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Selectins – potential pharmacological targets?

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Recent advances in our (patho)physiological understanding have underpinned the frequent involvement of the protein family of selectins in the progression of serious illnesses, including cancer and cancer metastasis, and immunological diseases, such as asthma, allergy and autoimmune reactions. Moreover, selectins seem to have a role in post-ischemic damage and during transplant failures (e.g. in graft-versus-host disease). Although the interplay between selectins and their counter-receptors and ligands is not always primarily involved in the development of these pathological conditions, selectins have been investigated as potential therapeutic targets for therapeutic intervention. This review focuses on the latest trends and developments in anti-selectin antibodies, anti-selectin receptor antibodies, recombinant selectin counter-receptors, low molecular weight selectin antagonists (glycomimetics), induction of selectin tolerance and selectin-targeted imaging agents.

Introduction to selectins

The selectin family features calcium-dependent type-I transmembrane glycoproteins with extracellular lectin-like domains that interact, for example, with sialylated carbohydrate determinants and mucin-like glycoproteins. In mammals, three structurally similar family members have been identified: E(ndothelium)-selectin, L(eukocyte)-selectin and P(latelet)-selectin (Figure 1). Common to all selectins is the mediation of initial tethering of circulating blood cells with endothelial cells of the intima or among each other [1]. Structural details of selectins and their expression profiles have been previously reviewed [1,2]; hence, only a brief description will be provided here.

Nature has developed an efficient mechanism to deliver immune cells to the site of inflammation or injury. Because circulating cells in the blood stream are subjected to high shear stresses, the emigration of leukocytes from the circulation requires a sophisticated and coordinated interplay controlled by multiple signalling and adhesion molecules, among which are selectins [1,3].

Inhibition of these interactions, which disrupts the recruitment cascade and consequently decreases the number of leukocytes in the tissues, might therefore result in alleviation of diseases caused by undesired immunological reaction (e.g. allergy, psoriasis, multiple sclerosis, inflammatory bowel diseases or rheumatoid arthritis) [4]. Furthermore, in cancer, the interaction of malignant cells with the endothelium and platelets is similar, thus selectins also have a key role in the development of metastasises [5].

E-selectin – role in (patho)physiology

E-selectin (CD62E, ELAM-1, LECAM-2) is specifically synthesized by endothelial cells. It is not constitutively present, but transcriptionally regulated by several transcription factors such as tumour necrosis factor α (TNFα), interleukin (IL)-1, nuclear factor κB (NF-κB) and activator protein 1 (AP-1) [6,7]. Once expressed on the cell surface, E-selectin is slowly internalized and directed to lysosomes for degradation [8]. A circulating form of E-selectin (soluble E-selectin or sE-selectin) might be released by enzymatic cleavage or result from shedding of damaged or activated endothelial cells. The concentration of sE-selectin appears to correlate with its expression on the surface of endothelial cells [9]. Therefore, plasma sE-selectin concentration might be a marker of endothelial cell damage or activation. E-selectin is involved in cardiovascular disease [10] and elevated levels of E-selectin and sE-selectin have been reported

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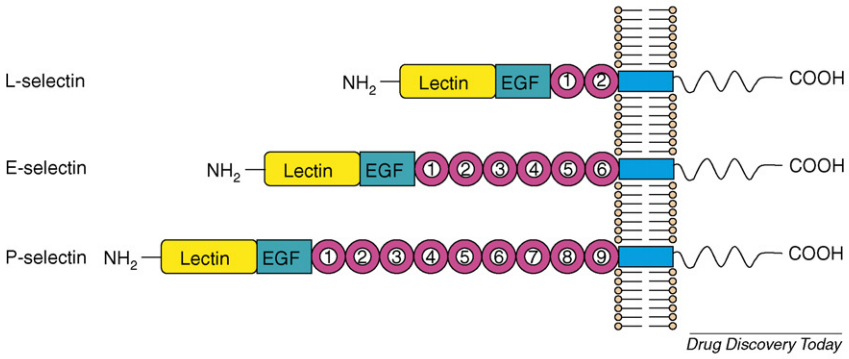

FIGURE 1

The extracellular N-terminus carrying the lectin-like domain is followed by an EGF-like domain and various numbers of consensus repeat (CR) domains. The selectins are anchored in the membrane by a single transmembrane domain and contain a small cytoplasmatic tail. The major structural difference between the three selectin types lies in the number of CR domains. In humans, P-selectin is the longest member of the family with nine CRs, E-selectin has six, and L-selectin contains only two CRs.

in Kawasaki disease, bronchial asthma, Guillain-Barre syndrome and Graves' disease [4,11].

L-selectin – role in (patho)physiology

L-Selectin (CD62L, LAM-1, LECAM-1) is expressed in peripheral blood leukocytes and involved in leukocyte trafficking in the systemic microcirculation [12]. A broad range of activating agents, including granulocyte/macrophage-colony stimulating factor (GM-CSF), interferon-α, and IL-8, have been reported for L-selectin, strongly depending on cell type [13]. In addition to transcriptional regulation, there is indication of activation through adrenalin (via β-adrenoceptors) or shear stress [13]. The soluble form of L-selectin (sL-selectin) is functionally active and inhibits leukocyte attachment to endothelium at high concentrations [14]. Protein kinase C, as well as tyrosine phosphorylation and dephosphorylation, is associated with an increase in sL-selectin [15]. Increased expression levels of L- selectin and sL-selectin have been identified in several diseases, among them allergy, HIV infections, insulin-dependent diabetes mellitus, meningeal leukaemia, multiple sclerosis and sepsis [11].

P-selectin – role in (patho)physiology

P-selectin (CD62P, LECAM-3) was originally purified from platelets and later found to be expressed also in endothelial cells. In both cell types, P-selectin is constitutively expressed and stored in secretory granules – α-granules in platelets and Weibel-Palade bodies in endothelial cells [16]. Upon stimulation (e.g. by histamine or thrombin) these secretory granules fuse with the plasma membrane, causing rapid surface expression. IL-4, IL-13, and oncostatin M all increase P-selectin mRNA synthesis and protein production in human endothelial cells [17,18]. By contrast, TNFα, lipopolysaccharide (LPS) and IL-1 do not increase P-selectin mRNA synthesis, probably because of the lack of a NF-κB binding site in the human P-selectin promoter [19]. It should be noted that the murine P-selectin promoter does contain a NF-κB binding site, and P-selectin expression is regulated by mediators such as TNFα and LPS in murine cells [19]. Once expressed on the surface of endothelial cells, P-selectin is rapidly internalized by endocytosis [20]. The physiological role of P-selectin is to work in concert with E-selectin in the mediation of initial leukocyte adhesion to activated endothelium during acute inflammation [21]. Expression of P-selectin on platelet surfaces stimulates recruitment of leukocytes to platelet aggregates, formation of platelet–leukocyte aggregates and has an important role in vascular haemostasis, atherosclerosis and inflammatory leukocyte extravasation [22].

A soluble form of P-selectin, which might represent a proteolytic fragment or a soluble splice variant lacking the transmembrane domain, is found in serum and plasma [23]. High expression of P-selectin or sP-selectin has been implicated in several inflammatory disorders, including adult respiratory distress syndrome, acute lung injury, ischemia-reperfusion injury, Gram-negative septic shock, thrombotic diseases, malaria, systemic sclerosis, connective tissue disease and rheumatoid arthritis. Furthermore, lung injury scores correlate significantly with sP-selectin in plasma of patients with acute lung injury [4,11].

Selectin ligands and counter-receptors

Selectin-binding structures with high affinity include primarily oligosaccharide and sulfopolysaccharide ligands that usually also represent the binding epitope of physiological selectin counter-receptors, including glycoproteins and glycolipids. P-selectin glycoprotein ligand-1 (PSGL-1, CD162) is a mucin-like transmembrane protein which forms homodimers by linking two 120 kDa chains via disulfide bridges (Table 1). PSGL-1 is a well-characterized receptor for selectins. It is expressed primarily on myeloid, lymphoid and dendritic cells, and can bind all three selectins, but with different binding strengths and association kinetics [24]. Sulfation of tyrosine residues and *O*-glycosylation in the mature NH_2-terminal region of PSGL-1 appear necessary for the high affinity of PSGL-1 to P-selectin, and the binding is regulated by different degrees and forms of glycosylation [25]. *In vitro* inhibition of PSGL-1 completely eliminates neutrophil rolling on P-selectin, consistent with the finding that genetic deletion of PSGL-1 attenuates P-selectin-mediated rolling of leukocytes *in vivo* [26]. The metastatic potential of cancer cells has been reported to correlate with their surface expression of

TABLE 1

Selectin counter-receptor interaction parameters [34]

Selectin	Ligand	K_D [μM]	IC_{50} [μM]
P-selectin			
	PSGL 1 (human)	0.2–0.32	
	rPSGL-IgG	0.06	
	sLex	7800	520–1300
	6-sulfo-sLex		220
E-selectin			
	ESL-1(mouse)	56–62	
	recombinant ESL-1	66	
	sLex	100–2000	100–750
L-selectin			
	GlyCAM1 (mouse)	108	
	sLex	3900	2300
	6-sulfo-sLex		800

PSGL-1 [27]. Cleavage of the protein from the cell surface is one mechanism involved in the deactivation process, when a soluble form of PSGL-1 is detectable in the circulation. sPSGL-1 is still capable of binding to P-selectin, thus representing a competitor for cellular PSGL-1 [28].

The small *O*-linked oligosaccharide-modified glycoprotein CD24 was identified as a ligand of P-selectin. CD24 is mainly expressed on neutrophils. In the absence of PSGL-1, CD24 can mediate the rolling on P-selectin [29].

Two major glycoprotein receptors for E-selectin have been identified, the E-selectin ligand-1 (ESL-1) [30] and PSGL-1 [24]. ESL-1 is a 150 kDa type-1 protein with a glutamine-rich NH_2-terminal segment of 70 amino acids followed by 16 cysteine-rich repeats, a transmembrane domain and a short cytoplasmic tail [31]. ESL-1 is expressed in leukocytes and binds specifically to E-selectin but not to P-selectin. However, there is currently no *in vivo* proof that ESL-1 is involved in E-selectin-mediated leukocyte tethering.

L-selectin binds to the sulfated sialyl-Lewis x (6-sulfo-sLex) epitopes present on *O*-glycans of various glycoproteins in high endothelial venules (HEV) [32]. Four L-selectin ligands have been identified in HEV of peripheral lymph nodes: three sulphated L-selectin ligands, Sgp50 (glycosylation-dependent cell adhesion molecule-1, GlyCAM-1), Sgp90 (CD34) and Sgp200, and the mucosal vascular addressin cell adhesion molecule-1 (MAdCAM-1) (Figure 2). The glycoprotein ligand GlyCAM-1 is secreted and might primarily transduce signals into leukocytes rather than support leukocyte adhesion [33]. Sgp90 is expressed on the surface of endothelial cells, whereas the third ligand, Sgp200, is both secreted and cell associated. Sgp90 and Sgp200 have been suggested to mediate the initial loose binding of lymphocytes to HEV [34]. MAdCAM-1 supports lymphocyte tethering and rolling through interactions with both L-selectin and $\alpha_4\beta_7$ integrin [35]. An interaction of L-selectin with PSGL-1 in the process of neutrophil aggregation has also been shown.

Pharmacological selectin targeting

Being crucial for the initial cell–cell interaction between platelets, leukocytes and the endothelium, the binding of selectin ligands

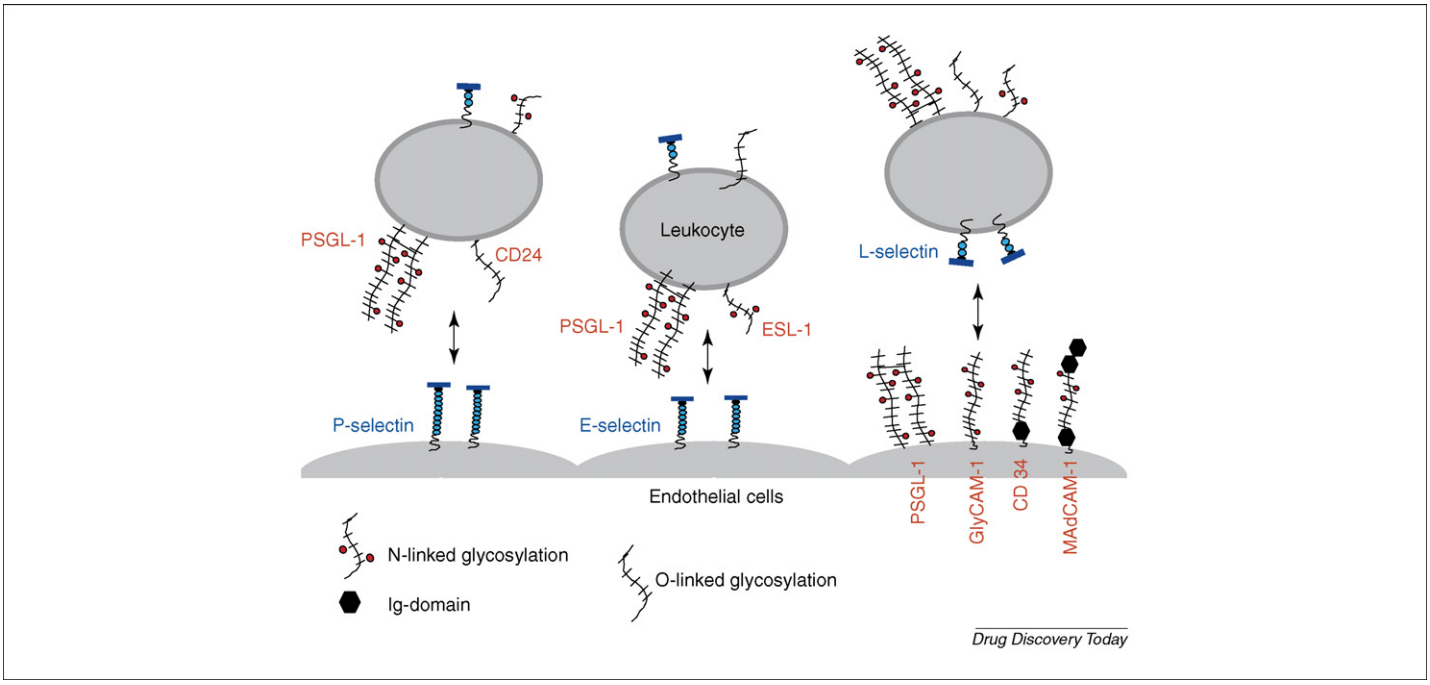

FIGURE 2

Interactions between selectins (blue) and their counter-receptors (red) mediate tethering of leukocytes to activated endothelial cells. Activated leukocytes can carry *N*-glycosylated P- and E- selectin glycoprotein ligands (PSGL-1, ESL-1), which interact with selectins expressed on activated endothelium (PSGL-1 with P- and E-selectin). In absence of PSGL-1, binding of *O*-glycosylated CD24 to P-selectin might be important. The lymphocyte homing receptor L-selectin can also be present on leukocytes and mediate interaction with the endothelium via glycosylated PSGL-1, CD34 and MadCAM-1 (mucosal vascular addressin cell adhesion molecule 1), whereas GlyCAM-1 (glycosylation-dependent cell adhesion molecule 1) is usually found as soluble molecule. Multiple of such interactions cause the initial tethering of the leukocyte to the endothelium, which is followed by firm attachment and often extravasation.

TABLE 2

Examples of *in vivo* evaluated selectin-targeting concepts

Category (name)	Target	Indication	Refs
Antibodies			
CDP850 (SPLAT-1)	E-selectin	Psoriasis	[36,37]
No name specified	E-selectin	Subarachnoid haemorrhage	[38]
RB40.34	P-selectin	Cerebral ischemia	[40]
		Restenosis	[39]
HuEP5C7.g2	E,P-selectin	Pharmacokinetic study	[41]
No name specified	L-selectin	Graft-versus-host-disease	[42]
RatPSG-huG1	PSGL-1	Post-ischemic damage (in transplantation)	[58]
4RA10	PSGL-1	Restenosis	[39]
Recombinant selectin counter-receptors			
rPSGL-Ig	E,P-selectin	Post-ischemic damage (myocardial infarction)	[45,49]
		Transplantation	[46]
		Thrombosis	[43]
		Restenosis	[47]
		Arthritis	[48]
Low molecular weight antagonists			
CY1503 (cylexin)	E,P-selectin	Post-ischemic damage	[50]
TBC1269 (bimosiamose)	E,P-selectin	Post-ischemic damage (myocardial infarction)	[53]
		Transplantation	[54]
		Psoriasis	[56]
		Asthma	[57]

represents an early step in the pathogenesis of diseases that are a result of excessive leukocyte recruitment and/or thrombosis. Interfering with the binding of selectin ligands has, therefore, great therapeutic potential. Several antagonists have been developed to either inhibit or to exploit platelet–leukocyte–endothelium interactions for therapeutic and diagnostic purposes (Table 2). A pharmaceutical approach that targets selectins or selectin ligands to achieve site-specific drug delivery has been reviewed elsewhere [11].

Anti-selectin antibodies

Although anti-selectin antibodies were initially employed to study the (patho)physiological role of selectins, they were later developed for therapeutic application to interrupt the selectin–receptor interactions involved in pathogenesis. Even if there have been failures in clinical trials, the therapeutic potential of such antibodies is highlighted by recent studies in various animal disease models.

SPLAT-1 (CDP850) represents an engineered human antibody to E-selectin. Initial characterization revealed its capacity to block leukocyte adhesion to E-selectin-presenting surfaces *in vitro*. Therefore, this antibody was thought to have the potential to reduce leukocyte attachment to activated endothelium, thereby inhibiting cell recruitment into the tissue. This could indeed be demonstrated for inflamed human skin grafted on SCID mice. Although no adverse reactions were observed in primates and this antibody was well tolerated in a randomized clinical trial, it failed to reduce the numbers of neutrophils and lymphocytes within the inflamed dermis of psoriasis patients and to improve the disease symptoms [36,37]. No further studies using CDP850 have been reported since the initial evaluation.

Another anti-E-selectin antibody was reported to reduce the inflammatory response when applied as bolus injection immediately after experimental subarachnoid haemorrhage in mice [38]. The therapeutic rational behind this treatment was seen in elevated levels of E-selectin within the cerebrospinal fluid of patients that developed vasospasms following haemorrhage. Unfortunately, the antibody used was not further characterized by the Taiwanese authors. Rat anti-mouse-P-selectin antibody (RB 40.34) significantly reduced neointima formation and vessel wall thickening after carotid injury in atherosclerosis-prone mice, suggesting a potential effectiveness against restenosis [39] (for more information see the 'Anti-selectin counter-receptor antibodies' Section).

The therapeutic rationale of single selectin inhibition might be limited by redundancy of P- and E-selectin and coexpression of non-selectin adhesion molecules. Exclusion (or attraction) of leukocyte subpopulations might also cause undesired reactions as exemplified by reduced survival (28% versus 71%) of Mongolian gerbils treated with rat RB 40.34 after global cerebral ischemia [40].

An advanced humanized and complementarity-determining region-grafted antibody with reactivity against not only E- but also P-selectin was engineered from murine mAb mEP-5C7 and termed HuEP5C7.g2 [41]. This antibody was able to effectively block binding of HL-60 leukemia cells to E- and P-selectin positive cells and possessed favourable pharmacokinetic properties in rhesus monkeys with an elimination half-life of approximately seven days.

Finally, acute graft-versus-host disease, which is a significant complication after bone marrow transplantation, could be delayed in transplanted mice when the tissue was pretreated with anti-L-selectin antibody [42].

Recombinant selectin counter-receptors

Selectin blockade using a recombinant derivative of the counter-receptor PSGL-1 represents an alternative therapeutic approach to blocking anti-selectin antibodies. In contrast to antibodies, the recombinant ligand targets all selectin subtypes, although with different affinities. It might therefore be particularly useful in the majority of cases, when different selectins that can complement each other, such as P- and E- selectin, are coexpressed in the diseased area.

A recombinant PSGL-1 immunoglobulin chimera (rPSGL-Ig) comprises the N-terminal 47 amino acids of the mature human PSGL-1 fused to the hinge region of human IgG1, which was modified for reduced complement activation and Fc receptor binding. This IgG chimera has a long circulation half-life of ~11 days in pigs, and production in mammalian cells assures correct carbohydrate modification for high-affinity binding to P- as well as E-selectin, and, with lower affinity, to L-selectin [43]. Evaluation of its safety in mice and rats showed good tolerability with no severe suppression of immune response to systemic bacterial infection [44].

Usefulness of this selectin inhibitor was evaluated for several potentially relevant conditions. In a porcine model of arterial thrombosis, coadministration with tissue plasminogen activator (tPA) reduced the time to thrombolysis to one-third [43].

Furthermore, rPSGL-Ig reduced post-ischemic damage after ischemia in rat liver and kidney, mouse intestine and pig myocard [45,46]. Consequently, organ survival after transplanatation might be increased by rPSGL-Ig, and doses of toxic sirolimus or cyclosporine for maintenance of long-term function of the transplant might be decreased.

PSGL-1–P-selectin binding is also involved in neutrophil and platelet adhesion to damaged vessel walls, inducing local inflammatory reactions that can ultimately lead to thrombosis and (re)stenosis. Indeed, rPSGL-1 reduced adhesion of neutrophils and platelets *in vivo* in injured pig arteries [47].

Finally, the biological effects of rPSGL-Ig were evaluated in a model of collagen-induced arthritis in mice. A reduction in cellularity and TNF levels in the synovium suppressed progression of the disease and protected from joint damage, suggesting further therapeutic potential of rPSGL-Ig [48]. Nevertheless, clinical development of rPSGL-Ig was discontinued by Wyeth Pharmaceuticals after disappointing results in myocardial infarction trials [49] and has been licensed to Y's Therapeutics that initiated a Phase II clinical trial, evaluating the potential of this molecule to prevent delayed graft function of kidney transplants (www.ysthera.com).

Low molecular weight selectin antagonists

Selectins bind to ligands via the interaction of the selectin's C-type lectin domain with carbohydrate modifications on the selectin ligand. As a consequence, the isolated sugar moiety was thought sufficient to antagonize selectin–ligand binding. Many of the pharmacokinetic properties of such low molecular weight selectin inhibitors might, unlike the antibodies discussed above, in principle be tailored to practical needs. Desired properties include oral availability, tissue distribution or predictable hepatic and renal elimination. In addition, their production should be considerably cheaper.

There is a long history of selectin-directed glycomimetics, a summary of which can be found in an excellent review by Kaila and Thomas [50]. Sialyl Lewis X, the obvious choice for such an antagonist, proved unsuccessful, presumably because of its low affinity for E- and P-selectin. CY1503 (cylexin), a pentasaccharide with sLex substructure, blocks selectin activity in a variety of animal models. Nevertheless, the candidate showed no benefit over the placebo in Phase II and III clinical trials for treatment of reperfusion injury, which was attributed to its high molecular weight (>100 kDa) and its extended structure causing low bioavailability and rapid degradation [50]. Modified trisaccharide sLex mimetics effectively suffered the same problems [50]. A new approach, involving careful molecular analysis of the multiple interactions involved in sugar binding to a relatively shallow groove on the selectin receptor, forms the basis for rational drug design [50]. The conjugated monosaccharide dimer bimosiamose (TBC1269, 1,6-bis[3-(3-carboxy-methylphenyl)-4-(2-a-D-mannopyranosyloxy)-pheny]hexane) [51] represents perhaps the most promising drug candidate obtained using this approach, and is currently in clinical development by Revotar Biopharmaceuticals [49]. Safety and pharmacokinetic properties after intravenous infusion proved to be suitable in a Phase I study in human [52]. Initial efficacy studies in experimental animals showed attenuated reperfusion injury after myocardial infarction in rats [53] and prolonged survival after allogeneic kidney transplantation [54]. Both effects were ascribed to inhibition of leukocyte extravasation at the level of tethering to the activated endothelium, albeit work by Hicks *et al.* [55] suggested that the *in vivo* effects of TBC1269 might be mediated through E-selectin and do not involve cell rolling. Latest clinical evaluation in human demonstrated that treatment with 600 mg over 14 days by subcutaneous injection improved skin lesions in psoriasis patients [56] and inhalation reduced the reaction of mild asthmatics following antigen challenge [57]. Long-term treatment and dose reduction might, however, be limited as selectin-mediated cell interactions are of high avidity, involving multiple low affinity binding events that need to be permanently inhibited.

Anti-selectin counter-receptor antibodies

The targeting of the selectin counter-receptor instead of the selectin itself might provide a viable alternative if equally effective. Inactivation of PSGL-1 expressed on leukocytes should, for example, not only reduce attachment to the endothelium and invasion of the target tissue but also reduce leukocyte interplay and selectin upregulation, while avoiding opsonization of the diseased tissue by anti-selectin antibodies [58]. Hence, this approach was primarily tested in experimental organ transplantation, where these potential effects might further reduce the risk of undesired side effects. In fact, pretreatment with a neutralizing anti-PSGL-1 antibody (Wyeth Pharmaceuticals) showed a protective effect against ischemia reperfusion injury in transplanted rat livers [58]. Infiltration with neutrophils, macrophages and dendritic cells, as well as the number of apoptotic cells, was markedly decreased in comparison with control animals, resulting in minimal vascular obstruction and necrosis.

Selectin–receptor interaction is also crucial following blood vessel injury. Exposure of the subendothelial basement membrane results in adhesion of P-selectin-presenting platelets and recruitment of leukocytes via PSGL-1, causing neointima formation, vessel wall thickening and a reduction in effective vessel diameter, if not its obstruction. When it follows balloon expansion of atherosclerotic vessels, this process is called restenosis. Although P-selectin antibodies are able to disrupt this inflammatory cascade, blocking PSGL-1 antibodies was equally effective in reducing inflammation and stenosis [39]. In this comparative study, mice with carotid injury were treated with a monoclonal anti-PSGL-1 antibody (4RA10) or an anti-P-selectin antibody (RB 40.34).

Induction of E-selectin tolerance

A completely different approach to achieve an anti-inflammatory effect at selectin-positive target sites is exploiting the host's own immune system. Induction of regulatory T-cells directed against selectin epitopes might provide a biologic means to suppress immune response at the inflamed epithelium by the release of inhibitory cytokines. As E-selectin is expressed for a prolonged time after pro-inflammatory endothelial stimulation, induction of mucosal tolerance against this member of the selectin family was examined. Repetitive administration of a low dose of E-selectin by nasal instillation not only induced tolerance to that antigen, but also reduced the incidence of ischemic and haemorrhagic strokes in spontaneously hypertensive SHR-SP rats [59]. It was further shown that E-selectin tolerance had the capacity to reduce the extent of post-ischemic damage to the infarcted brain tissue after experimental cerebral artery occlusion [60]. Tolerance was demonstrated in rat after two intranasal instillations of E-selectin by an almost absent delayed-type hypersensitivity reaction against E-selectin in the ear thickness test, unlike in control animals. Transfer of T-cell mitogen-treated cell suspensions isolated from the spleen of these rats also reduced infarct volumes in the naïve recipient. The applicability of this approach in the human species, as well as potential undesired effects associated with this more or less permanent knockout of a central leukocyte homeostasis mechanism, remains to be established. Recruiting for a Phase II study that evaluates the safety and effectiveness of an intranasal E-selectin spray in 60 healthy patients with previous stroke (NCT00012454, www.clinicaltrials.gov/ct) has been completed, but results of this study have not been made available yet.

Diagnostic selectin targeting

Finally, efforts are being made to exploit the specific cell surface expression of selectins for the detection and imaging of lymphoid tissues, inflamed vasculature and metastatic cancer cells. In the mid 90s, successful *in vivo* labelling of inflamed arthritic joints using the ^{111}Indium-conjugated monoclonal anti-E-selectin antibody 1.2B6 and its F(ab)$_2$-fragment was reported [61]. This approach using scintigraphy was later applied to assess the localization and extent of inflammation in patients with rheumatoid arthritis and inflammatory bowel disease [62,63].

These successes have stimulated the development of non-radioactive selectin-targeted *in vivo* diagnostics. Various new magnetic resonance contrast agents were described, in which targeting was achieved by monoclonal anti-E-selectin antibodies or sLex mimetics. The latter was reported to enable imaging of activated endothelium in the inflamed tissue of rats following focal ischemia of the brain and in hepatitis [64,65]. Furthermore, the monoclonal antibody MECA-79 with specificity for L-selectin, the major lymphocyte homing receptor, was conjugated to a near-infrared fluorescent dye to successfully stain lymph nodes of mice in whole-body fluorescence imaging [66]. In an attempt to increase binding specificity and signal strength, Funovics *et al.* [67] choose to attach E-selectin-binding peptides to fluorescent nanoparticles containing Cy5.

In an approach to improve the signal-to-noise ratio of selectin-targeted imaging agents, sLex was combined with an anti-ICAM-1 (intercellular adhesion molecule-1) antibody on microbubbles that can be imaged via ultrasound. ICAM-1 is coexpressed with E- and P-selectin on activated endothelium to enable integrin-mediated cell binding after initial tethering via selectins. *In vitro* evaluations of microbubbles targeted to both receptors under shear stress demonstrated superiority to constructs targeted to ICAM-1 or selectins alone [68].

Conclusions

In this review we have highlighted the potential of selectins and their ligands as target structures in the context of pharmacotherapy and diagnostics. Selectin–ligand interactions provide the initial binding in a multi-step process leading to adhesion between platelets, leukocytes and the endothelium. Hence, selectins are key players in inflammation and thrombosis, and therefore an ideal drug target. In addition, they are overexpressed at sites of inflammation and tumour growth, suggesting them also as potential targets for diagnostic markers. As glycosylation of the selectin counter-receptors is essential for selectin binding, enzymes involved in these modifications, such as fucosyltransferase, might provide additional targets that deserve further investigation.

Currently, only a few of selectin-targeted drugs have progressed into clinical trials and only a limited number has proven its potential *in vivo*. Nevertheless, it should be considered that the selectin–ligand interaction has not always been thoroughly validated as suitable drug target. Such target verification might have been hampered by technical problems imposed by the central physiological role of selectins, which might in itself bear the risk of undesired and unexpected effects of any selectin-targeting therapy.

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