P-selectin Glycoprotein Ligand-1 Is the Major Counter-receptor for P-selectin on Stimulated T Cells and Is Widely Distributed in Non-functional Form on Many Lymphocytic Cells*

Gloria Vachino‡§, Xiao-Jia Chang‡§, Geertruida M. Veldman‡, Ravindra Kumar‡, Dianne Sako‡, Lynette A. Fouser*, Michael C. Berndt, and Dale A. Cumming†

From the ‡Small Molecule Drug Discovery Group, and the ¶Department of Molecular Immunology, Genetics Institute, Cambridge, Massachusetts 02140 and the Department of Vascular Biology, Baker Medical Research Institute, Commercial Road, Prahran, Victoria, Australia

P-selectin glycoprotein ligand-1 (PSGL-1) is the high affinity counter-receptor for P-selectin on myeloid cells (Sako, D., Chang, X., 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. (1993) Cell 75, 1179–1186). Here we demonstrate that PSGL-1 is also widely distributed on T- and B-lymphocytic tumor cell lines, resting peripheral blood T and B cells, and on stimulated peripheral blood T cell and intestinal intraepithelial lymphocyte (IEL) lines. However, the majority of PSGL-1-positive resting peripheral blood lymphocytic cells and lymphoid tumor cell lines do not display significant P-selectin binding. In contrast, in vitro stimulated peripheral blood T cell and IEL lines avidly bind P-selectin, and PSGL-1 is the sole high affinity counter-receptor mediating this binding. During the course of in vitro stimulation, cell surface expression levels of PSGL-1 do not change as P-selectin binding increases. Rather, the activities of two glycosyltransferases reportedly involved in the production of functional PSGL-1 in myeloid cells are substantially higher in the stimulated T-lymphocytic lines than in resting T lymphocytes, consistent with the hypothesis that activation-dependent post-translational events contribute to the expression of functional PSGL-1 on lymphocytes.

The selectin family of adhesion molecules participates in the initial stages of leukocyte extravasation by tethering cells to the vascular endothelium (1). L-selectin, constitutively expressed on myeloid cells and the majority of lymphocytes, mediates cell binding to counter-receptors displayed on activated endothelia and high endothelial venules. Two other members of the selectin family, E- and P-selectin, are expressed on activated endothelia and are recognized by various leukocyte populations including neutrophils and monocytes (2) as well as by subpopulations of lymphocytes (3–8).

Selectin-mediated interactions have been studied frequently in the context of acute inflammation. However, the capacity of E- and P-selectin to bind primed and memory lymphocytes suggests that these adhesion molecules may also play a role in cell trafficking during chronic inflammation. E-selectin, for example, binds to memory T cells isolated from normal skin and activated T cells derived from dermatological lesions expressing the cutaneous lymphocyte antigen (4, 6, 9). P-selectin is detected on venules infiltrating chronically inflamed synovial membranes of rheumatoid arthritis (RA) patients (10). Furthermore, T lymphocytes derived from the synovial fluid of RA patients (11) and atopic dermatitis lesions (9) display marked P-selectin binding activity. Damle et al. (11) and Rossiter et al. (9) have also demonstrated that in contrast to resting T cells, in vivo activated T cells avidly bind P-selectin. Therefore, primed T lymphocytes may access chronic inflammatory lesions in vivo through selectin-mediated interactions.

Although the counter-receptor for P-selectin on stimulated T lymphocytes has not yet been fully characterized, recent studies by Alon et al. (8) suggest that the T cell ligand is the same as or similar to the myeloid P-selectin ligand. This myeloid glycoprotein, cloned from an HL-60 cDNA library and designated P-selectin glycoprotein ligand-1 (PSGL-1), has undergone extensive characterization (12–14). PSGL-1, a member of the mucin-like selectin counter-receptors (15), is expressed as a homodimer with an approximate molecular mass of 220 kDa and displays multiple sialylated, fucosylated, O-linked poly-N-acetyllactosaminylated oligosaccharides (16). Both protein and carbohydrate components of myeloid PSGL-1 are necessary for P-selectin binding with the terminal tetrasaccharide sialylLewisα (SLα) comprising a critical epitope for binding (17, 18). Proper post-translational modification of PSGL-1 requires the combined activities of a number of glycosyltransferases which are all expressed in myeloid cells, including Core 2 GlcNAc-transferase, a 2,3-sialyltransferase, and a fucosyltransferase (12, 16).

In the present study we evaluate the expression and function of PSGL-1 in P-selectin binding to cells of lymphocytic lineage, including T- and B-lymphocytic tumor cell lines, resting peripheral blood T and B cells and stimulated peripheral blood T cell, and intestinal intraepithelial lymphocyte (IEL) lines. Our studies show that although the vast majority of lymphocytes express cell surface PSGL-1, they display considerable varia-

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† To whom correspondence should be addressed. Tel.: 617-498-8919; Fax: 617-498-8993.

1 The abbreviations used are: RA, rheumatoid arthritis; PSGL-1, P-selectin glycoprotein ligand-1; IEL, intestinal intraepithelial lymphocyte; SLα, sialyl Lewis α; Core 2, Core 2 β1,6GlcNAc transferase; CHO, Chinese hamster ovary; CHO-PACE SOL, soluble PACE secreted CHO-cells; PACE, paired basic amino acid converting enzyme; EBV, Epstein-Barr virus; PBMC, peripheral blood mononuclear cells; IL, interleukin; mAb monodonal antibody; FITC, fluorescein isothiocyanate; Lec-1, P-selectin IgG1 Fc chimera; Ig, immunoglobulins; FACS, fluorescent-activated cell sorter; HBSS, Hank's balanced salt solution; BCECF-am, 2′,7′-bis(2-carboxyethyl)-5(6)-carboxyfluorescein acetomethylene ester; BSA, bovine serum albumin; PIPES, 1,4-piperazinediethanesulfonic acid; PA, protein A; PAGE, polyacrylamide gel electrophoresis; MOPS, 4-morpholinepropanesulfonic acid; α,1,3-FT, α(1,3)-fucosyltransferase.

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bility in P-selectin binding; only stimulated peripheral blood T cell and IEL lines show significant binding to P-selectin. The studies reported here further establish that PSGL-1 is the predominant counter-receptor for P-selectin on stimulated T-lymphocytic lines. We hypothesize that PSGL-1 undergos cell activation-associated post-translational modifications which enable high affinity binding to P-selectin. Consistent with this hypothesis, we observe that the activities of certain glycosyltransferases are significantly higher in stimulated T cells than in resting T lymphocytes.

EXPERIMENTAL PROCEDURES

Cells and Cell Lines—The tumor cell lines MOLT-4 (CRL 1582), CEM (TIB 195), THP-1 (TIB 202), HUT 78 (TIB 161), U-937 (CRL 1593), WIL2-NS (CRL 8155), Ramos (CRL 1596), HL-60 (CRL 240), and CCRF-SB (CCL 120) were obtained from the ATCC (American Type Culture Collection, Bethesda, MD) and maintained in RPMI 1640, 10% fetal calf serum (Sigma).

Chinese hamster ovary cells deficient in dihydrofolate reductase (CHO-DUKX) and stable transfectants of CHO-P-selectin cells were maintained as described previously (17). Stable transfectants of CHO-PACE SOL cells expressing a soluble form of paired basic amino acid converting enzyme (PACE; 19) were maintained in modified 11-AAU media as described previously (20). PACE-1 contains a cleavage site for PACE (12) evinced by the tetrapeptide consensus sequence RXRR (19). For co-culture experiments with lymphocytic cells, moderately confluent CHO-PACE SOL cells or CHO-DUKX cells were trypsinized, diluted 1:3, and plated in modified 11-AAU media. Shortly thereafter, 2 × 10^6 lymphocytic cells (i.e. B cell lines, CEM, or peripheral blood B cells) were added per 100-mm tissue culture plate, and these co-cultures were maintained overnight (except for peripheral blood B cells which were co-cultured for 4 h).

A peripheral blood T cell line was established by repeated co-stimulation with an Epstein Barr virus-transformed cell line JY. A human T cell line was co-cultured for 4 h). When 2 × 10^6 cells were added per 100-mm tissue culture plate, and these co-cultures were maintained for 4 h in methionine-free MEM (ICN), and lysed by sonication.

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either precipitated with trichloroacetic acid and analyzed directly by SDS-PAGE on 8% gels, or precipitated with Rb3026 or preimmune IgG precomplexed with PA-Sepharose, or recollected and reprecipitated with Lec-1 precomplexed with PA-Sepharose.

Construction of cDNAs for Soluble PSGL-1 Mutants—Two mutants of PSGL-1 with alterations in the PACE consensus sequence were generated from a previously described construct of soluble PSGL-1 (pE.D.SPSGL-1.177) (12) by site directed mutagenesis. In one construct, a point mutation was introduced at codon 61, substituting an alanine for an arginine and in the other, the recognition site for enterokinase cleavage (DDDDK) was substituted for the PACE cleavage site RDRR. The constructs were validated by DNA sequencing.

Glucosyltransferase Assay—Cell lysates were prepared from resting peripheral blood T cells and from the stimulated peripheral blood T cell and IEL lines as follows. Cells were washed with phosphate-buffered saline and lysed in 100 mM sodium cacodylate, pH 6.0, 150 mM NaCl, 25% glycerol, and 1% Nonidet P-40 (150 µl/10⁶ cells) on ice for 15 min. Cell debris was removed by centrifugation, and the supernatants were stored at −80 °C. Protein concentrations were determined using the BCA Protein assay (Pierce).

The α1,3-fucosyltransferase assay was performed essentially as described previously (26). Briefly, in a 50-µl reaction volume 30–100 µg of cell lysate protein was incubated with 50 mM MOPS buffer, pH 7.0, 5 mM MnCl₂, 100 mM NaCl, 2 nM of GDP-[¹⁴C]fucose (20,000 counts/min/nmol; Sigma) and 1 µm of the acceptor substrate LacNAc (Sigma) for 1 h at 37 °C. The reaction was stopped by the addition of 1 ml of ice-cold water, applied to a 1 ml column of Dowex 1X4 (Cl-form, Bio-Rad). The radiolabeled product was eluted with 3 ml of water and counted in a scintillation counter.

The Core 2 transferase assay was performed as described by Higgins et al. (27). Briefly, in a 50-µl reaction volume 30–100 µg of cell lysate protein was incubated in 50 mM sodium cacodylate, pH 6.7, 0.1% Triton X-100, 0.1% BSA, 0.1 mM GlcNAc, 10 mM UDP-[³⁵S]GlcNAc (20,000 counts/min/nmol) and 2 µM β Gal(1–3)α4-GalNAc-P-nitrophenol (Sigma) as a substrate for 1 h at 37 °C. The reaction was terminated by the addition of 1 ml of ice-cold water and applied to a C18 Sep-pak column (Water-Millipore). The column was washed with 5% acetonitrile in water-Millipore. The column was washed with 5% acetonitrile and the product eluted with 20% acetonitrile and counted in a scintillation counter. All assays were carried out in duplicate. Control reactions carried no substrate, and specific activity was determined as pmol/min/mg.

RESULTS

PSGL-1 Is Expressed by T- and B-lymphocytic Tumor Cell Lines—The expression of PSGL-1 transcripts by lymphocytic cell lines was examined by Northern blot analysis. The 2.5-kilobase PSGL-1 transcript previously detected in myeloid cell lines (HL-60, U-937, and THP-1) (12) is expressed in cells of T-lymphocytic (CEM, MOLT-4, and HUT 78) and B-lymphocytic (Ramos, SB) lineage. A third B cell line WIL2-NS expressed much lower levels of this 2.5-kilobase mRNA than the other lymphoid cell lines (Fig. 1).

Cell expression of PSGL-1 in lymphoid cell lines was assessed using PSL-275, a monoclonal anti-PSGL-1 antibody that was raised against a 15 amino acid peptide of PSGL-1 (Scheme 1). This peptide juxtaposes a PACE cleavage site, defined by the consensus sequence RXRR (19). Fig. 2A shows that soluble PSGL-1 (WT) expressed in COS cells could be immunoprecipitated by PSL-275. However, when PACE processing of recombinant PSGL-1 was prevented by either disrupting the consensus sequence with a point mutation (R61) or substituting RDRR with the consensus sequence for enterokinase cleavage (EK), PSL-275 was unable to bind PSGL-1. In contrast, a polyclonal anti-PSGL-1 antibody Rb3026 was able to immunoprecipitate both the wild type and mutant forms of PSGL-1. These data thus show the specificity of PSL-275 for PACE processed PSGL-1.

Flow cytometric analysis of PSL-275 binding by lymphocytic cell lines revealed that greater than 90% of T-lymphocytic cells bound PSL-275 although the mean fluorescence intensities varied among the different T cell lines; CEM and MOLT-4 cells were comparable to myeloid cells while HUT 78 was approxi-
expression of PSGL-1 protein and P-selectin binding by resting periph- 
ereal blood T and B lymphocytes were subsequently examined. Dual parameter flow cytometric analysis of PBMC revealed that PSGL-1 expression on resting peripheral blood B lymphocytes differs from the B-lymphocytic cell lines SB and Ramos. Between 30–50% of CD19+ peripheral blood B cells bound PSL-275 (Fig. 4A). A 4 h co-culture period of resting B cells with CHO-PACE SOL cells had no effect on either the percentage or fluorescence intensity of PSL-275-reactive B cells (data not shown). SB cells similarly co-cultured for 4 h showed a marked increase in PSL-275 binding. Attempts to measure P-selectin binding by B cells using flow cytometry were hampered by high nonspecific binding of protein A-FITC. Therefore, the cell-based adhesion assay was used exclusively to assess B cell/P-selectin interactions. As observed with the B cell tumor lines, the binding of resting B cells to adherent CHO-P-selectin cells was insignificant (data not shown).

The expression of PSGL-1 on resting peripheral blood CD3+ T cells was similar to CD14+ monocytes. The majority of both these cell populations bound PSL-275 (Fig. 4A), and expression levels of PSGL-1, as measured by the mean fluorescence intensities of PSL-275-reactive CD3+ and CD14+ cells, were comparable (data not shown). However, in contrast to monocytes, only a small percentage (less than 20%) of CD3+ T cells bound Lecy1 (Fig. 4B). Thus, analogous to the T cell lines CEM and MOLT-4, the majority of resting CD3+ T cells do not display marked P-selectin binding despite adequate expression of PSGL-1 at the cell surface.

Short term propagation of peripheral blood T cells in vitro led to augmented P-selectin binding activity. Between Day 0 and Day 7 of in vitro culture, the percentage of T cells binding Lecy1 increased from less than 20% to approximately 50% (data not shown).
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**In vitro ontogenies, peripheral blood T cells and IELs, were propagated** expression during T cell stimulation, T cells of two diverse species precipitated by Lec-gamma 

The lack of correlation between P-selectin binding and PSGL-1 expression remains constant during stimulation. Fig. 5 shows that the mean fluorescence intensity of PSL-275-reactive T cells (data not shown). Surprisingly, however, this increment in binding activity was not accompanied by a change in the mean fluorescence intensity of PSL-275-reactive T cells (data not shown) indicating that PSGL-1 expression levels remain constant during stimulation.

**In vitro stimulation of peripheral blood T lymphocyte and IEL lines increases P-selectin binding while cell surface expression of PSGL-1 remains constant**—To further evaluate the lack of correlation between P-selectin binding and PSGL-1 expression during T cell stimulation, T cells of two diverse ontogenies, peripheral blood T cells and IELs, were propagated in vitro for several weeks. Binding of Lec-gamma and PSL-275 was assessed on days 10, 24, and 38. Fig. 5A shows that the percentage of peripheral blood T lymphocytes binding Lec-gamma increased from 50% on day 10 to greater than 97% by day 38 while the majority of IELs consistently displayed Lec-gamma in- 

distinguish in electrophoretic mobility of the single predominant molecular species captured by Lec-gamma (data not shown). Nevertheless, a polyclonal anti-PSGL-1 antibody Rb3026, (but not preimmune antibody), was able to reprecipitate the Lec-gamma-binding cells increased by at least one order of magnitude. Yet, despite this marked increment in P-selectin binding by both IELs and peripheral blood T cells, expression levels of cell surface PSGL-1 did not increase during the course of stimulation. Fig. 5B shows that the mean fluorescence intensity of PSL-275-reactive cells was not appreciably over time in culture.

The Major High Affinity Counter-receptor for P-selectin on Stimulated Peripheral Blood T Cells and IEL Lines Is Immuno-cross-reactive with PSGL-1—The increase in P-selectin binding activity in the absence of increased PSGL-1 expression led us to hypothesize that during the process of lymphocyte acti-

**FIG. 3. P-selectin binding by myeloid and lymphocytic cell lines.** A, flow cytometric analysis of myeloid cell lines HL-60 and U-937, T cell lines CEM, MOLT-4, and HUT 78 and B cell lines, Ramos, SB and WIL2-NS reacted with the P-selectin-IgG chimera Lec-gamma (--), or control human IgG, (---) precomplexed with Protein A-FITC. B, cell adhesion of fluorescent-labeled tumor cells to adherent CHO-DUKX or CHO-P-selectin cells. Bound cells were quantitated using a microplate fluorometer. The level of fluorescence intensity per cell was similar for all tumor cell lines tested.

**FIG. 4. Flow cytometric analysis of PSGL-1 expression and P-selectin binding by subpopulations of resting peripheral blood mononuclear cells.** A, PBMC were incubated with mAb PSL-275, followed by FITC-conjugated anti-murine Ig antibody. The samples were subsequently reacted with phycoerythrin-conjugated antibodies against the cell-surface markers CD3 (T cells), CD14 (monocytes) or CD19 (B cells). B, PBMC were incubated with Lec-gamma precomplexed with Protein A-FITC, followed by incubation with phycoerythrin-conjugated anti-CD3 or anti-CD14 antibodies. Samples were analyzed by dual parameter flow cytometry. The data represent the mean of five samples from different donors.
suggesting, therefore, that there is no direct correlation between the electrophoretic mobility of PSGL-1 and its ability to mediate P-selectin binding.

To demonstrate that the P-selectin binding of IELs and T cells is mediated by PSGL-1 expressed at the cell surface, intact lymphocytes were treated with a metalloprotease displaying narrow substrate specificity. Mocarhagin, a protease derived from the cobra N. mocambique mocambique cleaves specifically near the amino terminus of mature PSGL-1 as the sole apparent proteolytic event on neutrophils and HL-60 cells. This cleavage results in the loss of both P-selectin binding and the PSL-275-reactive epitope. Thus, binding to CHO-P-selectin cell lines of mocarhagin-treated HL-60 cells, stimulated peripheral blood T cells, and IELs was reduced to 4, 9, and 6%, respectively, of untreated cells. Similarly, binding of PSL-275 by these cells, as measured by flow cytometry, was reduced to that of the isotype control (3–5%) following proteolysis by mocarhagin. The comparable behavior displayed by myeloid and lymphocytic cells in response to mocarhagin treatment further supports the finding that binding to P-selectin is mediated by the same cell surface ligand in both cell types. Thus, the increased P-selectin binding activity that accompanies lymphocyte activation is associated with an increase in PSGL-1 functionality rather than with the expression of another novel P-selectin ligand.

Glycosyltransferase Activity in in Vitro Stimulated T-lymphocytic Cell Lines Differs from Resting T Cells—While several mechanisms might account for the up-regulation of PSGL-1 binding to P-selectin, the time course for acquiring P-selectin reactivity in T cells suggests altered post-translational modifications of PSGL-1 during lymphocyte activation. Appropriate glycosylation of PSGL-1 is known to be essential for binding to P-selectin (12, 14), and two critical glycosyltransferases have been implicated: a fucosyltransferase capable of generating Lewisx-type structures (12) and Core 2 transferase (16). Thus, the activities of these two transferases were measured in resting T cells and in stimulated peripheral blood T cell and IEL lines over time in culture. Both transferases were elevated above those of resting T cells throughout the period of stimulation. Table I compares the activities of resting T cells with those of stimulated T-lymphocytic cell lines and shows that in contrast to stimulated T lymphocytes, resting T cells express no detectable Core 2 transferase activity. Also, fucosyltransferase activity, which is low in resting T cells, is increased 5-fold in stimulated T lymphocytes. Thus, increased P-selectin binding activity by T lymphocytes coincides with increased activities of fucosyltransferase and Core 2 transferase. However, although these observations are intriguing, a causal relationship between PSGL-1 function and transferase activity cannot be established from these data alone.

Even if these transferases are involved in lymphocyte PSGL-1 modification, the precise nature of the carbohydrate structures that confer P-selectin binding activity upon stimulated T cell PSGL-1 remains unclear. Monoclonal antibodies such as CSLEX-1 and HECA-452, which recognize the SLex5

3 M. C. Berndt, L. C. Dunlop, M. De Luca, J. Flannery, R. Ettling, D. A. Cumming, and G. M. Veldman, manuscript in preparation.
4 R. Kumar, R. Camphausen, and D. A. Cumming, manuscript in preparation.
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**TABLE I**

| Cells                        | α,(3,3)-Fucosyltransferase activity | Core 2 transferase activity |
|------------------------------|-------------------------------------|-----------------------------|
|                             | pmol/min/mg                         | Not detectable              |
| Resting peripheral blood T   | 8 ± 5                               |                             |
| blood T cell                 |                                     |                             |
| Stimulated peripheral        | 45 ± 11                             | 10 ± 2                      |
| blood T cell line            |                                     |                             |
| Stimulated IEL line          | 41 ± 4                              | 15 ± 3                      |

DISCUSSION

The present study assessed the expression and function of PSGL-1 on cells of T- and B-lymphocytic lineage and found no direct correlation between PSGL-1 expression and binding to P-selectin. On the contrary, PSGL-1 is expressed on the vast majority of lymphocytic cells while P-selectin binding is evident in some lymphocytic populations. In the present study only in vitro stimulated peripheral blood T cell and IEL lines displayed significant P-selectin binding. PSGL-1 is the major high affinity counter-receptor for P-selectin on these chronically stimulated T lymphocytic cells, and, thus, the functionality of lymphocyte PSGL-1 appears to be activation-dependent. P-selectin binding by stimulated and memory T lymphocytes has previously been reported by others. For example, Moore and Thompson (3) demonstrated that among the subpopulation of freshly isolated peripheral blood T lymphocytes that bind P-selectin, a significant percentage is of the CD45RO memory phenotype. In addition, our data show modest P-selectin binding by the T lymphoma cell line HUT 78. This cell line produces IL-2 constitutively (28), a behavior characteristic of stimulated T cells. The most striking display of stimulation-associated P-selectin binding, however, is seen in T lymphocytes propagated in vitro, where the degree of P-selectin binding reflects the extent of stimulation. This binding may be a general consequence of chronic T-cell stimulation as it occurs during both antigen-independent (this report) and alloantigenic (11) stimulation.

The correlation between P-selectin binding and T lymphocyte stimulation supports the notion that PSGL-1 is functionally up-regulated during activation. Such up-regulation alone provides an explanation for the lack of correlation between PSGL-1 expression and P-selectin binding among lymphocytic cells in general. Several alternative explanations for this lack of correlation can be excluded based on our data. For example, differences in P-selectin binding do not reflect differences in the density of PSGL-1 at the cell surface, as the expression levels of PSGL-1 on lymphocytes that bind poorly to P-selectin do not differ appreciably from those on myeloid cells. In fact, HUT 78 cells binding PSL-275 stain brighter than PSL-275-reactive HL-60 cells, yet most HUT 78 cells bind less well to P-selectin. Furthermore, the density of PSGL-1 on the surface of peripheral blood T cell and IEL lines undergoing in vitro stimulation does not change as P-selectin binding increases.

Another possible explanation for the lack of correlation between PSGL-1 expression and P-selectin binding is that another counter-receptor for P-selectin exists on lymphocytes and is induced during activation. However, analysis of stimulated T cell lines, employing the methodology previously utilized to identify the P-selectin counter-receptor on myeloid cells (i.e., affinity capture with immobilized P-selectin from detergent-solubilized cell membranes; 12, 13) revealed that the major high affinity ligand on stimulated peripheral blood T cell and IEL lines displays similar electrophoretic mobility to myeloid PSGL-1 and is recognized by polyvalent anti-PSGL-1 antibodies. It is important to note that this method of affinity capture precludes the detection of low affinity interactions. However, while other proteins may participate in P-selectin binding, PSGL-1 appears to be the sole high affinity counter-receptor for P-selectin on in vitro stimulated T lymphocytes.

Given these observations, it is reasonable to postulate that altered post-translational processing of PSGL-1 is responsible for the increase in P-selectin binding during T cell stimulation. Changes in glycosylation seem especially likely since previous studies have demonstrated the importance of appropriate O-linked glycosylation of PSGL-1 for P-selectin binding (12, 13). While many glycosyltransferases are involved in the biosynthesis of O-linked oligosaccharides, these studies suggested that at least two enzymes are critical: a fucosyltransferase capable of forming sialylated Lewis X (SLex) or related carbohydrate epitopes and Core 2 transferase which is required for the addition of blood group antigens such as SLex to O-linked oligosaccharides (29). Our evaluation of fucosyltransferase and Core 2 transferase activities revealed substantially elevated specific activities of both enzymes in stimulated T-lymphocytic cells. This is in agreement with findings by Piller et al. (30), who reported an activation-associated increase of Core 2 transferase activity in lymphocytes. While these observations are consistent with the notion that altered glycosylation regulates the ability of PSGL-1 to bind P-selectin, additional experimentation is clearly required both to further elucidate the nature of activation-associated glycosylation and to explore whether other post-translational modifications play an important role.

**TABLE II**

| Antibody                | % cells binding to CHO-P-selectin cells |
|-------------------------|----------------------------------------|
|                         | Stimulated IEL line | Stimulated peripheral blood T cell line | HL-60 |
| Non-immune rabbit serum | 100 ± 15             | 100 ± 17                             | 100 ± 12 |
| Rb3026 serum 1:15 dilution | 79 ± 21         | 72 ± 21                              | 20 ± 3  |
| Rb3026 affinity purified | 82 ± 8             | ND                     | 32 ± 3  |
| 14 µg/ml                | 85 ± 9               | ND                                  | ND      |
| 28 µg/ml                | 87 ± 15              | ND                                  | ND      |
| 56 µg/ml                | 104 µg/ml            | 62 ± 9                              | ND      |

* Not determined.
in modulating the ability of PSGL-1 to bind to P-selectin.

The display of carbohydrates on lymphocyte glycoproteins differs from those on myeloid cells (31, 32). For example, most stimulated T cells do not appreciably bind CSLEX-1 or HECA 452, two monoclonal antibodies which recognize the SLe\(^\alpha\) epitope on myeloid glycoproteins. Yet Ohmori et al. (33) have shown that these stimulated T cells do express a form of SLe\(^\alpha\) readily detectable by another anti-SLe\(^\alpha\) mAb designated 2F3. These authors have hypothesized that differences in SLe\(^\alpha\) antigenicity between cells of diverse ontology may reflect the linkage, length, and modifications of core structures which in turn are determined by the precise repertoire of cellular glycosyltransferases. Thus, while stimulated T lymphocytes and myeloid cells may both express glycosyltransferases involved in SLe\(^\alpha\) biosynthesis, the antigenicity of carbohydrate moieties could differ. Additional data consistent with this suggestion come from the differential neutralizing capacity of the polyclonal anti-PSGL-1 antibody (Rb3026). This antibody, which was generated against recombinant soluble PSGL-1 produced by COS cells co-expressing an αL(3,4)FT, effectively blocks myeloid cell binding to CHO-P-selectin cells but had only minimal effects on stimulated peripheral blood T cell or IEL binding. These results differ from those reported by Alon et al. (8) who found that Rb3026 significantly blocked P-selectin binding by a chronically stimulated T cell line. While differences in assay conditions may be responsible for these contrasting findings, the most notable distinction is the nature of the T cell lines studied. Alon et al. (8) investigated the effects of Rb3026 on a T cell line established from skin lesions of a patient with atopic dermatitis. These T cells bind HECA 452, similar to other skin-homing T-lymphocytes and myeloid cells. Thus, the presentation of carbohydrates expressed on skin T cells appears to differ from the display of oligosaccharides on the peripheral blood T cell and IEL lines examined in the present study. If at least part of the neutralization epitope for Rb3026 is carbohydrate in nature, then the differential effects of this anti-PSGL-1 antibody may reflect differences in oligosaccharide presentation among cells.

Consistent with the contention that T cells from different tissues express diverse carbohydrates, we have noted a distinction in the electrophoretic mobility of PSGL-1 between IELs and peripheral blood T cells. Fig. 6 shows that IEL PSGL-1 migrates at a slightly higher apparent molecular weight than peripheral blood T cell PSGL-1. Both cell lines had been propagated in vitro for 38 days at the time of analysis; however, the disparate electrophoretic mobilities of IEL/T cell PSGL-1 are evident irrespective of time in culture (data not shown). Based on these data and observations that the PSGL-1 expressed by the B cell line SB has a higher molecular weight than the PSGL-1 of U937 or the T cell line CEM (data not shown), we postulate that cell lineage-specific post-translational modifications influence the electrophoretic mobility of PSGL-1. However, there is no correlation between PSGL-1's electrophoretic characteristics and its ability to mediate P-selectin binding.

A possible role for PSGL-1 on stimulated and/or memory peripheral blood T cells is suggested by studies that have assessed the distribution of P-selectin in T cell-associated pathologies. P-selectin has been detected on chronically inflamed rheumatoid arthritic synovial endothelium (10) and can be induced on mesenteric lymph node high endothelial venules (33, 34). Potentially then, primed T cells may access synovial tissue or the cortex of lymph nodes through PSGL-1/P-selectin interactions.

It is more difficult to postulate a role for PSGL-1 on IELs. These cells are located at the basolateral surface of the epithelial layer throughout intestinal tissue. Although still controver-
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