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P-Selectin Glycoprotein Ligand-1 Negatively Regulates T-Cell Immune Responses

Masanori Matsumoto,* Masayuki Miyasaka,* and Takako Hirata2*†

Cell surface sialomucins often act as antiadhesive molecules by virtue of their extended structure and negative charge. CD43 is one such sialomucin, expressed on most leukocytes. P-selectin glycoprotein ligand-1 (PSGL-1) is another sialomucin expressed by leukocytes. It serves as a major selectin ligand, but no antiadhesive role for it has been described. In this study, we showed that PSGL-1-deficient T cells, like CD43-deficient T cells, exhibited increased adhesion and proliferation compared with wild-type cells. The loss of both PSGL-1 and CD43 led to a further increase in T cell adhesion and proliferation. The reexpression of full-length PSGL-1 or CD43 in double-deficient CD4+ T cells reversed their increased adhesion and proliferation phenotype. Using chimeric constructs of human CD8 and either PSGL-1 or CD43, we demonstrated that the intracellular domain of PSGL-1 or CD43 is required for suppressing proliferation but not adhesion. Furthermore, in a mouse model of inflammatory bowel disease induced by the adoptive transfer of naive T cells into RAG-deficient hosts, a PSGL-1 deficiency exacerbated the development of inflammation. These results reveal a novel regulatory role for PSGL-1 in T cell adhesion and proliferation and suggest that PSGL-1 negatively regulates T cell immune responses in vivo. The Journal of Immunology, 2009, 183: 7204–7211.

Cell surface sialomucins represent a family of glycoproteins that are highly glycosylated with sialylated O-linked glycans. Because of their extended structure and the negative charge imparted by their sialic acid residues, some sialomucins can provide a repulsive barrier around a cell that inhibits cell-cell interactions. CD43 is one such molecule (1). It is expressed by most hematopoietic cells, including T cells. The extracellular domain of both human and mouse CD43 contains more than 80 serine or threonine residues, most of which are glycosylated by O-linked glycans that are heavily sialylated; this domain has a rod-like structure that is predicted to extend 45 nm from the lipid bilayer (2). Supporting evidence for its antiadhesive role is observed in vitro by the enhanced homotypic adhesion and proliferation of CD43-deficient (CD43−/−) T cells (3), and in vivo by their increased migration into secondary lymphoid organs (4). CD43−/− neutrophils also show enhanced rolling and adhesion both in vitro and in vivo (5, 6). These antiadhesive functions, however, are not evident when CD43 interacts with its specific ligands (7). Several potential ligands for CD43 have been reported, including ICAM-1 (8), galectin-1 (9), and sialoadhesin (10). In particular, we and others reported that CD43 on mouse Th1 cells and human T lymphoblasts is modified with sialylated and fucosylated carbohydrate structures to interact with E-selectin (11, 12). This interaction plays an important role in mediating the migration of activated T cells to inflamed skin (13, 14).

P-selectin glycoprotein ligand-1 (PSGL-1;3 CD162) (3) is another sialomucin expressed by most leukocytes (15, 16). The extracellular domain of both human and mouse PSGL-1 contains >40 potential O-glycosylation sites. Like CD43, PSGL-1 is a highly extended molecule: its ectodomain is estimated to be 50–nm long (17). PSGL-1 was originally identified as the major ligand for P-selectin, and subsequent studies showed that it also binds the other two members of the selectin family, E- and L-selectin. Studies using PSGL-1-deficient (PSGL-1−/−) mice showed that, on activated T cells, PSGL-1 serves as the major ligand for P-selectin as well as being an E-selectin ligand (18, 19). Similarly, PSGL-1 on neutrophils binds P- and E-selectin to mediate their rolling on endothelial cells and migration into inflamed tissues (20, 21). These well-recognized functions of PSGL-1 are critically dependent on its glycosylation, which endows it with the ability to bind selectins. Thus, although PSGL-1 is abundantly expressed on most hematopoietic cells, its function in cells that are not equipped with the machinery to modify it to bind selectins, such as naive T cells, remains unknown.

To explore the function of PSGL-1 independent of its role as a selectin ligand, we exploited our previous findings that the related sialomucin CD43 has dual functions: it is usually antiadhesive, but when appropriately glycosylated in certain cell types, it can become a proadhesive selectin ligand (5, 6). We investigated whether the PSGL-1 expressed on T cells has antiadhesive or regulatory roles, using T cells isolated from wild-type (WT), PSGL-1−/−, CD43−/−, and PSGL-1/CD43 double-knockout (DKO) mice. We show that PSGL-1−/− T cells exhibited enhanced adhesion and proliferation. Loss of both PSGL-1 and CD43 resulted in a further increase in T cell adhesion and proliferation. The increased adhesion and proliferation of the DKO T cells were reversed by the reintroduction of PSGL-1 or CD43. The intracellular domain of

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3 Abbreviations used in this paper: PSGL-1, P-selectin glycoprotein ligand-1; WT, wild type; DKO, PSGL-1 and CD43 double-knockout; FL, full length.

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PSGL-1 or CD43 was required for the reversal of the increased proliferation, but not for reducing the adhesion. Moreover, in vivo, the PSGL-1 deficiency exacerbated the development of T cell-mediated colitis. Thus, in addition to its well-characterized role as a selectin ligand, these results point to a novel role for PSGL-1 in negatively regulating T cell adhesion and proliferation and in controlling T cell-mediated immune responses in vivo.

Materials and Methods

Mice

C57BL/6 (B6) mice were purchased from CLEA Japan. PSGL-1–/– mice on a B6 background were provided by B. Furie (Harvard Medical School, Boston, MA). Rag-1-deficient (Rag-1–/–) mice on a B6 background and CD43–/– mice on a B6 × 129S4/SvJae background were purchased from The Jackson Laboratory. CD43–/– mice were back-crossed more than six generations on the B6 genetic background. DKO mice were generated by breeding PSGL-1–/– mice with CD43–/– mice. All mice were used when 6 to 10 wk old. The mice were housed at the Institute of Experimental Animal Sciences at Osaka University Medical School. All studies and procedures were approved by the Ethics Review Committee for Animal Experimentation of the Osaka University Graduate School of Medicine.

Isolation of T cells

Total T cells and CD4+ T cells were isolated from the spleen and the peripheral and mesenteric lymph nodes using a BD IMag Mouse T Lymphocyte Enrichment Set and CD4 T Lymphocyte Enrichment Set (both from BD Biosciences). Isolated CD4+ T cells were stained with anti-CD45RB-Biotin (C363-16A11; BioLegend), anti-CD25-PE (PC61; BD Biosciences), and anti-CD4allophycocyanin (RM4-5; BD Biosciences), and naive CD4+ T cells (CD4+CD25−CD45RB+Biotin) were sorted by FACSaria (BD Biosciences).

Construction of retroviral vectors

Full-length mouse PSGL-1 (pGLS-1FL) cDNA was inserted into the pMXs-IG vector (22), provided by T. Kitamura (University of Tokyo, Tokyo, Japan), at the BamHI and XhoI sites. The viral long terminal repeats of this vector drive the expression of PSGL-1 and enhanced GFP via a bicistronic mRNA containing an internal ribosomal entry site element. cDNAs for full-length mouse CD43 (CD43FL) and full-length human CD8 (CD8FL) were amplified from mouse Th1-cell cDNA and human thymus RNA, respectively. Isolated CD4+ T cells were stained with anti-CD45RB-Biotin (C363-16A11; BioLegend), anti-CD25-PE (PC61; BD Biosciences), and anti-CD4allophycocyanin (RM4-5; BD Biosciences), and naive CD4+ T cells (CD4+CD25−CD45RB+Biotin) were sorted by FACSaria (BD Biosciences).

Retroviral infections

The improved retrovirus packaging cells, PLAT-E (23), were transfected by calcium phosphate precipitation with 20 μg of the pMXs-IG vector containing one of the constructs described above. At 12 h post transfection, the calcium phosphate-containing medium was replaced, and the cells were cultured for an additional 48 h. The virus-containing supernatant from the PLAT-E cells was harvested, added to a six-well plate coated with 20 μg/ml recombinant fibronectin fragment CH-296 (RetroNectin; Takara), and then centrifuged at 1,500 × g for 2 h. To infect T cells, purified CD4+ T cells from WT or DKO mice were stimulated with plate-immobilized anti-CD3e (1 μg/ml) and anti-CD28 (1 μg/ml) for 48 h. The cells were stained with anti-CD4-PE-Cy7 (RM4-5; BD Biosciences) and anti-CD8-Alexa Fluor 700 (BioLegend) for 1 h. The cells were washed, transferred to uncoated dishes, and cultured for an additional 3 days.

Homotypic aggregation assays

Isolated T cells (1 × 10^5 cells), naïve CD4+ T cells (1 × 10^5 cells), or retrovirus-transduced CD4+ T cells (1 × 10^6 cells) were added to 96-well culture plates and incubated with PMA (100 ng/ml) for 5 h in the presence of calcium or EDTA. In some experiments, T cells from DKO mice were added to the wells and incubated with PMA in the presence of 10 μg/ml anti-CD11a (M17/4; BD Biosciences), anti-ICAM-1 (KAT-1; provided by K. Kato, Juntendo University, Tokyo, Japan), anti-CD18 (GAME-46; BD Biosciences), anti-CD11b (M170; BD Biosciences), or isotype controls. To examine the effect of neuraminidase treatment, the isolated T cells from WT, PSGL-1–/–, CD43–/–, and DKO mice were incubated with or without neuraminidase from Clostridium perfringens (25 mU/ml; Sigma-Aldrich) for 1 h and cultured with PMA for 5 h. The number of unaggregated cells was determined from the samples before they were dissociated. To determine the total number of cells in each sample, the aggregates were dissociated to the single-cell level by the addition of EDTA. The percentage of cells that had aggregated (percent aggregation) was calculated as the number of cells in aggregates (total number of cells minus the number of single cells) over the total number of cells.

Cell adhesion assays

Isolated T cells or naïve CD4+ T cells (1 × 10^5 cells) were labeled with 3.3 μM CFSE for 10 min at 37°C and stimulated with plate-immobilized anti-CD3e (1 μg/ml) and anti-CD28 (1 μg/ml) for 48 h. The cells were stained with anti-CD4-PE-Cy7 (RM4-5; BD Biosciences) and anti-CD8-APC (53.6.7; BD Biosciences) and then analyzed on a FACS Calibur. Daughter generations of CD4+ and CD8+ T cells were determined by measuring the CFSE dilution. In some experiments, retrovirus-transduced CD4+ T cells (1 × 10^5 cells) were stimulated with plate-immobilized anti-CD3e (1 μg/ml) and anti-CD28 (1 μg/ml) in the presence of 1 μg/ml of [H]-thymidine (GE Healthcare) for 24 h. Cells were harvested onto a UniFilter-96 GF/C (PerkinElmer) using a UniFilter-96 Harvester (PerkinElmer), and the incorporated radioactivity was measured using a TopCount NXT scintillation counter (PerkinElmer).

Mouse colitis model

We adapted a mouse model for colitis in which colitis is induced in immunodeficient mice by the transfer of naïve T cells (24, 25). Naïve CD4+ T cells (5 × 10^4) were isolated from WT, PSGL-1–/–, CD43–/–, and DKO mice; they were injected via the tail vein into RAG-1–/– mice. The body weight of the recipient mice was monitored throughout the experiment. The mice were analyzed 6 wk after the T cell transfer. They were assigned a disease activity score that was the sum of four parameters: hunching and wasting were scored 0 or 1, colon thickening 0–3, and stool consistency 0–3, as described (26). Tissue samples from the middle colon of untreated or T cell recipient RAG-1–/– mice were prepared for histological staining with H&E. Individual sections were scored blindly, according to the following independent criteria: cell infiltration 0–3, crypt elongation 0–3, and number of crypt abscesses 0–3 (26).

Isolation of cells infiltrating the large intestine lamina propria

Cells in the large intestine lamina propria were prepared by digesting the intestinal tissue fragments depleted of intraepithelial lymphocytes with 400 U/ml collagenase D (Roche) and 10 μg/ml DNase I (Roche) for 30 min at 37°C. Lymphocytes were further enriched by centrifugation on 40/75% Percoll (GE Healthcare). Total cell numbers were determined with a hemocytometer. The percentage of CD4+ T cells was determined by staining the cells with anti-CD4-APC and analyzing them on a FACS Calibur (BD Biosciences).

Statistical analysis

Data are presented as the mean ± SEM. Statistical analyses were performed using the 2-tailed unpaired Student’s t test.

Results

T cells deficient in PSGL-1 and/or CD43 show an increase in homotypic aggregation and adhesion

To examine the role of PSGL-1 as an antiadhesive molecule on T cells, we first tested T cells isolated from WT, PSGL-1–/–, CD43–/–, and DKO mice for homotypic aggregation upon PMA.
Homotypic aggregation of PMA-stimulated DKO T cells. We examined the effect of Ab treatment on the homotypic aggregation of PMA-stimulated T cells. T cells from DKO mice were added to fibronectin-coated plates and incubated in the presence of anti-CD11a, anti-ICAM-1, anti-CD18, or isotype controls. Results represent one of three similar experiments. Data are presented as means ± SEM.

Effects of Ab treatment on the homotypic aggregation of PMA-stimulated T cells (Fig. 1). The percent aggregation of WT, PSGL-1−/−, CD43−/−, and DKO T cells was also increased, and the loss of both PSGL-1 and CD43 led to a further increase in aggregation (Fig. 1C). The addition of EDTA completely abolished the aggregation of cells of all genotypes (data not shown). These results suggest that the increased aggregation and adhesion of T cells deficient in PSGL-1 and/or CD43 is not due to alterations in the expression of other adhesion molecules, but rather is a direct effect of their deficiency.

T cells deficient in PSGL-1 and/or CD43 show enhanced proliferative responses. We next investigated the proliferative responses of T cells from WT, PSGL-1−/−, CD43−/−, and DKO mice. The isolated T cells were labeled with CFSE and stimulated with plate-immobilized anti-CD3ε and anti-CD28 for 48 h. Daughter generations of CD4+ (A) and CD8+ (B) T cells were determined by measuring the dilution of CFSE. Results represent one of three similar experiments. Data are presented as means ± SEM. **, p < 0.01 vs WT.

assays (Fig. 1A), the adhesion of PMA-stimulated PSGL-1−/− T cells to fibronectin was greater than that of WT cells (Fig. 1C). The adhesion of DKO T cells was further increased compared with cells deficient in either PSGL-1 or CD43 (Fig. 1C). We confirmed that the deficiency of PSGL-1 and/or CD43 also increased the binding of PMA-stimulated naive CD4+ T cells to fibronectin (supplementary Fig. S1B). The adhesion was largely mediated by αβ integrin, because it was blocked by treatment of the cells with an anti-αβ (CD49d) or anti-β2 (CD29) mAb (Fig. 1D).

To examine whether the deficiency of PSGL-1 and/or CD43 affects the expression of other adhesion molecules, the cell surface expression of PSGL-1, CD43, and integrin chains on T cells was measured by flow cytometry. The expression level of PSGL-1 on CD43−/− cells and of CD43 on PSGL-1−/− cells was comparable to that on WT cells (supplementary Fig. S2). The expression of integrin chains αL, αM, β1, β2, and β3, as well as ICAM-1, was also comparable among the four genotypes (supplementary Fig. S2 and data not shown). These results suggest that the increased aggregation and adhesion of T cells deficient in PSGL-1 and/or CD43 is not due to alterations in the expression of other adhesion molecules, but rather is a direct effect of their deficiency.

The extracellular/transmembrane domain of PSGL-1 or CD43 is responsible for the reversal of increased T cell aggregation. To verify that the deficiency of PSGL-1 and/or CD43 was responsible for the increased adhesion and proliferation, we reintroduced either PSGL-1 or CD43 into DKO T cells using a retroviral gene transfer system. DKO CD4+ T cells were infected with a GFP-containing control retrovirus vector (pMXs-IG) or with vectors encoding full-length PSGL-1 (PSGL-1FL) or CD43 (CD43FL). Transduced cells were sorted based on their GFP expression level,
so that the sorted cells would express levels of reintroduced PSGL-1 or CD43 that were similar to the endogenous expression levels in WT cells (Fig. 3 and data not shown). Consistent with the results of the homotypic aggregation assays of isolated T cells shown in Fig. 1A, DKO T cells infected with a control retrovirus showed an increase in aggregation compared with similarly infected WT cells (Fig. 4A). As shown in Fig. 4B, the reintroduction of PSGL-1FL or CD43FL reversed the hyperaggregation phenotype of the DKO T cells.

CD43 may regulate cell adhesion through steric hindrance, via its highly extended extracellular domain, which is extensively decorated with sialylated O-glycans (1). However, it is possible that the intracellular domain of CD43 is critical for its antiadhesive function (28). To determine which domain of PSGL-1 or CD43 mediates its antiadhesive function, we generated chimeric constructs, in which the extracellular and transmembrane domain of PSGL-1 or CD43 was fused to the intracellular domain of human CD8 (PSGL-1/CD8 or CD43/CD8), or the extracellular and transmembrane domain of CD8 was fused to the intracellular domain of human CD8 (PSGL-1/CD8 or CD43/CD8), of the extracellular and transmembrane domain of CD8 was fused to the intracellular domain of PSGL-1 or CD43 (CD8/PSGL-1 or CD8/CD43). DKO CD4⁺ T cells were infected with vectors encoding each of the chimeric proteins and sorted for GFP expression so that the sorted cells expressed similar levels of chimeric proteins to their full-length counterparts (Fig. 3).

DKO T cells transduced with the PSGL-1/CD8 chimeric construct showed levels of aggregation similar to those of PSGL-1FL-transduced cells (Fig. 4B), indicating that the extracellular/transmembrane domain but not the intracellular domain of PSGL-1 is important for attenuating cell aggregation. Similar, though less dramatic, results were obtained with CD43/CD8 expression (Fig. 4B). In contrast, the increased aggregation of the DKO cells was not reversed by the introduction of CD8/PSGL-1 or CD8/CD43 chimeric constructs or by the full-length CD8 (CD8FL) (Fig. 4B). These results suggest that the intracellular domain of PSGL-1 or CD43 is not required for the antiadhesive effect of these molecules.

The involvement of the extracellular/transmembrane domain but not the intracellular domain in the antiadhesive effect of PSGL-1 and CD43 is consistent with the barrier hypothesis that the extended and negatively charged extracellular domain prevents cell-cell interactions. Because both PSGL-1 and CD43 are highly sialylated and thus negatively charged, we examined the effect of the removal of sialic acids by neuraminidase on T cell aggregation and adhesion. Neuraminidase treatment indeed increased the homotypic aggregation of WT, PSGL-1⁻/⁻, CD43⁻/⁻, and DKO T cells (Fig. 5A). However, neuraminidase-treated DKO cells still showed an increase in aggregation compared with similarly treated WT cells (Fig. 5A). Similar results were obtained in adhesion assays (Fig. 5B). These results suggest that the antiadhesive role of PSGL-1 and CD43 involves mechanisms independent of the negative charge.

Intracellular domain of PSGL-1 or CD43 is responsible for the reversal of increased T cell proliferation

We next examined which domain of PSGL-1 or CD43 is involved in regulating T cell proliferation. DKO T cells transduced with each construct were stimulated with anti-CD3ε and anti-CD28 in

**FIGURE 3.** Isolation of retrovirus-transduced CD4⁺ T cells. CD4⁺ T cells isolated from DKO mice were transduced with the indicated constructs, and GFP⁺ CD4⁺ T cells in the rectangular gates were sorted by FACS-Aria. The gates were set so that the sorted cells would express similar levels of the PSGL-1, CD43, or CD8 epitope. The mean fluorescence intensity for CD43, PSGL-1, and CD8 is indicated in each histogram. Results represent one of three similar experiments.

**FIGURE 4.** Homotypic aggregation of retrovirus-transduced CD4⁺ T cells. A, Homotypic aggregation of WT and DKO CD4⁺ T cells transduced with a control retrovirus. B, Homotypic aggregation of DKO CD4⁺ T cells transduced with various constructs. The retrovirus-transduced CD4⁺ T cells were cultured with PMA for 5 h, and the percentage of cells involved in aggregates was determined. Results represent one of three similar experiments. Data are presented as means ± SEM. *p < 0.05.
the presence of [3H]thymidine for 24 h, and proliferative responses were determined by [3H]thymidine incorporation. The increased proliferation of DKO T cells was reversed by the reexpression of either PSGL-1FL or CD43FL (Fig. 6), indicating that this hyperproliferative phenotype was indeed due to the loss of PSGL-1 and/or CD43. In contrast, expression of PSGL-1/CD8 or CD43/CD8 had little effect (Fig. 6). These results suggest that the extracellular/transmembrane domain of PSGL-1 or CD43 does not play a major role in regulating T cell proliferation, and suggest that the intracellular domain of PSGL-1 or CD43 is involved in this process. Indeed, the introduction of CD8/PSGL-1 or CD8/CD43 decreased the proliferative response compared with CD8FL (Fig. 6). These results suggest that the intracellular domain of PSGL-1 and CD43 is required for the negative regulation of T cell proliferation.

**PSGL-1 and CD43 regulate the development of CD4+ T cell-mediated colitis in vivo**

The above results suggested that PSGL-1 and CD43 both regulate T cell adhesion and proliferation. To determine whether they regulate T cell-mediated immune responses, which require optimal T cell adhesion and proliferation in vivo, we used a mouse model of inflammatory bowel disease, which is caused by adoptively transferring naïve T cells into SCID or RAG-1−/− recipient mice (24, 25). For our experiments, we used CD4+CD25−CD45RBhigh T cells from WT, PSGL-1−/−, CD43−/−, and DKO mice for the adoptive transfer into RAG-1−/− recipient mice. The recipient mice were analyzed 6 wk after cell transfer. RAG-1−/− mice that received WT cells developed colitis, with clinical signs such as diarrhea and wasting. RAG-1−/− mice that received PSGL-1−/−, CD43−/−, or DKO cells also developed colitis. The body weight of the RAG-1−/− mice with colitis at 6 wk after cell transfer was reduced compared with untreated RAG-1−/− mice, but was comparable regardless of the T cell genotype that was transferred (data not shown). To quantify the severity of the colitis, we determined the disease activity as the sum of four parameters: hunching, wasting, colon thickening, and stool consistency. The disease activity score of the mice that received WT cells was 3.6 (n = 8), whereas that of the mice receiving PSGL-1−/−, CD43−/−, or DKO cells was 5.6 (n = 7), 5.0 (n = 10), or 5.7 (n = 10), respectively (Fig. 7A). Thus, the single and double knockout phenotypes all exacerbated the induced colitis.

We also performed histological analyses of colon cross-sections from untreated or RAG-1−/− mice or RAG-1−/− mice that...
received the transferred naive CD4⁺ T cells as described above. Six weeks after the transfer, typical pathologic changes of colitis, such as cell infiltration, crypt elongation, and crypt abscesses, were observed in mice that received the naive CD4⁺ T cells (Fig. 7B). For the histological colitis score, individual sections were scored blind as described in Materials and Methods. C, Histological colitis score. Individual sections were scored blind as described in Materials and Methods. D, The number of CD4⁺ T cells in the large intestine lamina propria. Cells were isolated from the large intestine lamina propria, enriched for leukocytes by percoll, and stained with anti-CD4-PE. The numbers of CD4⁺ T cells were determined. Values are means ± SEM for seven to ten mice in each experimental group. *, p < 0.05; **, p < 0.01.

FIGURE 7. Loss of PSGL-1 exacerbates T cell-mediated colitis. Naive CD4⁺ T cells were isolated from WT, PSGL-1⁻/−, CD43⁻/−, and DKO mice by FACS Aria, and transferred into RAG-1⁻/⁻ mice. The recipient mice developed colitis and were analyzed at 6 wk after T cell transfer. A, Disease activity score. The mice were scored for disease activity as described in Materials and Methods. B, Histological analysis of colon cross-sections. Tissue samples from the middle colon were prepared for histological staining with H&E. Crypt abscesses are indicated by arrowheads. C, Histological colitis score. Individual sections were scored blind as described in Materials and Methods. D, The number of CD4⁺ T cells in the large intestine lamina propria. Cells were isolated from the large intestine lamina propria, enriched for leukocytes by percoll, and stained with anti-CD4-PE. The numbers of CD4⁺ T cells were determined. Values are means ± SEM for seven to ten mice in each experimental group. *, p < 0.05; **, p < 0.01.
study, we provide evidence that PSGL-1 is a negative regulator of T cell adhesion and proliferation in vitro and of T cell immune responses in vivo.

Sialomucins often act as antiadhesive molecules and the sialomucin CD43 has both antiadhesive and proadhesive functions (7). Previous studies demonstrated that the targeted disruption of CD43 in mice leads to enhanced T cell adhesion (3). We speculated that PSGL-1, a sialomucin that shares many features with CD43, such as an extended extracellular structure, abundant expression on T cells, and microvillar localization, might also function as an antiadhesive molecule. Our data showed that a deficiency of PSGL-1 enhanced T cell adhesion and that the loss of both PSGL-1 and CD43 had an additive effect on T cell adhesion. Retroviral gene transfer experiments suggested that the antiadhesive function of PSGL-1 and CD43 was mediated by their extracellular/transmembrane domain. A similar requirement for the extracellular/transmembrane domain was reported for the antiadhesive function of CD34 on mast cells (29). These results are consistent with the barrier hypothesis that the highly extended and negatively charged extracellular domain of sialomucins provides a physical and electrostatic barrier around a cell. The observation that a PSGL-1 and/or CD43 deficiency enhanced T cell adhesion even after removal of sialic acids suggests that the antiadhesive role of their extracellular/transmembrane domain involves mechanisms independent of the electrostatic barrier. In contrast, Walker et al. (28) previously reported that the expression of a mutant CD43 that lacks the intracellular domain in a CD43<sup>+/−</sup> T cell line did not reverse its hyperaggregation phenotype. The reason for this inconsistency is unknown, although it is possible that the lack of the intracellular domain may have affected the configuration of the extracellular domain in the cell line used in their study.

Our study also demonstrated that a deficiency of PSGL-1 enhances the proliferative responses of T cells. Consistent with previous studies (3, 27), we also demonstrated that CD43<sup>+/−</sup> T cells show an increase in proliferative responses, and the loss of both PSGL-1 and CD43 additively increased proliferation. These results are in contrast to the report by Carlow et al. (30), which showed that a CD43 deficiency had no effect on T cell responsiveness. In fact, we did not observe the enhanced proliferation of CD43<sup>+/−</sup> T cells when the cells were stimulated with higher or lower concentrations of anti-CD3ε plus anti-CD28 (data not shown). In addition, when the proliferative response was assessed by [3H]thymidine incorporation, the role of PSGL-1 and CD43 as antiproliferative molecules was most prominent within 1 day after stimulation (data not shown), suggesting that PSGL-1 and CD43 regulate T cell proliferation during the early phase.

We also showed that the intracellular domain of PSGL-1 and CD43 is required for their antiproliferative effect. In support of this finding, two groups have published data demonstrating that a CD43 mutant lacking the intracellular domain is ineffective in reverting the hyperproliferative phenotype of CD43<sup>+/−</sup> T cells (28, 31). Although the intracellular domains of PSGL-1 and CD43 have no obvious homology, this domain is known to be involved in signal transduction. In addition, the intracellular domain interacts with the adapter proteins ezrin and moesin (32, 33), which function together to regulate T cell activation (34). The cytoplasmic domain of CD43 is also phosphorylated upon PMA stimulation (35). However, it remains unknown whether PSGL-1 and CD43 under physiological conditions bind to yet-undefined receptors to deliver an inhibitory signal for proliferation. Additional experiments will be required to clarify the mechanism of the antiproliferative effect mediated by the intracellular domains of PSGL-1 and CD43.

Besides PSGL-1 and CD43, several sialomucins, including CD34, podocalyxin, and endomucin, which can serve as L-selectin ligands when modified with selectin-binding glycans, are reported to have similar dual functions (36–38). We propose that these sialomucins generally function as antiadhesive molecules to prevent inappropriate cell adhesion and activation, but when they are modified to bind selectins in specific cells or tissues, they switch roles and become proadhesive, promoting cell-cell interactions.

We found that, in vivo, a deficiency of PSGL-1 as well as CD43 exacerbated the development of inflammation in a mouse colitis model induced by the adoptive transfer of naïve CD4<sup>+</sup> T cells into RAG<sup>−/−</sup> hosts. This colitis model is reported to be mediated by Th1 cells that migrate into the colon in a selectin-independent manner (24). Importantly, we showed that the numbers of CD4<sup>+</sup> T cells in the colon of DKO cell-recipient mice were significantly higher than in mice that received WT or singly deficient cells. These results are consistent with a role for PSGL-1 and CD43 in negatively regulating T cell adhesion and proliferation.

In conclusion, our study shows for the first time that PSGL-1 functions as a negative regulator of T cell adhesion and proliferation, adding this molecule to the expanding category of dual-functioning sialomucins. Together with CD43, PSGL-1 constitutes an additional layer of regulation for T cell adhesion and proliferation, and possibly for T cell-mediated immune responses.

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Disclosures

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