Editorial

Immune checkpoint regulator: a new assignment proposed for the classic adhesion molecule P-selectin glycoprotein ligand-1

Jiaxuan Chen[1,2], Carolyn A. Haller[1,2], Elliot L. Chaikof[1,2]

[1]Department of Surgery, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA 02215, USA; [2]Wyss Institute for Biologically Inspired Engineering at Harvard University, Boston, MA 02115, USA

Correspondence to: Elliot L. Chaikof, MD, PhD. Department of Surgery, Beth Israel Deaconess Medical Center, 110 Francis St, Suite 9F, Boston, MA 02115, USA. Email: echaikof@bidmc.harvard.edu.

Comment on: Tinoco R, Carrette F, Barraza ML, *et al*. PSGL-1 Is an Immune Checkpoint Regulator that Promotes T Cell Exhaustion. Immunity 2016;44:1470.

Submitted Aug 30, 2016. Accepted for publication Sep 02, 2016.
doi: 10.21037/tcr.2016.10.05
View this article at: http://dx.doi.org/10.21037/tcr.2016.10.05

P-selectin glycoprotein ligand-1 (PSGL-1) is a major selectin ligand expressed by hematopoietic cells and well known for its role in mediating leukocyte trafficking into inflamed tissue. A recent report by Tinoco *et al.* examines chronic viral infection and melanoma in PSGL-1 deficient mice (*Selplg*$^{-/-}$) and suggests a previously unknown and selectin-independent function for PSGL-1 as an immune checkpoint regulator that promotes T cell exhaustion (1).

PSGL-1 is expressed on the surface of all leukocytes. It is a ligand for L-, E-, and P-selectin; however, it binds with highest affinity to P-selectin to mediate leukocyte capture and rolling on inflamed vascular endothelium. PSGL-1 has also been described as having a trafficking role in adaptive immunity, but lymphocyte presentation of selectin-competent PSGL-1 is tightly regulated by the cellular activation state. Selectin ligation is strictly dependent on appropriate post-translational glycosylation of PSGL-1 by glycosyltransferases, such as core 2β-1,6-*N*-acetylglucosaminyl transferase I (C2GlcNAcT-I) and fucosyltransferase VII. In contrast to constitutive glycosyltransferase expression in innate immune cells, such as neutrophils and monocytes, T cell glycosyltransferase expression is activation-specific. Although PSGL-1 protein expression can be detected on both naïve and effector T cells, only effector T cells express fully glycosylated PSGL-1 with traditional selectin affinity (2-5). A selectin-independent PSGL-1 homing mechanism has been previously proposed by Veerman *et al.* for regulating the trafficking of naive T cells to secondary lymphoid organs (6). Specifically, a PSGL-1-dependent enhanced chemotactic response of resting T cells was observed to secondary lymphoid organ chemokines, CCL21 and CCL19. The mechanism for this selectin-independent function was not elucidated, however, the authors speculated that the extended mucin structure of PSGL-1 may have facilitated chemokine capture and that PSGL-1/chemokine interaction may have induced an intercellular signaling response. A subsequent study examined T cell homeostasis in PSGL-1 deficient (*Selplg*$^{-/-}$) mice (7). Despite the mild phenotype observed in healthy *Selplg*$^{-/-}$ mice, blood compartment specific T cell lymphopenia and increased T cell proliferation to homeostatic cytokines was observed, but a mechanistic understanding of these phenomena remain limited. Additional studies are needed to elucidate the selectin-independent functions of PSGL-1 in T cells, as well as the functional role of differentially glycosylated forms of PSGL-1.

In addition to a traditional role in mediating cell trafficking, several publications have also suggested an immunosuppressive role for PSGL-1. For example, PSGL-1 expression has been reported to suppress the proliferation of activated T cells (7,8), and antibody- or selectin-induced crosslinking of PSGL-1 has been reported to drive caspase-independent apoptosis of activated T cells (9). Likewise, PSGL-1 deficient regulatory T cells fail to inhibit T cell-dendritic cell interactions resulting in sustained T cell activation and increased severity of experimental autoimmune encephalomyelitis (10). Increased disease severity has also been noted in PSGL-1 deficient animal models of lupus and inflammatory bowel disease (8,11).

Immunoregulation is critical to the resolution of innate and adaptive immune responses so as to avoid excessive host tissue damage, it is well recognized that these same pathways are exploited in the pathogenesis of both cancer and viral disease to generate chronically immunosuppressed microenvironments. Indeed, checkpoint blockade of T cell suppressive pathways represents a promising approach in cancer immunotherapy, providing ample motivation to define the wide range of intrinsic mechanisms that lead to local T cell suppression. It is within this context that Tinoco *et al*. explore the hypothesis that PSGL-1 functions as an immune checkpoint regulator (1).

To examine the role of PSGL-1 in dysfunctional and exhausted T cell pathologies, chronic viral infection was induced in *Selplg*$^{-/-}$ and wild-type (WT) mice. Specifically, mice were infected with lymphocytic choriomeningitis virus clone 13 (LCMV Cl13), which displays prolonged viremia for up to 90 days post-infection (dpi) and is known to induce CD8$^+$ T cell exhaustion characterized by upregulation of immune checkpoints (12), increased apoptosis, decreased proliferation, and reduced expression of inflammatory cytokines (13). Although PSGL-1 expression is known to be constitutive in all T cells, the authors observed upregulated expression of PSGL-1 in WT virus-specific CD8$^+$ T cells in the spleen, lymph nodes, and lungs for up to 10 dpi. In contrast, *Selplg*$^{-/-}$ mice displayed increased frequencies and numbers of virus-specific T cells in the spleen, blood, lungs, liver, and kidneys at 10 dpi, which was sustained through 30 dpi in the spleen and blood. Noting that enhanced expansion or survival could explain increased numbers of virus-specific CD8$^+$ T cells in *Selplg*$^{-/-}$ mice, BrdU incorporation and the expression of survival molecules were analyzed. BrdU$^+$ positive CD4$^+$ and CD8$^+$ cells were increased in the spleen of WT mice at 9 dpi, suggesting no proliferation advantage in *Selplg*$^{-/-}$ mice. Characterization of survival markers, IL-7Rα and CD25, revealed enhanced expression for up to 10 dpi in virus-specific CD8$^+$ T cells of *Selplg*$^{-/-}$ mice, with increased IL-7Rα expression for up to 30 dpi. Moreover, PI$^+$/caspase-3$^+$ expression was reduced at 10 dpi in *Selplg*$^{-/-}$ CD8$^+$ T cells. Both of these observations were consistent with the notion that enhanced numbers of virus-specific CD8$^+$ T cells in *Selplg*$^{-/-}$ mice are a result of improved T cell survival. To confirm this observation, virus-specific CD8$^+$ T cells were sorted at 9 dpi and subject to RNA sequencing analysis. While WT cells displayed enhanced expression of proliferation markers and inhibitory receptors, including PD-1, PSGL-1 deficient cells displayed enhanced expression of pro-survival genes. *Ex vivo* cytokine production was also enhanced in *Selplg*$^{-/-}$ CD8$^+$ and CD4$^+$ T cells suggesting reduced T cell exhaustion. Significantly, less T cell exhaustion in *Selplg*$^{-/-}$ mice resulted in improved viral control. *Selplg*$^{-/-}$ mice displayed viral clearance at 15 dpi in the blood and 30 dpi in the liver, with WT mice exhibiting elevated viremia at 30 dpi. Despite enhanced viral control in *Selplg*$^{-/-}$ mice, heightened T cell responses were associated with increased circulating inflammatory cytokines, more severe pulmonary and hepatic pathology, and greater mortality beginning at 8 dpi.

In testing the hypothesis that PSGL-1 modulates T cell suppression, systemic PSGL-1 deficiency in *Selplg*$^{-/-}$ mice was acknowledged as a potentially confounding factor. To address this limitation, WT splenocytes were harvested at 9 dpi, when CD8$^+$ T cell exhaustion is evident. Cells treated with GP_{33-41} peptide along with IL-2 and anti-PSGL-1 antibody displayed decreased survival and increased PD-1 expression, as compared to cells treated in a similar manner but with a non-specific IgG antibody. While these results suggested that PSGL-1 engagement during T cell stimulation modulates T cell survival, the use of an anti-PSGL-1 blocking antibody (4RA10) represents only a surrogate for *in vivo* binding to an undefined ligand. The majority of CD8$^+$ T cells did not bind P-selectin and E-, L-, and P-selectin *in vivo* blocking experiments suggested that selectin binding played no role in the evolution of virus-specific T cells or Tregs. Within the context of chronic LCMV infection, unidentified binding partners were regulating PSGL-1-dependent T cell exhaustion.

PSGL-1-dependent T cell function was also examined in an inducible melanoma model. Yumm 1.5 murine melanoma cells were subcutaneously injected into WT and *Selplg*$^{-/-}$ mice. PSGL-1 deficient mice displayed better tumor control with slower growth and higher frequency of infiltrating CD8$^+$ and CD4$^+$ effector T cells. Significantly, effector T cells expressed less PD-1 and displayed higher IFN-γ, TNF-α, and IL-2 secretion.

Collectively, evidence provided from this study supports the notion that PSGL-1 modulates effector T cell responses during chronic viral infection and tumor growth. Remarkably, chronic Cl13 infection was inhibited in PSGL-1 deficient mice with significantly elevated numbers of virus-specific T cells, less T cell apoptosis, and less evidence of effector exhaustion. In addition, virus-specific T cells from PSGL-1 deficient mice expressed reduced levels of immune inhibitory receptors. Elevated levels of tumor localized effector T cells and slowed tumor growth were also observed in a PSGL-1 deficient model of melanoma.

These results provide motivation for the design of new therapeutics targeting PSGL-1 to alter immunosuppression pathways. For the rational design of such therapeutics, further study will be needed to better understand the mechanism and context of PSGL-1 activation and downstream signaling involved in T cell suppression. While selectin-independent activation of PSGL-1 was achieved using the anti-mouse PSGL-1 clone 4RA10, clones 2PH1 and 4RB12 were ineffective. Blocking activity of clone 2PH1 has been reported (14), but in our hands, clone 4RA10 is far superior in its PSGL-1 blocking activity and clone 4RB12 does not have reported blocking activity (15). The identification of a selectin-independent binding partner and associated intercellular signaling events remains critical missing components in understanding the significance of PSGL-1 as an immune checkpoint regulator.

With a limited understanding of selectin independent activators, a therapeutic approach to block immune suppressive functions of PSGL-1 may prove challenging. An intriguing strategy would involve the development a PSGL-1 mimetic to saturate the undefined activator of PSGL-1 and, therefore, prevent PSGL-1 engagement and subsequent signaling events. Our laboratory has developed a chemosynthetic approach to generate glycopeptide analogues of PSGL-1 as inhibitors of selectin ligation *in vitro* and *in vivo* (16). It would be interesting to investigate whether such a strategy could also block the immune suppressive function of PSGL-1. Such an approach would benefit from a detailed examination of PSGL-1 glycosylation patterns during the course of T cell exhaustion. The sialyl Lewis^X O-glycan modification governs the most well defined interactions between PSGL-1 and its binding partners including selectins and chemokines (6). Gaining a greater understanding of the glycosylation state of PSGL-1 and the role of the glycan and aglycone components of PSGL-1 in mediating its effect as a negative regulator of T cell responses will provide valuable insight into mechanism and therapeutic translation.

In summary, recent evidence suggests that PSGL-1 plays a role in T cell suppression. In mouse models of chronic virus infection and melanoma, PSGL-1 deficiency enhanced T cell survival and increased effector function with concomitant reduced expression of immune inhibitory receptors that promote T cell exhaustion. Functional inhibition of T cell PSGL-1 may serve as a novel anti-viral therapy or checkpoint blockade for cancer immunotherapy. However, pursuing further studies to gain additional mechanistic insight will be of great value to this endeavor.

Acknowledgments

Funding: This work was supported by National Institutes of Health (R01 DK107405, R01 HL128237, U01GM116196).

Footnote

Provenance and Peer Review: This article was commissioned and reviewed by the Section Editor Chen Qian (Center for Inflammation & Epigenetics, Houston Methodist Hospital Research Institute, Houston, USA).

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at http://dx.doi.org/10.21037/tcr.2016.10.05). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

References

1. Tinoco R, Carrette F, Barraza ML, et al. PSGL-1 Is an Immune Checkpoint Regulator that Promotes T Cell Exhaustion. Immunity 2016;44:1470.
2. Wagers AJ, Waters CM, Stoolman LM, et al. Interleukin 12 and interleukin 4 control T cell adhesion to endothelial selectins through opposite effects on alpha1, 3-fucosyltransferase VII gene expression. J Exp Med 1998;188:2225-31.
3. Blander JM, Visintin I, Janeway CA Jr, et al. Alpha(1,3)-fucosyltransferase VII and alpha(2,3)-sialyltransferase IV are up-regulated in activated CD4 T cells and maintained after their differentiation into Th1 and migration into inflammatory sites. J Immunol 1999;163:3746-52.
4. Haddad W, Cooper CJ, Zhang Z, et al. P-selectin and P-selectin glycoprotein ligand 1 are major determinants

for Th1 cell recruitment to nonlymphoid effector sites in the intestinal lamina propria. J Exp Med 2003;198:369-77.
5. Hirata T, Merrill-Skoloff G, Aab M, et al. P-Selectin glycoprotein ligand 1 (PSGL-1) is a physiological ligand for E-selectin in mediating T helper 1 lymphocyte migration. J Exp Med 2000;192:1669-76.
6. Veerman KM, Williams MJ, Uchimura K, et al. Interaction of the selectin ligand PSGL-1 with chemokines CCL21 and CCL19 facilitates efficient homing of T cells to secondary lymphoid organs. Nat Immunol 2007;8:532-9.
7. Veerman KM, Carlow DA, Shanina I, et al. PSGL-1 regulates the migration and proliferation of CD8(+) T cells under homeostatic conditions. J Immunol 2012;188:1638-46.
8. Matsumoto M, Miyasaka M, Hirata T. P-selectin glycoprotein ligand-1 negatively regulates T-cell immune responses. J Immunol 2009;183:7204-11.
9. Chen SC, Huang CC, Chien CL, et al. Cross-linking of P-selectin glycoprotein ligand-1 induces death of activated T cells. Blood 2004;104:3233-42.
10. Angiari S, Rossi B, Piccio L, et al. Regulatory T cells suppress the late phase of the immune response in lymph nodes through P-selectin glycoprotein ligand-1. J Immunol 2013;191:5489-500.
11. He X, Schoeb TR, Panoskaltsis-Mortari A, et al. Deficiency of P-selectin or P-selectin glycoprotein ligand-1 leads to accelerated development of glomerulonephritis and increased expression of CC chemokine ligand 2 in lupus-prone mice. J Immunol 2006;177:8748-56.
12. Barber DL, Wherry EJ, Masopust D, et al. Restoring function in exhausted CD8 T cells during chronic viral infection. Nature 2006;439:682-7.
13. Wherry EJ. T cell exhaustion. Nat Immunol 2011;12:492-9.
14. Borges E, Eytner R, Moll T, et al. The P-selectin glycoprotein ligand-1 is important for recruitment of neutrophils into inflamed mouse peritoneum. Blood 1997;90:1934-42.
15. Pendl GG, Robert C, Steinert M, et al. Immature mouse dendritic cells enter inflamed tissue, a process that requires E- and P-selectin, but not P-selectin glycoprotein ligand 1. Blood 2002;99:946-56.
16. Krishnamurthy VR, Sardar MY, Ying Y, et al. Glycopeptide analogues of PSGL-1 inhibit P-selectin in vitro and in vivo. Nat Commun 2015;6:6387.

Cite this article as: Chen J, Haller CA, Chaikof EL. Immune checkpoint regulator: a new assignment proposed for the classic adhesion molecule P-selectin glycoprotein ligand-1. Transl Cancer Res 2016;5(Suppl 4):S668-S671. doi: 10.21037/tcr.2016.10.05

