cell clone 4G3 recognizes the ovomucin epitope SIINFEKL in combination with the major histocompatibility complex molecule K+ (24). It was cultured in Dulbecco's modified Eagle's medium with 10% fetal calf serum and 45 mM β-mercaptoethanol (Sigma, München, Germany) and restimulated every other week with irradiated EG7-OVA cells and 5% supernatant of concanavalin A (ICN, Costa Mesa, CA)-stimulated rat spleen cells, as described before (25). Both cell lines were kindly provided by Dr. P. Walden (Charité, Humboldt Universität zu Berlin, Germany). Hybridoma cell lines were grown in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum.

**Absorptions and Selectin-IgG Chimera**—The antibody-like fusion protein of mouse PSGL-1 and human IgG1 containing essentially all of the extracellular domain of mouse PSGL-1 has been described (16). In analogy to Yang et al. (15), the rabbit anti-mouse PSGL-1 antisera was raised against a peptide covering amino acids 42–60 of the sequence of murine PSGL-1. The rabbit antiserum 703, recognizing human as well as mouse PSGL-1, was raised against the peptide CREDGDILTHFSPLP, starting with an artificial cysteine and covering the C-terminal 16 amino acids of human PSGL-1. Peptides were conjugated to a carrier protein and used for immunization as described by Weller et al. (43). Specific antibodies were purified by affinity isolation on the bovine serum albumin-conjugated peptides linked to CNBr-Sepharose as described (26). The antibody-like fusion protein of mouse PSGL-1 (PSGL-IgG) was affinity-purified on PSGL-IgG conjugated to CNBr-Sepharose (Pharmacia, Uppsala, Sweden). Control rabbit antibodies were purified from a nonimmune serum with protein A-Sepharose (Pharmacia). The mAbs HECA452 (anti-carbohydrate rat IgM) (27), CSLEX-1 (anti-sLex mouse IgM) (28) were purchased from the American Type Culture Collection (ATCC). The mAbs 2F3 (anti-sLex mouse IgM) (29) and 2H5 (anti-sLex mouse IgM) (30) were purchased from Becton Dickinson (San José, CA). The activation peptides P-selectin-IgG, E-selectin-IgG, and VE-cadherin-IgG have been produced as described elsewhere (26, 31). Conjugated antibodies and streptavidin were purchased from Dianova (Hamburg, Germany).

**Flow Cytometry**—Adherent 4G3 cells were harvested by cooling the plates at 4 °C followed by gentle pipetting. Cells were incubated with 25 μg/ml selectin-IgG chimera, 10 μg/ml rabbit antibodies, or 50 μg/ml purified mouse IgM mAb in Hanks' balanced salt solution (Biochrom, Berlin, Germany) supplemented with 3% fetal calf serum and 0.04% azide or with rat hybridoma supernatant for 30 min on ice. Subsequently, cells were stained with phycoerythrin-conjugated F(ab')2 donkey anti-human IgG, FITC-labeled goat anti-rabbit IgG, FITC-labeled rabbit anti-mouse IgM, or FITC-labeled goat anti-rat IgG and IgM, respectively. Analysis was performed on a Becton Dickinson (San Jose, CA) FACsCalibur with CellQuest analysis software.

**Fucosyltransferase Assays**—Assays were basically performed as described (32) using detergent-extracted 4G3 cells and 3′-sialyllactosamine as acceptor oligosaccharide. To obtain values solely due to fucosylation of acceptor substrate, total counts of the control (without acceptor substrate) were subtracted from total counts of samples with acceptor. The activity of the fucosyltransferase-containing cell extract was calculated as pmol/min/mg.

**Cell Adhesion Assay**—Adhesion assays were performed in 96-well flat bottom plates coated with 5 μg/ml selectin-IgG chimera or human IgG in Hanks' balanced salt solution (Biochrom). 5 × 104 cells were incubated for 20 min at 4 °C under mild rotating conditions (80 rpm). Plates were washed three times with Hanks' balanced salt solution, fixed with 2% glutaraldehyde (Sigma), and evaluated by computer-aided image analysis with the NIH Image 1.55 software (17). For inhibition studies, cells were preincubated with 5-fold concentrated supernatant for 30 min on ice and washed once before the addition to the microtiter plates.

**Immunoprecipitation**—Cells were surface-biotinylated with 0.5 mg/ml Sulfo-NHS-biotin (Pierce) in phosphate-buffered saline, lysed, and subjected to immunoprecipitation as described (17, 18). Immunoprecipitated proteins were separated by electrophoresis on 6% SDS-PAGE and transferred to nitrocellulose (Schleicher & Schuell). Filters were blocked for 1 h in PBS containing 0.1% bovine serum albumin (Dianova) and the ECL system (Amersham, Braunschweig, Germany). Alternatively, nonlabeled cells were subjected to immunoprecipitations, and precipitated antigens were analyzed by immunoblotting.

**Western Blot Analysis**—Total cell extracts (5 × 106 cells/lane) or affinity-isolated material were separated by electrophoresis on 6% SDS-PAGE and transferred to nitrocellulose (Schleicher & Schuell) by semi-dry blotting (Bio-Rad). Filters were blocked with 4% defatted milk powder (Saliter, Oberguzenburg, Germany) in Tris-buffered saline (TBS) and incubated with 10 μg/ml antibodies in 4% milk powder/TBS or with hybridoma supernatant. Blots were washed with 4% milk powder/TBS and incubated with a peroxidase-conjugated secondary antibody in 4% milk powder/TBS. After washing with TBS, blots were developed with the ECL system (Amersham). Alternatively, an alkaline phosphatase-conjugated second antibody was used and detected with nitro blue tetrazolium and 5-bromo-4-chloro-3-indolylphosphate (Promega, Madison, WI).

**Depletion Experiments**—Total cell extracts (3 × 107 cells) were lysed in 200 μl of lysis buffer (Tris-Cl, pH 8.0, 0.5 mm Tris(2-carboxyethyl)phosphine, 1 mm CaCl2). For depletion, half of the cell extract was incubated at 4 °C with 20 μg of antibody bound to 20 μl of protein A-Sepharose. Incubations were repeated twice, and each round of incubation was for 8 h. Depletions were done with a mixture of affinity-purified anti-PSGL-1 antibodies from either antisera 703 and 002 or antisera 703 and 124. For negative control depletions, IgG from a nonimmune rabbit serum was used. Depleted lysates were subjected to electrophoresis on 6% SDS-polyacrylamide gel electrophoresis and analyzed in immunoblots with mAb HECA452.

**RESULTS**

**Antigen-specific Activation of the T Cell Clone 4G3 Induces Transient Expression of the HECA452 Carbohydrate Epitope**—The mouse CD8+ T cell clone 4G3 recognizes an ovomucin epitope in combination with the major histocompatibility complex molecule K+. 4G3 cells were restimulated every other week in an antigen-specific manner with EGT-OVA cells, which were derived by ovalbumin transfection of EL4 cells. The activation cycle peaks in an activation maximum after 4 days when cells are blasted, highly cytotoxic, and proliferate at maximal rates. The activation level gradually declines in the following 8 days to base-line levels before they have to be restimulated. Cells at day 12 represent the cells that are restimulated at day 0. At days 4, 8, and 12 after stimulation, cells were analyzed by flow cytometry for binding of the mAb HECA452, affinity-purified rabbit antibodies (antisera 124) against the N terminus of mouse PSGL-1, and the antibody-like selectin fusion proteins E-selectin-IgG and P-selectin-IgG. At 4 days after stimulation, cells strongly expressed the HECA452 epitope as well as ligands for E- and P-selectin (Fig. 1). In contrast, cells at days 8 and 12 showed strongly reduced binding for HECA452 as well as for E-selectin-IgG, while binding of P-selectin-IgG was unchanged. The mean fluorescence intensity for HECA452 decreased from 721 at day 4 to 76 at day 8 and 40 at day 12, and for E-selectin-IgG it decreased from 609 at day 4 to 109 at day 8. The expression level of PSGL-1 was similar on cells of all three activation states (mean fluorescence intensities of 921, 751, and 590 at days 4, 8, and 12, respectively). The observed slight decrease of the mean fluorescence intensity for PSGL-1 was probably due to a decrease of cell size at days 8 and 12. Binding of the mAb HECA452 to 4G3 cells at the three activation states was compared with the binding of three other mouse IgM antibodies against sLeα. While the CSLEX-1 antibody did not show significant binding, the antibodies 2F3 and 2H5 stained cells at day 4 (Fig. 2). No staining with any of the antibodies was observed at days 8 and 12. These data indicate that the sLeα-related HECA452 epitope is different from the carbohydrate.
CSLEX-1 epitope but similar to the sLe\(^\alpha\)-related epitopes defined by 2F3 and 2H5. These data agree with published evidence that the CSLEX-1 epitope is absent in the mouse (30, 33) and that the mAb 2H5 recognizes its epitope on mouse neutrophils (33).

The induction of sLex-like structures requires the expression of an \(\alpha\)-(1,3/1,4)-fucosyltransferase activity. Therefore, we analyzed the expression level of such enzymes in \(\text{in vitro}\) fucosyltransferase assays using detergent extracts of 4G3 cells at days 4 and 8 and the acceptor oligosaccharide 3\(^\text{9}\)-sialyllactosamine. Cells at day 4 expressed 6.6-fold higher enzyme levels (5.0 \(\pm\) 1, 7 pmol/min/mg) than cells at day 8 (0.76 \(\pm\) 0.05 pmol/min/mg).

Binding of 4G3 Cells to Immobilized E-selectin-IgG Is Only Observed at 4 Days after Activation, whereas Cells at Days 4 and 8 Bind to P-selectin-IgG—Cells at days 4 and 8 were analyzed in static cell adhesion assays in 96-well microtiter plates for their ability to bind to immobilized E- and P-selectin-IgG. Correlating with the expression of the HECA452 epitope (Fig. 1) only cells at day 4 bound to E-selectin-IgG, while cells at day 8 did not bind (Fig. 3A). This binding could be blocked by 80% \((\pm 6.6%)\) when the cells were preincubated with the mAb HECA452, demonstrating the relevance of this carbohydrate modification as E-selectin ligand on 4G3 cells (Fig. 3B). In contrast to the binding to E-selectin, cells at day 4 bound with similar efficiency to P-selectin-IgG as cells at day 8 (Fig. 3A), indicating that the HECA452 epitope is not necessary for cell binding to P-selectin. The mAb HECA452 could partially inhibit the binding of cells at day 4 to P-selectin-IgG by 41% \((\pm 10.8%)\), indicating either that this carbohydrate epitope is overlapping with the carbohydrate epitope necessary for binding to P-selectin or that the HECA452 epitope can be involved in P-selectin binding if it is expressed.

PSGL-1 Is Precipitated by E- and P-selectin-IgG from 4G3 Cells at Day 4 but Only by P-selectin-IgG from Cells at Day 8—Activated 4G3 cells were surface-labeled by biotinylation and subjected to immunoprecipitations with E-selectin-IgG, P-selectin-IgG, and affinity-purified polyclonal antibodies 124 against the N terminus of mouse PSGL-1 (aPSGL-1). The thin line represents negative control staining with an irrelevant rat IgM, with VE-cadherin-IgG, or with total IgG of a rabbit nonimmune serum. Antibodies or fusion proteins were detected as described under “Experimental Procedures.” The depicted experiment represents one of five similar experiments.

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**Fig. 1.** Flow cytometry of activated 4G3 cells. The mouse T cell clone 4G3 was stimulated with antigen-expressing target cells and analyzed by flow cytometry at days 4, 8, and 12 after stimulation with mAb HECA452, the fusion proteins E-selectin-IgG (E-sel-IgG) and P-selectin-IgG (P-sel-IgG) and affinity-purified rabbit antibodies 124 against an N-terminal peptide of mouse PSGL-1 (aPSGL-1). The thin line represents negative control staining with an irrelevant rat IgM, with VE-cadherin-IgG, or with total IgG of a rabbit nonimmune serum. Antibodies or fusion proteins were detected as described under “Experimental Procedures.” The depicted experiment represents one of five similar experiments.
against the C terminus of PSGL-1, and precipitated material was analyzed in immunoblots with mAb HECA452. As shown in Fig. 5, only PSGL-1 from cells at day 4 carried the HECA452 carbohydrate epitope, while PSGL-1 from cells at day 8 was only very faintly positive and PSGL-1 from cells at day 12 was negative for this epitope. In conjunction with the data of Fig. 4, these data indicate that PSGL-1 binds to E-selectin only when it expresses the HECA452 epitope, while the binding to P-selectin does not correlate with the expression of this carbohydrate epitope.

**PSGL-1 Is the Major Glycoprotein on Activated 4G3 Cells Carrying the HECA452 Epitope**—In immunoblots of total cell extracts of 4G3 cells at day 4, mAb HECA452 reacted with one broad band at 140 kDa (Fig. 6A), which appeared as a doublet in some experiments. Similarly, the 140-kDa monomeric form of PSGL-1 appeared as a doublet, especially when the sample was intensively boiled before electrophoresis, as shown in the immunoblot in Fig. 6B. To examine whether the HECA452-reactive band represents PSGL-1 or also represents other proteins, we depleted total cell extracts for PSGL-1 by three successive rounds of incubation with affinity-purified anti-PSGL-1 antibodies using a mixture of either antibodies 703 and 002 or of antibodies 703 and 124. Immunoblots of the residual proteins of the depleted extracts revealed that all of the HECA452-reactive material had been removed during PSGL-1 depletion (Fig. 7A). The same blot was reprobed with the anti-PSGL-1 antibodies 703 to confirm complete depletion (Fig. 7B). Thus, PSGL-1 is the major glycoprotein carrier of the HECA452 epitope in the cell.

**DISCUSSION**

We have analyzed the ability of PSGL-1 on activated T cells to bind to P-selectin and to E-selectin. We found that antigen-specific activation transiently stimulates expression of the E-selectin-binding carbohydrate epitope HECA452 on PSGL-1 and enables PSGL-1 to bind to E-selectin. In contrast, binding of PSGL-1 to P-selectin was independent of this carbohydrate modification. Furthermore, PSGL-1, known to be the major ligand for P-selectin on most leukocytes, was the only ligand that could be affinity-isolated with E-selectin-IgG and was the major glycoprotein carrier of the HECA452 epitope in the cell. Our results suggest the following. First, the binding of PSGL-1 to E-selectin requires posttranslational modifications that are not necessary for the binding to P-selectin. Second, if the cutaneous lymphocyte antigen is indeed a glycoprotein, it is mainly represented by PSGL-1 on highly activated 4G3 cells.

Binding of PSGL-1 to E-selectin has been reported before (8, 9, 18, 19, 34). In a detailed study it was shown that PSGL-1 requires core 2 O-linked glycans that are sialylated and fucosylated to bind P- and E-selectin (19). Interestingly, sulfation of tyrosine residues was only essential for the binding to P-selectin but not necessary for the binding to E-selectin (19). These studies may suggest that fewer or less complex posttranslational modifications are necessary for the binding to E-selectin than for the binding to P-selectin. In agreement with this, more glycoprotein ligands have been described for E-selectin than for P-selectin (35, 36), suggesting that the criteria that define an E-selectin ligand would be somewhat less stringent than those that define a P-selectin ligand.

Our results provide evidence for a different scenario, suggesting that the recognition motif of E-selectin is not necessarily simpler than that of P-selectin. We describe isoforms of PSGL-1 that carry all of the posttranslational modifications necessary for the binding to P-selectin but that still lack necessary modifications for the binding to E-selectin. Thus, each of the two endothelial selectins requires structural elements for binding of PSGL-1 that are not required for the other selectin. Although our data do not provide direct evidence, it is likely that at least some of the modifications specifically necessary for the binding to E-selectin are represented by the carbohydrate epitope HECA452.

A decisive step in the generation of the HECA452 epitope and the ligand structures recognized by E- and P-selectin is the expression of an α-(1,3/1,4)-fucosyltransferase. A central question arising from the current study is how the E-selectin and the P-selectin binding activities of PSGL-1 could be differentially regulated and what the structural differences between

**FIG. 2. Flow cytometry with four mAbs against sLeα-related carbohydrate epitopes.** Activated 4G3 cells at days 4 (thick line), 8 (thin line), and 12 (dotted line) were analyzed by flow cytometry with mAbs HECA452, 2F3, 2H5, and CSLEX-1 (as indicated). Antibodies were detected as described under "Experimental Procedures." The depicted experiment represents one of two similar experiments.
both recognition motifs are. Since we found a high activity of a fucosyltransferase in 4G3 cells at day 4 but low levels at day 8, a simple answer would be that the expression of the E-selectin binding activity (and the HECA452 epitope) might require higher levels of a fucosyltransferase than the expression of the P-selectin binding activity. The sLex/sLea-related HECA452 epitope might only be formed if a certain density of correctly modified carbohydrate side chains has been generated, while the ability to bind to P-selectin might already be generated if a lower density of similar carbohydrate motifs is expressed on the PSGL-1 polypeptide motif. In this way a quantitative difference of fucosylation could generate qualitative differences in the recognition motif. An alternative explanation for the differences between the E- and P-selectin recognition motifs would be that the E-selectin binding epitope might consist of further components in addition to fucosylated sialolactosamine. Indeed, the HECA452 epitope on 4G3 cells is different from sLe\(^a\) as it is defined by the mAb CSLEX-1, since highly activated 4G3 cells were negative for CSLEX-1. Whatever explanation might be correct, our results establish that the binding activity of PSGL-1 for P- and E-selectin can be independently regulated for each of the two selectins.

It has been reported that phorbol 12-myristate 13-acetate activation of Jurkat cells and stimulation of human T lymphoblasts induce HECA452 and CSLEX-1 epitopes as well as E-selectin binding activity (37), which could be linked to the induction of FucTVII. Induction of the HECA452 epitope on human T cells was found upon stimulation with transforming growth factor-\(\beta\)1 and interleukin-2 (38, 39) and with superantigen (40). Another study described different human CD4\(^+\) T cell clones, which expressed different levels of the HECA452 epitope correlating with the ability of these cells to attach to E-selectin (41, 42). The current study is the first to show that the HECA452 epitope can be induced by antigen-specific activation and that this epitope can be expressed on mouse cells. The remarkable selectivity with which PSGL-1 was modified with the HECA452 epitope in 4G3 cells is reminiscent of what we described recently for another ligand of E-selectin, the E-selectin ligand-1 in Chinese hamster ovary cells (32). In highly activated 4G3 cells, the level of ESL-1 was too low to be recognized with the mAb HECA452 in immunoblots (data not shown). The mechanism that allows us to selectively generate the HECA452 epitope on only very few glycoproteins will be of
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FIG. 6. The mAb HECA452 recognizes one protein of 140 kDa in total cell extracts of activated 4G3 cells at day 4. A, total cell extracts of activated 4G3 cells at days 4 (4d) and 8 (8d) and of the target cell EG7-OVA (EG7) were analyzed in immunoblots with mAb HECA452. A specific signal with mAb HECA452 was only seen with activated 4G3 cells at day 4. B, for comparison, total cell extracts of activated 4G3 cells at day 4 were immunoblotted with affinity-purified rabbit antibodies 124 against the N terminus of mouse PSGL-1 (aPSGL) or with rabbit control antibodies (Co). Intensive boiling sometimes caused the monomeric form of PSGL-1 to appear as a broad double band, as shown in A and B. Molecular weight markers (in kDa) are indicated on the left.

FIG. 7. The HECA452-reactive glycoprotein can be completely removed with antibodies against PSGL-1. Detergent extracts of activated 4G3 cells at day 4 were depleted by three rounds of incubation with protein A-Sepharose loaded either with affinity-purified rabbit antibodies against mouse PSGL-1 (aPSGL) or with control rabbit antibodies (Co). As a further negative control, cell lysate buffer that had been incubated with protein A-Sepharose loaded with rabbit IgG was electrophoresed on the same gel (lysate buffer), immunoblotted, and analyzed with mAb HECA452. Note that the HECA452-reactive cellular material was completely removed upon depletion with anti-PSGL-1 antibodies. The blot that was used in A was reprobed in B. The depicted experiment represents one of four similar experiments. Molecular weight markers (in kDa) are indicated on the left.

central importance in understanding the regulation of E-selectin ligand expression.

The fact that PSGL-1 is the only glycoprotein ligand that could be isolated with E-selectin-IgG as affinity probe suggests that PSGL-1 represents a significant ligand for E-selectin. This is further supported by the finding that PSGL-1 is the major glycoprotein carrier of the HECA452 epitope, a carbohydrate structure that is likely to be involved in E-selectin binding. Unfortunately, no antibodies against mouse PSGL-1 are available that could specifically block the interaction between PSGL-1 and E-selectin. Therefore, direct evidence is still lacking that would allow us to determine to what extent the HECA452-modified isoform of PSGL-1 is involved in cell attachment to E-selectin. The best evidence that PSGL-1 can indeed mediate cell rolling on E-selectin was reported by Patel et al. (34), who showed that the mAb PL1 against human PSGL-1 can inhibit rolling of HL60 cells and human neutrophils on E-selectin by about 70%. In agreement with these results, we found that PSGL-1 isolated from HL60 cells reacts with mAb HECA452 in immunoblots (data not shown). In summary, the current study suggests that PSGL-1 is a good candidate for an important presenter of functionally relevant HECA452 epitopes on highly activated T cells. In the future, it will be important to directly examine the role of PSGL-1 in leukocyte interactions with E-selectin.

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