Published in final edited form as:

Transl Res. 2017 May ; 183: 1–13. doi:10.1016/j.trsl.2016.11.007.

TARGETING PSGL-1/P-SELECTIN INTERACTIONS AS A NOVEL THERAPY FOR METABOLIC SYNDROME

Madhukar S. Patel, MD, MBA, ScM^{a,b,c}, David Miranda-Nieves, BS^{b,c,d}, Jiaxuan Chen, PhD^{b,c}, Carolyn A. Haller, PhD^{b,c}, and Elliot L. Chaikof, MD, PhD^{b,c}

^aDepartment of Surgery, Massachusetts General Hospital, Boston, MA

^bDepartment of Surgery, Beth Israel Deaconess Medical Center, Boston, MA

^cHarvard Medical School, Boston, MA

^dDivision of Health Sciences and Technology, Massachusetts Institute of Technology, Cambridge, MA

Abstract

Obesity-induced insulin resistance and metabolic syndrome continue to pose an important public health challenge worldwide as they significantly increase the risk of type 2 diabetes and atherosclerotic cardiovascular disease. Advances in the pathophysiologic understanding of this process has identified that chronic inflammation plays a pivotal role. In this regard, given that both animal models and human studies have demonstrated that the interaction of P-selectin glycoprotein ligand-1 (PSGL-1) with P-selectin are not only critical for normal immune response, but also are upregulated in the setting of metabolic syndrome, PSGL-1/P-selectin interactions provide a novel target for preventing and treating resultant disease. Current approaches of interfering with PSGL-1/P-selectin interactions include targeted antibodies, recombinant immunoglobulins that competitively bind P-selectin, and synthetic molecular therapies. Experimental models as well as clinical trials assessing the role of these modalities in a variety of diseases have continued to contribute to the understanding of PSGL-1/P-selectin interactions and have demonstrated the difficulty in creating clinically relevant therapeutics. Most recently, however, computational simulations have further enhanced our understanding of the structural features of PSGL-1 and related glycomimetics, which are responsible for high affinity selectin interactions. Leveraging these insights for the design of next generation agents has thus led to development of a promising synthetic method for generating PSGL-1 glycosulfopeptide mimetics for the treatment of metabolic syndrome.

*Address correspondence to: Elliot L. Chaikof, M.D., Ph.D., Department of Surgery, Beth Israel Deaconess Medical Center, 110 Francis St, Suite 9F, Boston, MA 02115, Tel: +1 (617) 632-9581, echaikof@bidmc.harvard.edu.

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INTRODUCTION

The metabolic syndrome, characterized as a collection of risk factors for atherosclerotic cardiovascular disease and type 2 diabetes, is driven by excess energy intake and obesity [1]. The five interrelated factors comprising the syndrome are atherogenic dyslipidemia, elevated blood pressure, glucose intolerance and insulin resistance, a pro-thrombotic state, and a pro-inflammatory state [2]. Primarily, management of metabolic syndrome focuses on lifestyle modifications, such as weight reduction and increased physical activity [3]. In patients with persistent risk factors, further treatment with lipid lowering agents, anti-hypertensives, and antiplatelet agents help reduce the risk of cardiovascular disease, whereas drugs to reduce serum glucose and improve insulin sensitivity can be used to treat resultant diabetes [2]. Currently, despite a prevalence of 20–30%, therapies to prevent the development of cardiovascular disease and diabetes due to obesity-induced metabolic syndrome are lacking [2].

Mechanistically, a state of chronic inflammation has been suggested to underlie metabolic syndrome [4]. Specifically, obesity-induced immune cell infiltration of adipose tissue has been found to be a significant factor in the development of insulin resistance, type 2 diabetes, hepatosteatosis, and atherosclerosis [5–11]. Broadly, the inflammatory response includes monocytes [8, 12–16], neutrophils [17, 18], T cells [19–22], B cells [23, 24], mast cells [25], and eosinophils [26], with the extent of metabolic dysfunction directly correlating with the activation of pro-inflammatory cytokines and chemokines [27–29], as well as the modulation of inflammatory pathways such as the c-Jun N-terminal kinases (JNK) and nuclear factor- κ B (NF- κ B) transcription factor [30, 31].

In view of this, attempts to develop targeted therapies that modulate the inflammatory cascade as it pertains to metabolic syndrome, are ongoing [4]. Examples of such anti-inflammatory agents include statins and angiotensin converting enzyme inhibitors (ACE-I), which suppress the production of the pro-inflammatory Th1 and Th17 cells [32, 33]; apolipoprotein C-III inhibitors that prevent toll-like receptor 2 (TLR2) activation [4]; omega-3 fatty acids that can be converted to specialized pro-resolving mediators (SPMs) [34, 35]; and peroxisome proliferator-activated receptor alpha (PPAR- α) agonists, which promote suppression of monocyte chemoattractant protein 1 (MCP-1), intracellular adhesion molecule 1 (ICAM), vascular cell adhesion protein 1 (VCAM) [36], and NF- κ B [37]. Additionally, in randomized clinical trials, the anti-inflammatory drug salsalate has been found to improve insulin sensitivity and inflammatory parameters [38], as well as glucose and triglyceride levels [39]. In a subsequent multicenter trial, a reduction in blood glucose, diabetes medication, and markers of cardiovascular risk were noted over a 48-week interval in patients with type 2 diabetes [40]. A sustained improvement in insulin sensitivity, along with a reduction in markers of systemic inflammation have also been reported in response to an IL-1 receptor antagonist [41]. Although the magnitude of glucose lowering has been modest in response to both salsalate and IL-1 β blockade, these studies suggest that targeting inflammation is a valid strategy for the prevention and treatment of the adverse metabolic effects of obesity. With the inflammatory pathway continuing to evolve as a focus for the prevention and treatment of obesity-induced insulin resistance, diabetes, and cardiovascular disease, new promising targets have been identified and warrant review.

In this article, targeting the interaction of P-selectin glycoprotein ligand-1 (PSGL-1) with selectin will be discussed as a novel therapeutic strategy for metabolic syndrome. Specifically, PSGL-1 and selectin interactions in inflammation will be reviewed, with a specific emphasis on their role in the pathophysiology of obesity-induced metabolic syndrome. Importantly, current strategies of blocking PSGL-1/P-selectin interactions will be discussed and next generation synthetic approaches of creating PSGL-1 glycosulfopeptide (GSP) mimetics will be considered.

PSGL-1/P-SELECTIN INTERACTIONS AS MEDIATORS OF OBESITY-INDUCED INFLAMMATORY RESPONSES

PSGL-1 is a glycoprotein that is expressed on the surface of all leukocytes and supports leukocyte recruitment as a component of a range of inflammatory responses [42–46]. Structurally, PSGL-1 is a membrane protein with a disulfide-linked homodimer that has a mucin ectodomain with serine, threonine, and proline repeats that are sites for potential O-glycan modification [46]. PSGL-1 is a ligand for endothelium-selectin (E-selectin, CD62L), platelet-selectin (P-selectin, CD62P) and leukocyte-selectin (L-selectin, CD62L), but binds with highest affinity to P-selectin [47, 48].

P-selectin, which as its name implies, was originally isolated from platelets but later noted to be expressed by endothelial cells, has a membrane bound and a soluble form [48, 49], both of which bind leukocytes [50]. P-selectin is stored in the α -granules in platelets and in the Weibel-Palade bodies of endothelial cells. As a cell adhesion molecule, in the setting of inflammation, P-selectin translocates to the plasma membrane where it can interact with ligands. P-selectin is structurally made up of three characteristic protein motifs including a lectin, an epidermal growth factor (EGF) domain, and a number of short consensus repeats (SCRs)[51]. The soluble form of P-selectin may be produced from alternatively spliced mRNA that does not code for the transmembrane domain [49], and may also arise from damaged platelet membranes expressing P-selectin [52, 53]. The interaction of PSGL-1 with P-selectin on activated platelets leads to leukocyte-platelet aggregates that promote the adhesion and infiltration of inflammatory cells [54–57]. Similarly, the ligation of P-selectin on endothelial cells by PSGL-1 comprises the initial capture and rolling step in leukocyte-endothelial cell interactions (Figure 1) [58, 59]. Given this critical role in inflammatory response, and in turn its importance in the pathophysiology of obesity-induced metabolic syndrome, animal and clinical studies have been performed to better characterize PSGL-1/P-selectin interactions in this context.

In animal models of obesity, the influx of leukocytes into visceral adipose tissue is mediated by P-selectin [60] and PSGL-1 [12]. Particularly, in high-fat diets, excess free fatty acid formation increases endothelial cell (EC) expression of P-selectin [61–63]. Myeloperoxidase (MPO), which is released upon adherence of leukocytes to P-selectin, is also increased in obese mice [17]. MPO impairs adiponectin signaling [64], which increases leukocyte trafficking to visceral fat and further increases inflammation [65–67]. It is noteworthy that genetic deletion of PSGL-1 is protective against visceral fat inflammation in both diet-induced and leptin receptor mutant obese mice [68]. Leukocyte-EC interactions and

macrophage content are both reduced in these animal models, as are circulating levels of P-selectin and MCP-1 [68]. These results confirm that PSGL-1/P-selectin interactions mediate leukocyte-EC and leukocyte-platelet interactions, which contribute to the adverse metabolic consequences of obesity. Furthermore, obese mice that are deficient in PSGL-1 display enhanced insulin sensitivity, improved lipid metabolism, decreased hepatic steatosis, and are protected from endothelial dysfunction and adipose inflammation [68–70]. Upon analysis, these mice showed a reduction in leukocyte accumulation and expression of pro-inflammatory genes with a concomitant reduction in adipocyte hypertrophy and free fatty acid levels [69]. Importantly, compared to transgenic mice expressing the entire human *SELP* gene responsible for coding P-selectin, P-selectin-deficient mice have significantly reduced atherogenesis [71].

More translationally, clinical studies have been conducted to better understand the role of PSGL-1 and P-selectin in obesity-induced disease. Through the study of gene expression levels, the *SELP* gene has been found to be upregulated 1.5 fold in the omentum of morbidly obese diabetic patients [72]. When stratified further, those patients with hyperlipidemia were noted to have a 2.9 fold increase in gene expression relative to those with normal lipid panel [72]. Accordingly, P-selectin is upregulated after consumption of a single high-fat meal [73] and elevated lipid and triglyceride levels are associated with increased concentrations of P-selectin at follow-up [74]. Moreover, in a study of obese women without other cardiovascular risk factors, visceral body fat was found to correlate with endothelial dysfunction and elevated circulating P-selectin levels [75]. In this group, reduction of body weight (of at least 10%) was associated with a decrease in cytokine levels reflecting a reduction in endothelial activation [75]. Lastly, in evaluation of patients with insulin resistance and metabolic syndrome, platelet expression of P-selectin, platelet-leukocyte aggregates, and plasma P-selectin were all found to be increased [76–83], including a cohort of 2,570 participants in the Framingham Heart Study who, in spite of having metabolic syndrome, were free of both overt diabetes and cardiovascular disease [84].

Taken together, the evidence in both animal models and human studies demonstrating the critical role of chronic inflammation in obesity-induced insulin resistance and metabolic syndrome suggest that novel therapies for preventing and treating resultant disease are possible through specific targeting of PSGL-1/P-selectin interactions.

APPROACHES TO BLOCKADE OF PSGL-1/P-SELECTIN INTERACTIONS

Current therapeutic approaches targeted at interfering with PSGL-1/P-selectin interactions have led to the development of PSGL-1 and/or P-selectin antibodies, the creation of recombinant immunoglobulins to competitively bind P-selectin (rPSGL-Ig), and the design of molecular therapies (Figures 2A and 2B). In this section, results from prior literature focusing on each of these approaches in various disease models will be discussed, along with foreseeable challenges with applying these approaches to the development of new treatments for obesity-induced metabolic syndrome.

Antibody-mediated therapies

Antibody-mediated therapies targeted at PSGL-1 and P-selectin have been developed not only to aid in understanding their role in cellular interactions, but also as potential treatments for clinical disease. This therapeutic modality makes use of an antibody specific to either PSGL-1 or P-selectin that after binding renders them inactive, and in turn impacts underlying disease that relies on PSGL-1/P-selectin interaction for initiation and/or progression [85]. In studies relevant to metabolic syndrome, for instance, mice with diet-induced obesity and resultant increase in mesenteric perivascular adipose tissue macrophage content, as well as vascular oxidative stress, were administered weekly injections of PSGL-1 blocking antibody [70, 86]. In these investigations, this antibody was noted to be effective in preventing endothelial dysfunction and reducing visceral adipose inflammation [70, 86]. Furthermore, through chronic inflammation and adipokine production abrogation, PSGL-1 blockade maintained local nitric oxide (NO) levels and preserved the vessel wall in a non-activated, quiescent state [70, 86]. As endothelial dysfunction is predictive of later atherosclerosis and cardiovascular complications [87–90], it is encouraging that PSGL-1 blocking antibody has been shown to inhibit visceral fat-induced atherosclerosis [91]. Further efforts investigating antibody-mediated therapies targeting PSGL-1 are summarized in Table 1.

Although the breadth of experience in the development of PSGL-1 and P-selectin blocking antibodies is extensive, major challenges have been described for antibody-mediated therapies. These drawbacks include high cost, the effectiveness of monoclonal agents as clinical therapies, limited shelf-life [92], and the potential development and consequences of acquired immunogenicity given the likely need for repeated administration [93–99].

Recombinant therapies

Recombinant strategies have also been explored to suppress PSGL-1/P-selectin interactions. This approach uses synthetic biology to develop competitive inhibitors of P-selectin [100]. To this end, researchers have expressed a soluble form of PSGL-1 through the co-transfection of Chinese hamster ovary (CHO) cells with PSGL-1 complementary DNA (cDNA) and a sialyl Lewis X (SLe^X)-forming fucosyltransferase [101], yielding a functional PSGL-1 mimetic that specifically binds to P-selectin [100]. Further modifications to increase half-life and binding were performed through the addition of recombinant immunoglobulin to ultimately create rPSGL-Ig [102]. Despite reports that have found inhibitory antibodies to PSGL-1 to be more effective than rPSGL-Ig, a discrepancy attributed to dosage effect, improper timing of drug delivery, or lack of species specificity given that rPSGL-Ig is structurally based on human rather than rodent PSGL-1 [103], a number of encouraging studies in animal models using recombinant therapy have been reported (Table 2).

Given the positive evidence in animal studies, human clinical trials have been conducted to evaluate the effect of rPSGL-Ig in transplant and myocardial infarction patients. In a multi-center phase IIA study in renal allograft recipients, rPSGL-1 was noted to have no impact on the primary outcome measure of dialysis within the first week post-transplant [104]. In a separate single-center phase II study evaluating the role of rPSGL-Ig in deceased-donor liver transplant recipients, treatment was associated with a non-statistically significant trend

towards decreased ischemia reperfusion injury and improved early liver function [105]. With regards to myocardial infarction, the P-Selectin Antagonist Limiting Myonecrosis (PSALM) phase II trial assessed patients with ST-segment elevation acute myocardial infarction who were treated with intravenous rPSGL-Ig as an adjunctive therapy to thrombolysis [106]. Due to a lack of significant benefit with regards to myocardial blood flow, ST-segment change resolution, left ventricular ejection fraction, and Thrombolysis In Myocardial Infarction (TIMI) flow grade, this trial was stopped early [106]. Taken together, trials evaluating rPSGL-Ig as a competitive inhibitor of P-selectin highlight the difficulty in translating convincing data from animal experiments to yield clinical benefit. Furthermore, additional challenges with continued development rPSGL-Ig therapies involve their failure to accurately recapitulate the native glycosylation pattern of PSGL-1 and their relatively low potency with IC₅₀'s in the high micromolar range [102, 107].

Synthetic molecular therapies

Within the realm of synthetic molecular therapies, multiple mechanisms have been exploited to interfere with PSGL-1/P-selectin interactions, including the inhibition of enzymes required to make selectin ligands, the creation of aptamers to directly bind P-selectin, and the synthesis of glycomimetics to the N-terminus of PSGL-1 to competitively bind selectins.

Glycosyltransferase inhibitors

Structurally, ideal selectin ligands are glycoconjugates composed of protein or lipid scaffolds that contain a sLe^X or sialyl Lewis A (sLe^A) [108]. In order for sLe^X or sLe^A to be properly linked to these glycoconjugates, a series of glycosyltransferase reactions are performed by N-acetylglucosaminyl-, galactosyl-, sialyl-, and fucosyl-transferases [48]. As such, inhibitors have been developed in order to block the necessary steps of conjugating these moieties to the underlying glycan chain and thereby interfere with PSGL-1/P-selectin interactions by preventing the endogenous production of PSGL-1. Considering that these glycosyltransferase reactions take place in the endoplasmic reticulum and Golgi apparatus, along with the inherent drug design challenge of creating a molecule that is able to reach these structures, such inhibitors have been difficult to advance to *in vivo* studies. Nonetheless, progress has been reported by a number of investigators. Examples include the development of a cell permeable sialyltransferase (ST) inhibitor, 3F_{ax}-Neu5Ac, which enters into the cell in a peracetylated form after which it is deacetylated by native cellular esterases and is subsequently able to inhibit the action of ST [109]. Although these inhibitors have been found to be beneficial in experimental models of cancer [109–111], their lack of selectivity is concerning as off-target effects have been noted, leading to significant morbidity such as irreversible renal injury [112]. Thus, recent efforts have focused on the encapsulation of these inhibitors in nanoparticles, such as poly(lactic-co-glycolic acid) (PLGA), displaying cancer targeting antibodies [113]. However, as this drug delivery based solution requires knowledge of a specific antigen to be targeted, further progress in the development of specific glycosyltransferase inhibitors for clinical use will require increased structural knowledge of native and pathogenic glycosyltransferases.

P-selectin aptamers

Representing a distinct synthetic approach to interfering with PSGL-1/P-selectin interactions, aptamers, or single-stranded oligonucleotides that bind target proteins, have been created with affinity for P-selectin [114]. Therapeutically, anti-mouse P-selectin aptamers have been shown to prevent adhesion of sickle red blood cells and leukocytes to endothelial cells in mice models of sickle cell disease [114]. Further, in a baboon model of venous thrombosis, anti-P-selectin aptamer (ARC5692) has been noted to promote vein recanalization, preserve valve competency, and decrease vein wall fibrosis [115]. Currently, however, such aptamers have not been used clinically, potentially due to challenges with cross-reactivity, interaction with intracellular targets, and degradation[116].

Glycomimetics

Most generally, glycomimetics represent a class of synthetic compounds that have structures similar to native carbohydrates. Given that PSGL-1 is a glycoconjugate, creating a glycomimetic of this ligand affords an opportunity to interfere with PSGL-1/selectin interactions. As one example, GMI-1070 is a pan-selectin inhibitor designed, in part, on the conformation of sLe^x in the carbohydrate binding region of E-selectin as delineated by nuclear magnetic resonance techniques [117–119]. This glycomimetic has been tested in mice models of multiple myeloma and has been shown to decrease tumor growth and progression, as well as restore sensitivity to chemotherapeutics such as bortezomib, which are challenged by drug resistance [120]. In patients with sickle cell disease, recent results of a prospective multicenter phase 2 study suggest that this compound may yield clinically meaningful reductions in vaso-occlusive crises with reduced use of opioid analgesia [121]. Although this pan-selectin inhibitor is potent for E-selectin, it requires relatively high concentrations to inhibit P-selectin, thus, limiting its use clinically for interfering with PSGL-1/P-selectin interactions [119, 121, 122]. Bimosiamose (TBC1269) is another pan-selectin inhibitor that has previously been shown to be effective in psoriasis [123] and has recently been used in patients with chronic obstructive pulmonary disease with evidence from a phase II trial suggesting that when administered as a nebulizer treatment along with standard bronchodilator therapies decreases pulmonary inflammatory cell infiltrate and cytokine production [124].

In addition to pan-selectin inhibitors, targeted small molecule glycomimetic inhibitors of P-selectin have also been developed, primarily for the management of thrombosis [125–131]. Examples include an orally administered, C2 benzyl substituted, quinolone salicylic acid [131]. PSI-697 was the first of two drugs in this class and in clinical trials did not show beneficial inhibition on platelet-monocyte aggregation [132]. Further modification of this compound has yielded PSI-421, which demonstrated improved pharmacokinetics and has been found to be effective in animal models of both arterial and venous injury [131]. Clinical trial data is unavailable.

While first generation glycomimetics of PSGL-1/selectin interactions have helped validate targets, they have demonstrated limited clinical benefit, which may be related to their low binding affinity to particular selectins. Specifically, the pan-selectin inhibitor GMI-1070 has been noted to have low activity against P-selectin with an IC₅₀ of 423 μM [119], and

bimosiamose (TBC1269) has similarly been noted to have an IC_{50} of 70 μM [133]. Likewise, even selective P-selectin inhibitors such as PSI-697 and PSI-421 have weak binding affinity with K_d 's of approximately 200 μM [131, 134].

NEXT GENERATION APPROACHES TO TARGET PSGL-1/P-SELECTIN INTERACTIONS

Previous strategies aiming to interfere with PSGL-1/P-selectin interactions provide a compelling rationale for the development of more potent inhibitors that could be effective as disease-modifying agents for patients suffering from metabolic syndrome, in order to prevent the onset of diabetes as well as limit the induction of coronary atherosclerosis. Fundamentally, high affinity PSGL-1/P-selectin binding requires stereospecific interactions with clustered tyrosine sulfates (Tyr-SO₃H), as well as a core-2 O-glycan, which has a sLe^x containing hexasaccharide (C2-O-sLe^x) [135–137]. To date, the design of most existing P-selectin inhibitors has focused on mimicking the C2 O-glycan bearing sLe^x moiety and in so doing, these approaches have been largely unsuccessful in accounting for the contribution of critical clustered tyrosine sulfates [119, 124, 130, 131, 138, 139]. Further synthetic challenges have included suboptimal selectivity in glycosylation [140], incompatible protecting groups for the synthesis of oligosaccharides [141], and the acid lability of tyrosine sulfates [142, 143], all of which have contributed to low yield of PSGL-1 GSP mimetics.

Recent advances have been achieved to address these limitations. Specifically, molecular dynamic simulations have been used to account for structural elements of PSGL-1/P-selectin interactions as determined by both crystallographic and experimental data, and guide the design of glycomimetics [144]. With added insight, a stereoselective scheme has been developed in order to achieve a multi-gram, preparative scale (>50g) synthesis of the C2 O-glycan [144]. Furthermore, in order to address the acid lability of tyrosine sulfate, discovery that these groups can be replaced with hydrolytically stable isoteric sulphonates (Fmoc-Phe (*p*-CH₂SO₃H) [145, 146] and Fmoc-Phe (*p*-SO₃H) [147]) has led to an overall increase in GSP yield from < 0.5% when tyrosine O-sulfates were used to 24% when the corresponding sulfonate analogues were synthesized [144].

Through use of these approaches, a novel PSGL-1 glycomimetic model compound, GSnP-6, has been identified as a low nanomolar antagonist of PSGL-1/P-selectin interactions both in vitro and in vivo [144, 148]. GSnP-6 was the result of a screening program intended to inform the rational design of PSGL-1 mimetics in which critical, high-affinity, carbohydrate and peptide recognition epitopes were preserved [144]. Currently, GSnP-6 has the highest known binding affinity to human P-selectin with a K_d of 22 nM, comparable to that of native PSGL-1, which has a K_d of approximately 70 nM [149]. Furthermore, the compound has been noted to be stable at low pH (~5) as well as high temperature (60°C) [144]. In vivo experimentation characterizing platelet-leukocyte adhesion and platelet aggregation, confirmed that GSnP-6 inhibited early thromboinflammatory events [144].

Given the important physiologic role of PSGL-1/P-selectin interactions, potential side effects secondary to blockade will need to be considered. Nonetheless, studies in established

models of bacterial sepsis have found that recombinant therapy can be given without worsening of infection, immune clearance, or increased mortality [150]. In murine models of lupus, exacerbation of nephritis has been noted [151, 152]. As treatment for metabolic syndrome would likely require chronic therapy, it will be important to assess these potential risks.

Although further studies are needed, it is encouraging that GSnP-6 potentially blocks the adhesion of leukocyte subsets implicated in obesity-induced metabolic syndrome [144]. Most importantly, as a synthetic molecule, GSnP-6 is amenable to further modification, which provides for flexibility in drug design and delivery. Ultimately, GSnP-6 provides a unique structural scaffold for the synthesis of a range of highly potent selectin-specific antagonists, including even simpler analogues with comparable or higher selectin binding affinity.

CONCLUSIONS

Evidence in both humans and animal models have validated chronic inflammation as a promising target for the prevention and treatment of metabolic syndrome. Given the significant role of PSGL-1/P-selectin interactions in inflammation, targeting these interactions has the potential to reduce the risk of type 2 diabetes and cardiovascular disease. Although various different approaches to blocking PSGL-1/P-selectin have been the focus of prior studies, recent advancements in the synthesis of glycomimetic analogues provides a novel strategy for creating highly potent selectin-specific antagonists. As synthetic compounds can be manufactured in a cost-effective manner with a high degree of quality control and reproducibility, they warrant continued investigation as therapeutics for the increasingly prevalent, obesity related insulin resistance and metabolic syndrome.

ABBREVIATIONS

PSGL-1	P-selectin glycoprotein ligand-1
JNK	c-Jun N-terminal kinases
NF-κB	nuclear factor- κ B
ACE-I	angiotensin converting enzyme inhibitors
TLR2	toll-like receptor 2
SPM	specialized pro-resolving mediator
PPAR-α	peroxisome proliferator-activated receptor alpha
MCP-1	monocyte chemoattractant protein 1
ICAM	intracellular adhesion molecule 1
VCAM	vascular cell adhesion protein 1
GSP	glycosulfopeptide

EGF	epidermal growth factor
SCR	short consensus repeat
EC	endothelial cell
MPO	myeloperoxidase
rPSGL-Ig	recombinant P-selectin glycoprotein immunoglobulin
NO	nitric oxide
CHO	Chinese hamster ovary
cDNA	complementary DNA
sLe^X	sialyl Lewis X
PSALM	P-Selectin Antagonist Limiting Myonecrosis
TIMI	Thrombolysis In Myocardial Infarction
sLe^A	sialyl Lewis A
ST	sialyltransferase
PLGA	poly(lactic-co-glycolic acid)
C2	core-2

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Figure 1.

Interaction of P-selectin on activated endothelial-cells and platelets with PSGL-1 promotes capture and rolling of inflammatory cells. PSGL-1: P-selectin glycoprotein ligand-1. Modified from Kolaczkowska E, Kubes P. Neutrophil recruitment and function in health and inflammation. *Nat Rev Immunol.* 2013;13:159–75.

Figure 2.

Approaches targeted at interfering with PSGL-1/P-selectin interactions act on either **(A)** P-selectin or **(B)** PSGL-1. Different therapies include the following: 1. anti-P-selectin antibodies, 2. P-selectin aptamers, 3. glycomimetics, 4. recombinant immunoglobulins that competitively bind P-selectin (rPSGL-Ig), 5. glycosyltransferase inhibitors, and 6. anti-PSGL-1 antibodies. PSGL-1: P-selectin glycoprotein ligand-1; EGF: epidermal growth factor domain; SCR: short consensus repeat.

Table 1

Summary of animal studies evaluating anti-PSGL-1 based antibody-mediated therapies that target PSGL-1/P-Selectin interactions.

	Disease	Model	Effect	References
Inflammation	Pancreatitis	Mouse – Caerulein induced acute pancreatitis	Reduction in leukocyte rolling	[¹]
	Uveitis	Mouse – Uveitis induced by systemic injection of lipopolysaccharide	Reduction of retinal leukostasis and severe uveitis manifestations	[²]
	Encephalomyelitis (EAE)	Mouse – Myelin basic protein injection	Reduction in EAE incidence and severity	[³]
	Peritonitis	Mouse – Intraperitoneal thioglycollate bouillon	Decreased neutrophil accumulation	[⁴]
	Chronic colitis	Mouse – Oral dextran sodium sulfate	Reduced disease activity index and mucosal injury	[⁵]
	Crohn's disease	Mouse – Spontaneous ileitis	Improvement in ileitis	[⁶]
Infection	Sepsis	Mouse – Cecal ligation and puncture	Decreased leukocyte accumulation and pulmonary edema, and improved pulmonary function	[^{7, 8}]
T-cell mediated	Graft versus host	Mouse – Bone marrow transplant	Induced apoptosis of abnormally activated T-cells	[⁹]
	Type I diabetes	Mouse – Non-obese diabetic		
Neurological	Epilepsy	Mouse – Systemic pilocarpine injection	Prevented of disease development	[¹⁰]
Ischemia-Reperfusion	Intestinal	Mouse – Superior mesenteric artery clamping	Reduced leukocyte and platelet adhesion	[^{11, 12}]
	Cerebral	Mouse – Bilateral common carotid artery occlusion	Decreased leukocyte-endothelial-platelet cell interactions	[¹³]
Malignancy	Gastric cancer	Mouse – Human gastric carcinoma tissue implant	Decreased metastatic rate of tumor cells	[¹⁴]
	Multiple Myeloma (MM)	Mouse – Intravenous injection of MM cell line	Decreased proliferation and adhesion of tumor cells; increased sensitivity to chemotherapeutics	[¹⁵]
Vascular disease	Thrombosis	Mouse – Inferior vena cava ligation Mouse – Systemic IL-1 β administration and carotid photochemical injury Baboon – Inferior vena cava balloon occlusion	Decreased thrombus mass and vein wall inflammation	[¹⁶⁻¹⁸]
	Neointimal Formation	Mouse – Carotid artery wire injury	Reduced neointimal formation and plaque macrophage content	[¹⁹]
Hepatic	Acute obstructive cholestasis	Mouse – Bile duct ligation	Reduced elevation in liver enzymes and decreased leukocyte rolling	[²⁰]
Transplantation		Rat – Liver	Reduced leukocyte infiltration and improved hepatic function	[²¹]

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Table 2

Summary of animal studies evaluating recombinant PSGL-1 (rPSGL-Ig) that targets PSGL-1/P-Selectin interactions.

Disease		Model	Effect	References
Inflammation	Traumatic	Rat – Noble-Collip drum shock	Attenuated pathophysiological consequences and decreased leukocyte rolling	[¹]
	Pulmonary	Rat – Acid aspiration	Reduced bronchoalveolar lavage neutrophil counts and pulmonary vascular permeability index	[²]
		Mouse – Lipopolysaccharide induced lung injury	Decreased recruitment of inflammatory cells and improved lung repair	[³]
	Arthritis	Mouse – Collagen-induced arthritis	Suppressed disease progression	[⁴]
Ischemia-Reperfusion	Intestinal	Rat – Superior mesenteric artery occlusion	Attenuated neutrophil-endothelial cell adherence	[⁵]
	Renal	Rat –Warm and in-situ perfused cold ischemia	Decreased leukocyte infiltration and renal dysfunction	[⁶]
	Cardiac	Cat – Reversible left anterior descending artery occlusion	Preserved vascular endothelial function and decreased myocardial injury	[⁷]
		Canine – Coronary artery balloon occlusion	Reduced neutrophil infiltration, myeloperoxidase activity, and myocardial injury	[⁸]
		Pig – Reversible left anterior descending artery occlusion	Decreased infarct size and improved myocardial perfusion	[⁹]
Vascular Disease	Thrombosis	Pig – Iliac artery	Accelerated thrombolysis through inhibition of leukocyte accumulation	[¹⁰]
	Neointimal Formation	Pig – Coronary artery balloon injury	Decrease neointimal hyperplasia	[¹¹]
		Pig – Double common carotid artery balloon injury	Decreased platelet and leukocyte adhesion and reduced restenosis	[¹²]
Transplantation		Rat – Liver	Decreased hepatocyte injury as well as neutrophil adhesion and migration	[¹³]
		Rat – Kidney	Decreased tubular cell damage and reduced amount of maintenance immunosuppression required	[^{14, 15}]
		Rat – Cardiac	Reduced infiltration of mononuclear cells and decreased myocardial infarction and apoptosis	[¹⁶]

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