==========REVIEWS==========

PSGL-1: A UNIVERSAL SELECTIN LIGAND OR A SIGNALING MOLECULE?

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Received April 24, 2024

Revised July 10, 2024

Accepted July 12, 2024

Abstract. Interactions of intercellular adhesion molecules of the selectin family with glycoconjugates of cell membranes mediate the initial stage of the adhesion cascade, which recruits leukocytes, circulating in the bloodstream, to the sites of infection or damage. The formation of heterotypic cell aggregates between individual cells of hematopoietic and non-hematopoietic origin may be involved in processes, leading to inflammation, thrombosis and metastasis. A key protein, plays an important role in the binding of selectins, which serves as a ligand for all three selectins, dimeric glycoprotein, P-selectin glycoprotein ligand – PSGL-1. PSGL-1 combines signals activating various biochemical pathways during binding and rolling of leukocytes. The integration of these signals leads to activation of leukocytes, integrin-mediated arrest, restructuring of the cytoskeleton of interacting cells, polarization and subsequent diapedesis of leukocytes into surrounding tissues. The multilevel effect of PSGL-1 on cellular traffic in the physiological and inflammatory states is largely determined by posttranslational modifications, among which an important place is given to specific O- and N-glycosylation and sulfation. In this review, we discuss modifications of PSGL-1 associated with the initiation of biochemical pathways, as well as its interactions, which make it possible to classify this molecule as signaling, paying special attention to the mechanisms leading to pathology, including cardiovascular.

Keywords: *glycoprotein ligand of selectins, PSGL-1, adhesive function of the endothelium, selectins*

DOI: 10.31857/S00268984250103e8

INTRODUCTION

Simple and complex carbohydrates, called glycans, constitute an important supramembrane complex of the apical surface of endotheliocytes – the endothelial glycocalyx (EG), which mediates

physiological and pathological processes [1]. Glycans have a three-dimensional structure that determines their adaptation to perform physiological functions [2]. Normally, the components of the intact EG provide protection against cell adhesion molecules, preventing pathological intercellular interactions; meanwhile, under normal conditions, blood cells interact both with each other and with endotheliocytes. Glycans can store information, the volume of which exceeds that contained in nucleic acids and proteins. This information is decoded by glycoproteins called lectins, in particular, molecules of the selectin family [3]. In the last 20 years, carbohydrate-protein specificity has been considered as a complement to the genetic code: glycoproteins, glycolipids, polysaccharides, proteoglycans have the highest potential for encoding biological information. In carbohydrate complexes, information can be encoded not only by the sequence of monomers, which is characteristic of protein molecules, but also by the anomeric configuration and position of bonds, as a result of which carbohydrate chains have unique coding abilities.

Selectins are cell adhesion receptors that include Ca^{2+}-dependent domains for recognizing and binding glycans. Three representatives of this family are known – P, E, and L-selectins, which mediate the initial stages of leukocyte binding on damaged endothelium with the prospect of subsequent rolling and transvazation into inflammatory foci and their emigration to lymphoid tissues. Cell attachment and rolling along the endothelial surface occurs in the vascular bed under the influence of shear stress, which requires selectins to possess unique biophysical properties. With high binding kinetics, selectins provide the formation of temporary bonds and a dynamic contact zone that moves along the vascular endothelium during cell rotation [4].

Currently, the participation of selectins in the immune response, hemostasis, tissue healing process, as well as in inflammatory reactions and carcinogenesis has been discovered, which is why their contact with ligands may also have therapeutic significance [5].

It is known that a large number of various molecules bind to selectins. However, not all of them are involved in biological processes. In this regard, a number of characteristics have been proposed that a true selectin ligand must meet. These include the following:

1) the ligand molecule must be present, as they say, in the right place at the right time; for example, a true P-selectin ligand should be located on the surface of a mature circulating leukocyte when P-selectin itself is actively expressed on the surface of endothelial cells or platelets in the vascular bed;

2) inactivation of the putative ligand on the non-activated cell excludes biochemically relevant interactions;

3) each selectin binds to the ligand with high, but not identical, affinity, and with specific stoichiometry [6].

As it turned out later, the protein PSGL-1 meets all these requirements (*P-selectin glycoprotein ligand 1; SELPLG; CD162; CLA; PSGL-1; PSGL1*).

STRUCTURE OF PSGL-1

The search for a ligand to the first of the three known selectins - P-selectin, led to the discovery of PSGL-1 in neutrophils in 1993. PSGL-1 - is a type I bitopic transmembrane glycoprotein, expressed in lipid rafts at the top of leukocyte microvilli [7]. Initially, PSGL-1 was identified in human neutrophil-like HL-60 (human leukemia cell) cells. Currently, it is known that PSGL-1 is expressed by all leukocytes: neutrophils, monocytes, and most lymphocytes (including B cells, natural killer cells, naive cells, memory T cells), as well as platelets and some endothelial cells. In addition, there is evidence of PSGL-1 presence in chronically inflamed human prostate tissue, on activated human umbilical vein endothelial cells (HUVEC), as well as on endothelial cells of the small intestine and mesenteric lymph nodes of mice with chronic ileitis [8, 9]. PSGL-1 is a homodimer whose monomers are connected by a disulfide bond, with the monomer molecular weight being 120 kDa. The PSGL-1 precursor has a molecular weight of 43.2 kDa and consists of 412 amino acid residues: signal peptide (1-17) and propeptide (18-412); it includes three domains: N-terminal extracellular (303 residues, 18-320), transmembrane (21 residues, 321-341), and a short C-terminal cytoplasmic domain (71 amino acid residues, 342-412). PSGL-1 is activated by partial proteolysis: the mature protein consists of 371 amino acid residues. After final maturation and glycosylation, the PSGL-1 dimer has a molecular weight of 240 kDa.

The extracellular domain, an N-terminal protein segment 50-60 nm in length [10], contains branching sites for O- and N-glycosylation, and terminal sites that undergo final modifications leading to the ability to bind with selectins. PSGL-1 is enriched with amino acid residues such as Thr, Pro, Ser. Also, the N-terminal region contains three Tyr residues at positions 46, 48, and 51, which undergo sulfation; and 14-16 decameric repeats carrying O-linked glycans [11]. The amino acid sequences of the transmembrane and cytoplasmic domains are considered highly conserved, implying important functions.

On the extracellular domain, there are multiple glycans connected to the protein via O-glycosidic bonds at Ser and Thr residues. Near the transmembrane domain, there is a Cys residue that forms a disulfide bond, which is believed to stabilize the dimeric form of the protein.

The short cytoplasmic tail of the PSGL-1 molecule contains a juxtamembrane cytoplasmic sequence of 18 amino acids (RLSRKGHMYPVRNYSPTE in humans) [12]. Conservative residues in

the cytoplasmic region of PSGL-1 can be associated with ezrin and moesin, which belong to the ezrin/radixin/moesin (ERM) family of adapter proteins responsible for membrane-cytoskeletal connections [13, 14].

POST-TRANSLATIONAL MODIFICATIONS OF PSGL-1

Protein functioning does not have a clear dependence on the expression level; important post-translational modifications are necessary for the normal functioning of the PSGL-1 ligand. These modifications include the following transformations: glycosylation [15], tyrosine sulfation [16, 17], and the formation of branched carbohydrate side chains involving the core protein [18]. The key O-glycan in PSGL-1, containing the smallest recognition epitope – the tetrasaccharide sialyl-Lewis X (sLe ^{x}), and its analog sialyl-Lewis A (sLe ^{A}), is located near the N-terminus in human PSGL-1 at Thr-57. All selectins recognize sLe ^{X}, however, exclusively sLe ^{X} alone may be sufficient only for interactions with E-selectin. The other two selectins require a ligand modified by additional sulfation, for example, GlcNAc-6-sulfation for L-selectin; P-selectin, besides sLe ^{X}, requires at least one additional O-sulfated tyrosine residue of the polypeptide chain. Such ligand modifications increase the affinity of their binding to selectins. Carbohydrate structures that are determinants of selectin binding are formed as a result of post-translational modifications of proteins or lipid scaffolds using enzymes necessary for glycosylation – glycosyltransferases, enzymes that catalyze the transfer of a monosaccharide residue from a donor to a hydroxyl group of an acceptor, thereby forming a new glycosidic bond. There is reason to believe that each glycosidic bond has its own glycosyltransferase [19]. Donors in this reaction are nucleotide sugars, for example, UDP-GlcNAc, UDP-GalNAc, UDP-Glc, UDP-Gal, GDP-Fuc, GDP-Man, CMP-NeuAc. Side groups of synthesized glycans can be chemically "refined"; for example, sulfotransferase modifies the hydroxyl OH6 of GlcNAc by adding a sulfate group and forms 6-sulfo-sLeX – the L-selectin ligand.

Enzymes that actively participate in post-translational modifications of PSGL-1 are presented in Table 1.

Table 1. Enzymes involved in post-translational modifications of PSGL-1

Enzyme	Gene name	Modification
Fucosyltransferase-IV or VII	*FUT4* or *FUT7*	α-1,3-fucosylation
Core 2-β-1,6-glucosaminyltransferase-I	*GCNT1*	formation of branched carbohydrate side chains

β-1,4-galactosyltransferase-I	*B4GALT1*	β-1,4-galactosylation
Sialyl-3-transferase IV	*ST3GAL4*	α-1,3-sialylation
Tyrosylprotein-O-sulfotransferase 1 or 2	*TPST1* or *TPST2*	tyrosine-O-sulfation

Post-translational processing of PSGL-1 includes sulfation of N-terminal tyrosine residues, which can be carried out by at least one of the two tyrosine sulfotransferases. It is known that myeloid cells constitutively express the enzymes necessary for PSGL-1 glycosylation. In contrast, $CD4^+$ T cells synthesize significant amounts of PSGL-1 but do not contain fucosyltransferase VII and 2-β-1,6-glucosaminyltransferase-I C2GlcNAcT-I, which are necessary for the appropriate modification that can enable binding to P-selectin [20]. Additionally, sialyl-3-transferase IV is expressed at low levels. Naive T cells proliferate and differentiate only after presentation of a cognate antigen. Expression of the enzymes mentioned above is induced immediately after T cell differentiation into type I T-helper cells (Th1) and their activation, which leads to the modification of PSGL-1 for successful binding to selectins, i.e., the ability to bind to selectins is acquired only during the proliferation and differentiation of effector T cells [21]. In naive T cells, the incompletely glycosylated protein can bind structurally homologous chemokines CCL21 and CCL19, which stimulate leukocyte migration to secondary lymphoid organs through enhanced chemotactic T-cell response.

Thus, among the post-translational modifications of PSGL-1, the most important for binding are α-1,3-fucosylation, α-2,3-sialylation, β-1,4-galactosylation, and 2β-1,6-N-glycosylation by acetylglucosaminyltransferase.

INTERACTION OF PSGL-1 WITH THE CELL CYTOSKELETON

PSGL-1 is capable of performing its adhesive function only when it is connected to the cell cytoskeleton [22]. Cells expressing PSGL-1 lacking the intracellular domain, as well as cells with actin cytoskeleton damaged by pharmacological agents, were characterized by a significant decrease in rolling efficiency. As noted above, the cytoplasmic domain of PSGL-1 is connected to the actin cytoskeleton through adapter molecules - ezrin, radixin, and moesin (ERM) proteins. These ERM proteins regulate the redistribution of PSGL-1 across the plasma membrane of cells from the tips of leukocyte microvilli to the rear protrusions of polarized cells - uropods. Phosphorylation of ERM leads to enhanced translocation of PSGL-1 to T-cell uropods. After binding to ERM proteins, PSGL-1 becomes connected to the cell's actin cytoskeleton and to signaling molecules, such as MAPK (mitogen-activated protein kinases), whose functions in cells are diverse and include control of gene

transcription, metabolism, proliferation, motility, and cell apoptosis.

Additionally, the FERM domain of radixin (found in ERM proteins) is associated with PSGL-1, consisting of three subdomains: A, B, and C. Interaction with PSGL-1 occurs through the C-domain; the β-chain of PSGL-1 forms an antiparallel β-β structure with the β5C chain of the C subdomain, with 12 amino acid residues participating in hydrogen bond formation. The PSGL-1 binding site in the FERM domain of radixin overlaps with the binding site of the adhesion molecule ICAM-2 (Intercellular adhesion molecule 2, CD102). The ITAM-like motif (immunoreceptor tyrosine-based activation motif, ITAM, amino acid residues 191-208) acts as a hinge region connecting subdomains B and C, and the two phosphorylation sites Y191 and Y205 are hidden by subdomains B and C, respectively. Such embedding of the Y191 and Y205 sites suggests that the steric hindrance effect exists both when the phosphorylation sites affect the ITAM-like motif and when recruiting the non-receptor tyrosine kinase Syk of the spleen, preventing excessive activation of leukocytes through the PSGL-1 signaling pathway under hemodynamic conditions [23].

Nine residues of PSGL-1 that are part of the nonpolar region directly contact subdomain C. As in ICAM-2, the conserved nonpolar residues Tyr10 and Val12 of PSGL-1 are located in a large hydrophobic pocket formed by Ile245, Ile248, Leu281, His288, and Met285. In addition, Tyr10 forms, like His288, a hydrogen bond in both proteins.

There is another highly conserved protein associated with cell membranes and localized in lipid rafts - flotillin-2 (Flot-2), which may also play a role in recruiting PSGL-1 to uropods in T-cells, thereby promoting their activation [24]. Additionally, Flot-2 contacts PAR-1, one of the mediators of the main signaling pathways involved in cell growth and metastasis, and may contribute to tumor progression.

Besides ERM proteins, the PSGL-1 molecule is linked through its cytoplasmic domain to the cytoplasmic non-receptor tyrosine kinase Syk and the metalloproteinase ADAM8, with ERM acting as adapters in the latter case. Urzainqui A. et al. showed that the interaction of PSGL-1 with P-selectin leads to Syk phosphorylation and direct impact on the amino acid sequence of the N-terminal portion of the immunoreceptor, which is the activation site of ITAM in moesin or ezrin in human T-lymphoblasts. Subsequently, these proteins undergo phosphorylation at tyrosine residues, leading to the formation of the PSGL-1-Syk-moesin complex and, ultimately, to the activation of the proto-oncogene cFos *Fos* [25]. The association of PSGL-1 with the enzyme from the ADAM8 metalloproteinase family causes cleavage of the extracellular domain of PSGL-1 and blocks neutrophil rolling. At the same time, the association of the decameric repeat domain of PSGL-1 with ADAM28 enhances binding to P-selectin, which provides regulation of immune cell recruitment to

inflammation sites through the binding of P-selectin to its ligand PSGL-1 [26].

Matsumoto M. and Hirata T. using intravital microscopy showed that moesin may partially participate in PSGL-1-mediated activation of β2-integrin and slowing of leukocyte rolling after P-selectin binding to PSGL [27]. Thus, in moesin gene knockout mice, a slight increase in the rolling speed of neutrophils in the cremaster muscle venules is observed under the influence of tumor necrosis factor α (TNF-α) [27]. The binding of neutrophils observed with decreased moesin synthesis may be due to compensatory interaction of PSGL-1 with ezrin. PSGL-1, by interacting with ERM proteins through phosphorylation of extracellular signal-regulated kinase (ERK), participates in the activation of the MAPK kinase pathway, which is not associated with Syk phosphorylation. It has been noted that the activation of two pathways, ERK and Syk, induced by PSGL-1, depends on the integrity of lipid rafts [28]. The role of lipid rafts in the redistribution of PSGL-1 is also being studied. For instance, Xu T. et al. showed that lipid raft integrity is necessary for PSGL-1-mediated rolling of neutrophils on P-selectin. Ligation of PSGL-1 in HL-60 cells causes rapid phosphorylation of β-adducin protein and mediates ligation-dependent distribution of PSGL-1 through dissociation from the actin cytoskeleton [29].

BINDING OF PSGL-1 TO SELECTINS P, E, L

As already mentioned, PSGL-1 is expressed on the surface of lymphoid and myeloid cells, including platelets and activated T-cells, and through interactions with selectins participates in the binding of leukocytes to endothelial cells, facilitating subsequent transvasculation into tissues. Currently, PSGL-1 is recognized as the most studied physiological ligand that meets all previously proposed criteria for a true ligand. It can bind with high affinity through the N-terminal portion of the polypeptide chain to all three selectins, but its affinity depends on the type of selectin: K_D of P-selectin is 320 nM, E-selectin – 15 μM, L-selectin – 5 μM [30].

When describing the interactions of PSGL-1 with selectins, it is necessary to return to the structure of the extracellular domain, which is endowed with conformational flexibility that facilitates binding to counter-receptors. This domain contains Ser, Thr residues and Pro-rich decameric repeats, which vary among different species and protrude above the cell surface. In general, the extracellular domain, unlike the transmembrane domain, may have some variability. However, despite this, the O-glycosylation site Thr58, located near the N-terminus, is conservative. It is this domain that carries sLe^X and its analog sLe^A, which mediates binding with selectins. Tyrosine sulfation (Tyr46, Tyr48, and Tyr51) provides high binding affinity of human PSGL-1 with selectins. Additionally, sulfated

tyrosine promotes the binding of chemokines CCL19 and CCL21 to the N-terminus of PSGL-1, whereas branching at O-glycan attachment points and sialylation reduce binding [31]. The key event in selectin binding is the electrostatic interactions between Ca^{2+} ions and hydroxyl groups at the C3 and C4 atoms of the fucose residue in the tetrasaccharide sLe^X. The formation of hydrogen bonds between the galactose residue of sLe^X and Tyr4 and Glu92, between sialic acid and Tyr48, provides stabilization of the selectin-ligand complex [32]. After ligation with selectins, PSGL-1 is localized in membrane microdomains called microvilli, at the trailing edge of migrating leukocytes, which ensures its close proximity to other adhesive and signaling molecules, such as CD43 and CD44, and supports leukocyte binding to the endothelium.

Leppanen A. et al. investigated the role of tyrosine sulfation by synthesizing glucosulfopeptides that model the binding region of PSGL-1 [33]. It was established that sulfation of at least one tyrosine is necessary for binding to P-selectin, and sulfation of Tyr48 ($K_D \sim 6$ μM) promotes higher affinity than sulfation of Tyr46 ($K_D \sim 10$ μM) or Tyr51 ($K_D \sim 10$ μM). The model with all sulfated tyrosine residues (Tyr46, Tyr48, and Tyr51) showed higher binding affinity ($K_D = 0.65$ μM), while the non-sulfated model had significantly lower affinity ($K_D \sim 25$ μM). This study also showed that the contribution of fucose to binding is more significant than that of sialic acid, and all interactions depend on charge magnitude. It was also shown that sulfation of the GlcNAC sLe^X residue leads to the formation of 6-sulfo-sLe^X, which increases binding affinity with L-selectin. At the same time, recognition and binding with E-selectin do not depend on sulfation [34].

Sladek V. et al. [35] showed that mutations in E-, L-, and P-selectins, for example, at positions 46, 85, 97, and 107, differently affect ligand functioning. Furthermore, it is suggested that sulfation of the tyrosine residue at position 51 leads to interaction with Arg85 in P-selectin, which is a significant factor for the increased affinity of P-selectin to PSGL-1 compared to E- and L-selectins [35].

Currently, it is known that single nucleotide or complex polymorphisms in selectin genes and PSGL-1 can cause differences in the structure of these proteins, which may lead to functional effects and the development of vascular and metabolic diseases. For example, the variable number of tandem repeats (VNTR) *variable number of tandem repeats, VNTR* polymorphism is relatively common and affects the length of the extracellular domain of the PSGL-1 molecule, including the distance from the P-selectin binding site to the cell surface. In humans, three allelic variants are known: A, B, and C, which contain 16, 15, and 14 decameric repeats, respectively. Allele B lacks repeat 2, while C contains this repeat but lacks repeats 9 and 10. The frequencies of allele A were the highest, and those of C were the lowest. Homozygous carriers of the short alleles B and C had a lower risk of premature

myocardial infarction due to lower adhesive capacity [36].

Decameric repeats contain recurring segments of 10 amino acids, containing about 30% O-glycosylated threonine residues, 10% proline, which lengthen and strengthen the protein framework and separate the N-terminal selectin binding sites from the cell membrane. Glycosylated decamers constitute almost two-thirds of the molecular mass of PSGL-1 and contain at least 50 potential O-glycosylation sites. Deletions in genes encoding decameric repeats disrupt P- and especially L-selectin-dependent interactions with PSGL-1 [37]. Studying recombinant decamers, Tauxe C. et al. were the first to show that decamers directly interact with E-selectin, unlike P-selectin, and provide up to 80% of leukocyte rolling on E-selectin. They found that the main function of decamers is to extend the N-terminus of PSGL-1 and "remove" it from the cell surface to present it to flowing leukocytes, activated endothelial cells and platelets, attached platelets or leukocytes, and to limit the flexibility of the peptide backbone. They also increase the ability of the N-terminus of PSGL-1 to maintain L- and P-selectin-dependent binding, play a major role in stabilizing L-selectin-dependent rolling interactions; directly interact with E-selectin and act as an adhesion receptor [38].

SIGNALING CASCADES TRIGGERED BY PSGL-1

In addition to its adhesive function, PSGL-1 is characterized by participation in the initiation of several signaling cascades. Non-receptor tyrosine kinases of the Src family phosphorylate numerous cytosolic, nuclear, and membrane proteins and play a central role in PSGL-1-mediated signal transduction. Disruption of their functioning may contribute to the progression of cellular transformation and oncogenic activity. A special role is played by the already mentioned non-receptor tyrosine kinase Syk, which, when activated by phosphorylation, binds to the ITAM-like motif in moesin and thus indirectly connects with PSGL-1 and induces transcription of the early response gene cFos *Fos* . C-Fos, in turn, is part of the activator protein-1 transcription factor, which regulates the expression of other cell adhesion molecules – ICAM-1 and VCAM, necessary for subsequent stages of the adhesion cascade. Thus, by controlling the expression of ICAM-1 and VCAM-1, c-Fos also controls the transendothelial migration of leukocytes [39].

It has also been shown that PSGL-1 is required for independent activation of integrins mediated by G protein-coupled receptors. Continuous binding of PSGL-1 to E-selectin induces signaling events that increase the affinity of LFA-1 (integrin αLβ2; *Lymphocyte function-associated antigen 1*) for ICAM-1 and mediates slower binding of ICAM-1 to E-selectin.

Recently, a new regulatory mechanism has been identified in which cytosolic myeloid proteins

(MRP) 8 and 14, secreted by myeloid cells, as well as PSGL-1, are used as pro-inflammatory mediators [40]. It has been shown that the E-selectin-PSGL-1 interaction triggers the release of Mrp8/14 and, in turn, activates the small GTPase Rap1 via Toll-like receptor 4 (TLR4) [40]. This leads to rapid activation of β2 integrins. As a consequence, this pathway enhances leukocyte adhesion and transendothelial migration. The binding of P-selectin to its ligand also enhances integrin-dependent activation and adhesion to immobilized ICAM-1.

It is assumed that, acting through PSGL-1, P-selectin induces activation of Src-kinase, which affects processes of cellular differentiation, proliferation, and adhesion, the dysregulation of which can lead to progression of cellular transformation and oncogenic activity; hyperactivation of PI3 is also associated with the development of oncopathology. This, in turn, leads to activation of macrophage-1 antigen (Mac-1, αMβ2, CD11b/CD18, CR3) and LFA-1 on leukocytes [41].

INVOLVEMENT OF PSGL-1 IN PATHOLOGY

It is known that selectins and PSGL-1 participate in physiological processes such as hemostasis, immune response, and wound healing. They are also involved in metastasis processes and, consequently, in the pathogenesis of oncological diseases. Over the past three decades, knowledge about the functions of selectins and PSGL-1 in the development and progression of various pathologies has significantly expanded.

Thus, the study of cellular interactions in inflammatory processes continues. For example, it has been shown that PSGL-1-P-selectin interaction through the Syk/Ca^{2+}/PAD4 signaling pathway induces the formation of neutrophil extracellular traps in acute pancreatitis. Targeting the selectin-PSGL-1 interaction in mice modeling acute pancreatitis reduces the severity of histopathological manifestations, which could become a promising treatment strategy for this pathology [42].

The involvement of PSGL-1 and selectins in cardiovascular system pathology is being actively studied. For instance, experiments on mice have shown that suppression of GALNT4, one of the enzymes involved in post-translational O-glycosylation of PSGL-1, reduces atherosclerotic plaque formation, monocyte adhesion, and transmigration through the Akt/mTOR and NF-κB pathways. Therefore, GALNT4 can also be considered as a potential therapeutic target for atherosclerosis [43].

Furthermore, co-signaling of PSGL-1 and CXCR2 increases the frequency and size of thrombi in mice, promoting deep vein thrombosis, partly by stimulating the release of neutrophil extracellular traps. Unlike neutrophils, blocking PSGL-1 or CXCR2 signaling in monocytes did not affect their recruitment to thrombi or tissue factor expression [44].

Increased expression of PSGL-1 has been detected in patients with aortic aneurysm. At the same time, PSGL-1 deficiency significantly reduced the frequency and severity of aortic aneurysm, as well as decreased elastin fragmentation, collagen accumulation, and smooth muscle cell degeneration. This protective effect of PSGL-1 inhibition was mediated by decreased adhesion molecule content and subsequent reduction in leukocyte adhesion to the endothelium via the NF-κB pathway, ultimately leading to reduced inflammatory cell infiltration and expression of inflammatory factors [45].

It was established that neutrophil activation caused by knockout of the *KLF2* (*Kruppel-like factor, KLF2*) gene or administration of antiphospholipid antibodies (APS) leads to PSGL-1 clustering through cortical actin remodeling, enhancing adhesion at thrombosis sites. The use of nanoparticles with anti-PSGL-1 antibodies replicates the PSGL-1 clustering situation and leads to reduced thrombosis manifestations, demonstrating the importance and feasibility of targeting activated neutrophils to prevent immunothrombosis [46].

Attempts have also been made to study PSGL-1 involvement in the pathogenesis of vascular diseases with the aim of further investigating the mechanisms regulating its interactions with selectins. Differences were found in the concentration of PSGL-1 in the blood serum of healthy donors and patients with lower extremity vascular diseases such as varicose veins, acute venous thrombosis, and peripheral atherosclerosis [47-49].

González-Tajuelo R. et al. [50] studied the effect of PSGL-1 on pulmonary vessel wall remodeling and concluded that PSGL-1 deficiency in leukocytes is associated with decreased numbers of regulatory T cells (Treg), NO production, and estrogen receptor (ERα) expression with subsequent increase in angiotensin II levels in the lungs of female mice, contributing to the development of pulmonary arterial hypertension [50]. The absence of PSGL-1 promotes endothelial dysfunction characterized by decreased vasodilation response due to impaired NO generation, caused by reduced phosphorylation of endothelial NO synthase at the activation site Ser ^{1176} .

The study of PSGL-1's role in immune response continues. For instance, the adverse effect of this ligand on the course of HIV infection has been described. It has been shown that PSGL-1 inhibits the process of HIV RNA reverse transcription and inhibits the infectivity of HIV virions produced by cells expressing PSGL-1. It has been established that PSGL-1 expression negatively affects immune cell functions, especially T-cells, which are crucial participants in protection against HIV infection. Indeed, PSGL-1 expression and signaling provoke T-cell exhaustion. Additionally, PSGL-1 can also mediate the capture of viral particles and their transfer into cells. Thus, PSGL-1 negatively affects immune cells during HIV infection and potentially can predict its progression [51].

The possible involvement of PSGL-1 in the pathogenesis of the novel coronavirus infection COVID-19 has also been studied. The results of this study revealed increased activation of the P-selectin-PSGL-1 pair with enhanced efficiency of primary leukocyte recruitment, which contributes to tissue damage and immunothrombosis. It was concluded that endothelial cell activation and excessive leukocyte migration involving the P-selectin-PSGL-1 pair play a central role in the pathogenesis of COVID-19 [52].

CONCLUSION

Disruption of selectin interactions with their main ligand PSGL-1 is observed in inflammatory diseases, arterial and venous thrombosis, atherosclerosis, and alterations in immune response. Due to active involvement in such serious pathologies, the interaction of selectins with PSGL-1 becomes a therapeutic target in these conditions. PSGL-1 is now considered in a broader context: it is not just a ligand for adhesion molecules, but a signaling molecule capable of triggering a series of biochemical cascades. In this regard, it is important to search for mechanisms underlying the regulation of activity in interactions between cell adhesion molecules with each other and with their ligands, and PSGL-1 can serve as a marker molecule, targeting which in the future may have therapeutic applications.

FUNDING

Writing this review did not require special funding.

ETHICS DECLARATION

This article does not contain any studies involving humans or animals as research subjects.

CONFLICT OF INTERESTS

The authors declare no conflict of interest.

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