Targeting the PSGL-1 pathway for immune modulation

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PSGL-1 can regulate leukocyte migration & differentiation

Immune responses depend on coordinated mechanisms that guide responding cells to inflammatory sites where they can exert their effector functions. Specific combinations of receptors and ligands that are temporally activated in tissues during the response ensure the responding leukocytes' traffic to the correct location. P-selectin glycoprotein ligand-1 (PSGL-1; *Selplg*), which is highly expressed on most hematopoietic cells, is a critical regulator of cell migration during homeostasis and disease [1]. PSGL-1 is expressed on cells as a homodimer that is post-translationally glycosylated and sulfated to bind its primary ligand, P-selectin, although it can also bind to E- and L-selectin [2–4]. Naive T cells express the nonselectin binding form of PSGL-1, which can engage chemokines and potentially other currently unknown ligands [5]. While T-cell activation induces the enzymatic machinery that modifies PSGL-1 to engage selectins [6], myeloid cells constitutively express these enzymes and are therefore poised to quickly become recruited into a response by engaging selectins that are expressed on inflamed endothelium [7]. Importantly, PSGL-1 not only regulates cell migration, but also modulates signaling pathways that impact cellular differentiation and function. Recently, we discovered that PSGL-1-regulated inhibitory mechanisms induce T-cell exhaustion during chronic viral infection within melanoma tumors that were independent of selectin binding [8]. These findings provide a rationale to therapeutically modulate the PSGL-1 pathway to augment or dampen immune function, which may be relevant in a variety of disease settings.

PSGL-1 is a negative regulator of immune function

PSGL-1 is a highly conserved protein expressed in both humans and mice. The transmembrane and cytoplasmic domains are similar, however, the mucin-like extracellular domains differ in the lengths of decameric repeats that elongate and strengthen this protein region [9,10]. Initial signaling studies in neutrophils showed that the cytoplasmic domain of PSGL-1 engages adaptor proteins that anchor PSGL-1 to the cytoskeleton to recruit Src family kinases [11]. Despite extensive studies of neutrophils showing that downstream signals promote cytoskeletal remodeling to facilitate cell migration, the PSGL-1 signaling pathways in T cells and other immune cells continue to be under investigation. Initial studies however, have shown that crosslinking PSGL-1 on murine dendritic cells (DCs) can induce the immunosuppressive molecules c-Fos, IDO, IL-10 and TGF- β , which lead to Treg induction and limit effector T-cell proliferation [12]. Additionally, crosslinking PSGL-1 on T cells extinguished T-cell receptor signals, reduced IL-2 signals and cell survival and upregulated inhibitory receptors [8]. These studies reveal a fundamental role for PSGL-1 in regulating the DC-T-cell axis, acting to limit the effector T-cell response. Interestingly, engaging PSGL-1 on human monocytes instead promoted a proinflammatory phenotype [13]. These findings show that PSGL-1 can function in different immune cells to trigger signaling pathways that modulate their effector function.

Studies in *Selplg*^{-/-} mice also revealed a PSGL-1 inhibitory function in DCs and T cells. *Selplg*^{-/-} DCs expressed increased levels of MHC II, CD86 and CD40 and were better at stimulating T-cell proliferation [12]. Naive *Selplg*^{-/-} CD8⁺ T cells were found to have delayed entry and egress in lymph nodes suggesting a role for PSGL-1 in their

homeostatic regulation. In support of this concept, naive *Selplg*^{-/-} CD8⁺ T cells were hyper-responsive to the cytokines IL-2, IL-4 and IL-15, with increased proliferation compared with wild-type (WT) cells [14]. During an immune response, *Selplg*^{-/-} T cells differentiated into more potent effectors in both colitis and experimental allergic encephalomyelitis (EAE) models of autoimmunity and produced enhanced levels of the effector cytokines IFN- γ , IL-4 and IL-17. An immune regulatory function was further revealed in *Selplg*^{-/-} mice infected with chronic lymphocytic choriomeningitis virus. We found that *Selplg*^{-/-} mice had increased accumulation of antiviral T cells that was not due to proliferation but due to sustained T-cell survival. Additionally, *Selplg*^{-/-} CD4⁺ and CD8⁺ T cells had enhanced effector function, producing increased levels of IFN- γ , TNF- α and IL-2 compared with WT cells. Furthermore, antiviral *Selplg*^{-/-} T cells downregulated PD-1 and other inhibitory receptor levels. The sustained effector function resulted in early viral control that prevented establishment of a persistent infection in *Selplg*^{-/-} mice. This enhanced T-cell response in *Selplg*^{-/-} mice resulted in T-cell-dependent immunopathology, further underscoring its role in restraining the effector T cells. The inhibitory function of PSGL-1 was also observed in melanoma tumor models wherein *Selplg*^{-/-} mice exhibited increased antitumor immunity. *Selplg*^{-/-} T cells accumulated in higher numbers in melanoma tumors displayed increased effector function and downregulated PD-1, leading to tumor growth control [8]. Together, these studies showed that PSGL-1 can function as an immune checkpoint inhibitor on T cells. Additionally, these studies highlight an important regulatory axis placing PSGL-1 as a regulator of immune responses in autoimmune and chronic disease settings.

Targeting PSGL-1 during acute & chronic disease

Since PSGL-1 is expressed on the cell surface, the molecule is an attractive target for therapeutics, particularly in the context of chronic antigen stimulation of T cells, which can occur during immunostimulatory environments as in autoimmune disease, as well as in immunosuppressive environments such as during persistent viral infection and metastatic cancer. Inhibitory coreceptor signaling that leads to aberrant immune responses therefore differs in these two disease contexts. In an immunosuppressive environment, inhibitory receptor signaling through PD-1 and many others predominates to effectively turn off T-cell function [15]. In contrast, during autoimmune flares, inhibitory receptors are active but outpaced by costimulatory signaling pathways [16]. This dynamic interplay between inhibition and stimulation can determine the extent of T-cell function. Since PSGL-1 signaling can inhibit T-cell function by directly turning off T-cell receptor signaling in mice, blocking PSGL-1 signaling could promote better effector T-cell responses.

To date, efforts to therapeutically target PSGL-1 have primarily focused on blocking PSGL-1 counter receptors, all three selectins, and in acute inflammatory disease settings to impede cell migration. Targeting *in vivo* selectins by antibodies has been tested in preclinical models of psoriasis, subarachnoid hemorrhage, cerebral ischemia, restenosis and graft-versus-host-disease [17]. Only a few strategies have targeted PSGL-1 itself. These include: using antibodies in models of postischemic damage in transplantation and restenosis, [18,19] and using rPSGL-Ig to block PSGL-1-binding receptors in models of myocardial infarction, transplantation, thrombosis, restenosis and arthritis [17]. These studies provided the *in vivo* proof of concept that preventing recruitment of cells by blocking PSGL-1 or its ligands can limit acute inflammation. However, a caveat is that our studies of *Selplg*^{-/-} mice showed that under inflammatory conditions, *Selplg*^{-/-} T cells effectively migrated into sites of widespread virus infection and did not have aberrant infiltration into melanoma tumors. These findings highlight that in the context of inflammation, adhesion molecules other than PSGL-1 (e.g., CD44, VLA-4, LFA-1) may be utilized for extravasation that could limit the efficacy of treatment. Thus, in settings where targeting PSGL-1-dependent cellular recruitment is ineffective, engaging this molecule to modulate intracellular signaling in T cells may offer a therapeutic approach to inhibit T-cell function in autoimmunity and allergy or reinvigorate their responses during chronic infection or cancer to mediate antigen control.

PSGL-1 potential for immunotherapy

Targeting inhibitory coreceptors has shown great clinical benefit in cancer patients. Checkpoint blockade inhibiting PD-1/PDL-1 and CTLA-4 has had promising results for several different cancers, with some patients having durable responses leading to prolonged progression-free survival [20]. However, the reality today is that most patients will not respond to current immunotherapies, even in combination, implying that additional targets need to be identified, developed and tested to increase the clinical benefit for many more patients that currently do not have treatment options. An important aspect of PSGL-1-dependent regulation of T cells is that signaling inhibits T-cell receptor signals, and promotes expression of several other inhibitory receptors, as noted above. Currently, the mechanisms

of how PSGL-1 signaling modulates T-cell function are not clearly defined. However, its potential to impact multiple pathways of inhibition make it an attractive candidate to more broadly target suppressive mechanisms to alleviate T-cell dysfunction. Thus, future studies to identify the signaling pathways that can be engaged by PSGL-1 are warranted to aid in drug discovery efforts. It is conceivable that antibodies or agents specific for PSGL-1 with agonistic function can be generated to suppress immune responses of T cells and myeloid cells in autoimmune disease. In contrast, such therapeutics with antagonistic or neutralizing function can be developed to prevent PSGL-1 inhibitory signaling to reverse T-cell exhaustion. In addition, targeting PSGL-1 in the setting of cancer could provide a treatment option for patients who are unresponsive to current therapies, and in the future be combined with tumor targeted therapies, or additional checkpoint blockade strategies to increase the efficacy of patient antitumor responses. Considerations in PSGL-1 target design must account for binding affinity, the glycosylation state of PSGL-1, the cell types targeted and potential impacts on cell migration.

With the current recognition that the capacity to modulate immune responses through coinhibitory and costimulatory pathways is feasible, the development of therapeutics to target PSGL-1 may represent a new line of therapy to promote and inhibit inflammatory processes that underlie immune dysfunction in diverse human diseases.

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