Carbohydrate sulfotransferases: novel therapeutic targets for inflammation, viral infection and cancer

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Effective direct inhibition of adhesion receptors by small molecules has been hampered by extended receptor–ligand interfaces as well as the entropic penalties often associated with inhibition of cell adhesion. Therefore, alternative strategies have targeted enzymes that are centrally involved in the biosynthesis of recognition epitopes, which are crucial for productive adhesion. Two classes of enzymes shown to play a pivotal role in cell–cell and cell–matrix adhesions are the protein-tyrosine and carbohydrate sulfotransferases, which impart crucial sulfate moieties onto glycoproteins. The carbohydrate sulfotransferases will be discussed in terms of target validation and small-molecule inhibitor discovery.

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▼ The modulation of biological adhesion events has been a longstanding goal of pharmaceutical intervention in pathological processes ranging from inflammation, arteriosclerosis and thrombosis to viral infection and tumour metastasis[1–7]. In principle, extracellular or cell-surface-associated cell adhesion molecules (CAMs) are attractive drug targets because of their accessibility, in that a drug does not have to traverse plasma or intracellular membranes. With certain exceptions such as the integrins[8–10], inhibition of cell adhesion has been hampered by a number of drawbacks:

- adhesion receptor–ligand pairs often exhibit extended and relatively flat contact interfaces[11];
- multivalent interactions provide high avidity, which strongly increases the intrinsic affinity of the adhesion receptor for its recognition epitope[12,13]; and
- each small-molecule CAM antagonist pays an individual entropic penalty upon binding to its target, whereas many CAM–ligand interactions are gained for every CAM–ligand binding event that occurs in the process of CAM-mediated annealing of cell surfaces (Fig. 1). Thus, direct inhibition of cell–cell or cell–matrix adhesion by small molecules produces a net loss of entropy, which must be overcome by very high binding affinity of the antagonist and/or presentation of the antagonist in a multivalent fashion[14–16].

In order to overcome these obstacles, alternative strategies for functional inhibition of cell–cell, cell–matrix or cell–virus adhesion have been sought.

One possible alternative is the targeting of enzymes that are selectively involved in the biosynthesis of recognition epitopes crucial to the adhesive interaction of interest. This strategy capitalizes on the reasonable chemical tractability of enzyme targets, in addition to the reduced entropic penalty associated with inhibition of an enzyme versus direct inhibition of cell adhesion (Fig. 1). A post-translational modification important in cell adhesion is sulfation. Sulfation can occur either on tyrosine residues or on carbohydrate moieties in glycoproteins. Tyrosine sulfation is catalysed by a family of at least two tyrosylprotein sulfotransferases (TPST-1 and TPST-2)[17,18] and is important in P-selectin-mediated adhesion[19] and chemokine–chemokine receptor (CCR) binding[20]. A role for tyrosine sulfation of CCR5 in viral entry of certain HIV isolates has been established[20], and therefore inhibitors of tyrosine sulfotransferases might be useful as anti-retroviral therapeutics. Sulfation of carbohydrates is widespread in the extracellular matrix and on cell surfaces, and might significantly affect the physicochemical character of proteoglycans and mucins, particularly by imparting a net negative charge at physiological

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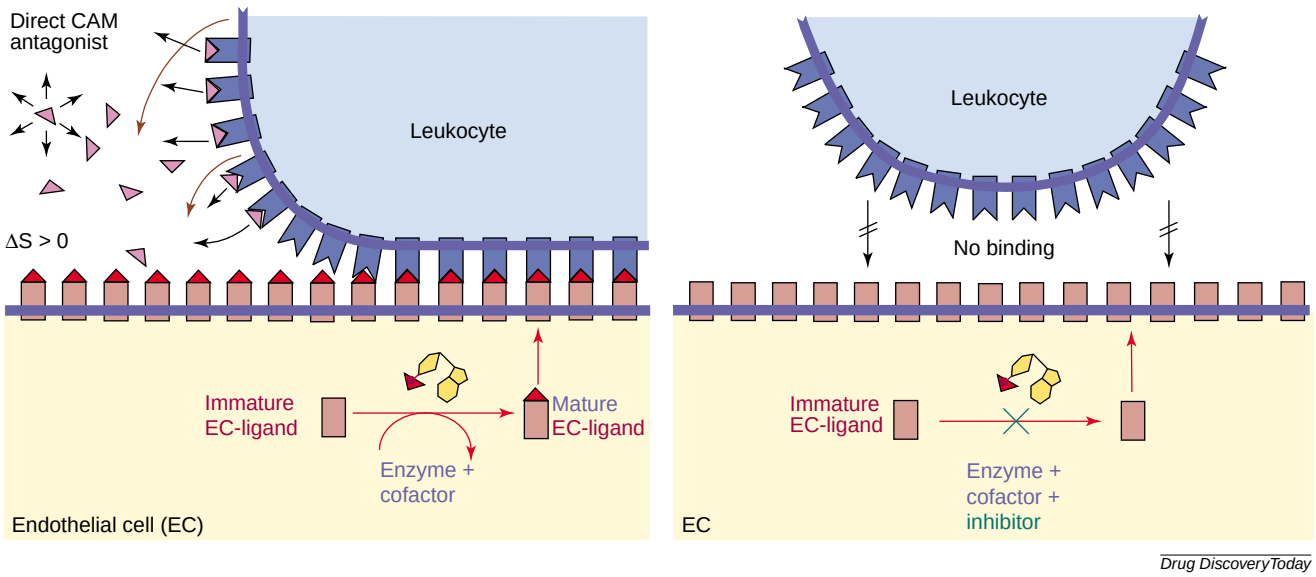

Figure 1. Entropic penalty inherent to inhibition of cell adhesion by direct cell adhesion molecule (CAM)-antagonists. As illustrated for leukocyte endothelial adhesion **(a)**, the cell adhesion receptor (dark blue) and ligand (pale red) are embedded in the lipid bilayer membranes and are therefore restricted to two degrees of freedom. Thus, the entropic cost (ΔS) of a monovalent adhesion receptor–ligand interaction is essentially the same as that of a multivalent CAM–ligand interaction. Small-molecule antagonists, however, must inhibit each CAM individually, and therefore each molecule pays the entropic price corresponding to a loss of one degree of freedom (restriction of movement from three dimensions to two dimensions). Consequently, entropy is gained when small-molecule inhibitors are displaced from membrane-bound CAMs by cognate counter-receptors in a second membrane or other kind of matrix. Furthermore, productive binding of one CAM receptor ligand-pair brings the neighbouring CAMs into close proximity with their cognate adhesion receptors, thus greatly increasing the probability of a second productive bond. Many adhesion receptor–ligand pairs exhibit relatively low intrinsic affinity at the level of monomeric binding (K_d for L-selectin ~100 μM, Ref. 75). Thus, a major component of the free energy driving cell adhesion might reside in cooperative binding as well as gain of entropy, putting direct small-molecule antagonists at a disadvantage. Inhibition of an appropriate enzyme within the pertinent biosynthetic pathway results in expression of non-adhesive ligand and therefore blocks adhesion with reduced negative entropy balance **(b)**. In this case, the loss of freedom suffered by the enzyme inhibitor is compensated by the gain of freedom enjoyed by the displaced substrate (PAPS). In addition, because many CAMs can be processed by a small number of enzyme molecules, effective blocking of adhesion would require inhibition of fewer enzyme molecules than CAM molecules in order to achieve the same effect.
Abbreviation: ΔS, entropy change (ΔS>0 = gain of entropy; ΔS<0 = loss of entropy).

pH. In addition, defined regioselectively sulfated carbohydrate epitopes comprise recognition determinants for many receptors[21] and are of crucial importance in processes as diverse as L-selectin-mediated adhesion[22], blood clotting[23] and herpes simplex virus entry[24]. These sulfation reactions are catalysed by a class of enzymes known as carbohydrate sulfotransferases. This review will focus on carbohydrate sulfotransferases as potential drug targets, with particular emphasis on a novel family implicated in lymphocyte migration and inflammation.

Carbohydrate sulfotransferases

The carbohydrate sulfotransferases identified to date comprise two major families and there are also several additional enzymes that cannot be assigned to particular subgroups[25]. The two major families are the heparin sulfotransferases[26] and the galactose (Gal)/*N*-acetylgalactosamine (GalNAc)/*N*-acetylglucosamine (GlcNAc) 6-*O*-sulfotransferases (GSTs)[27]. Most carbohydrate sulfotransferases are transmembrane glycoproteins with type II topology that reside in Golgi vesicles. In general, these enzymes exhibit a relatively short N-terminal cytoplasmic tail that might play a role in localization and/or retention of the polypeptide in the Golgi membrane[28]. The cytoplasmic tail is followed by a single transmembrane domain and a large C-terminal luminal domain, which imparts catalytic activity (Fig. 2). The catalytic domains can be expressed in recombinant soluble form for use in homogenous-phase enzyme-activity assays and crystallographic studies[29–31].

Enzymatic catalysis

Like the well-established cytosolic sulfotransferases that are involved in detoxification and steroid and neurotransmitter metabolism[32], the carbohydrate sulfotransferases

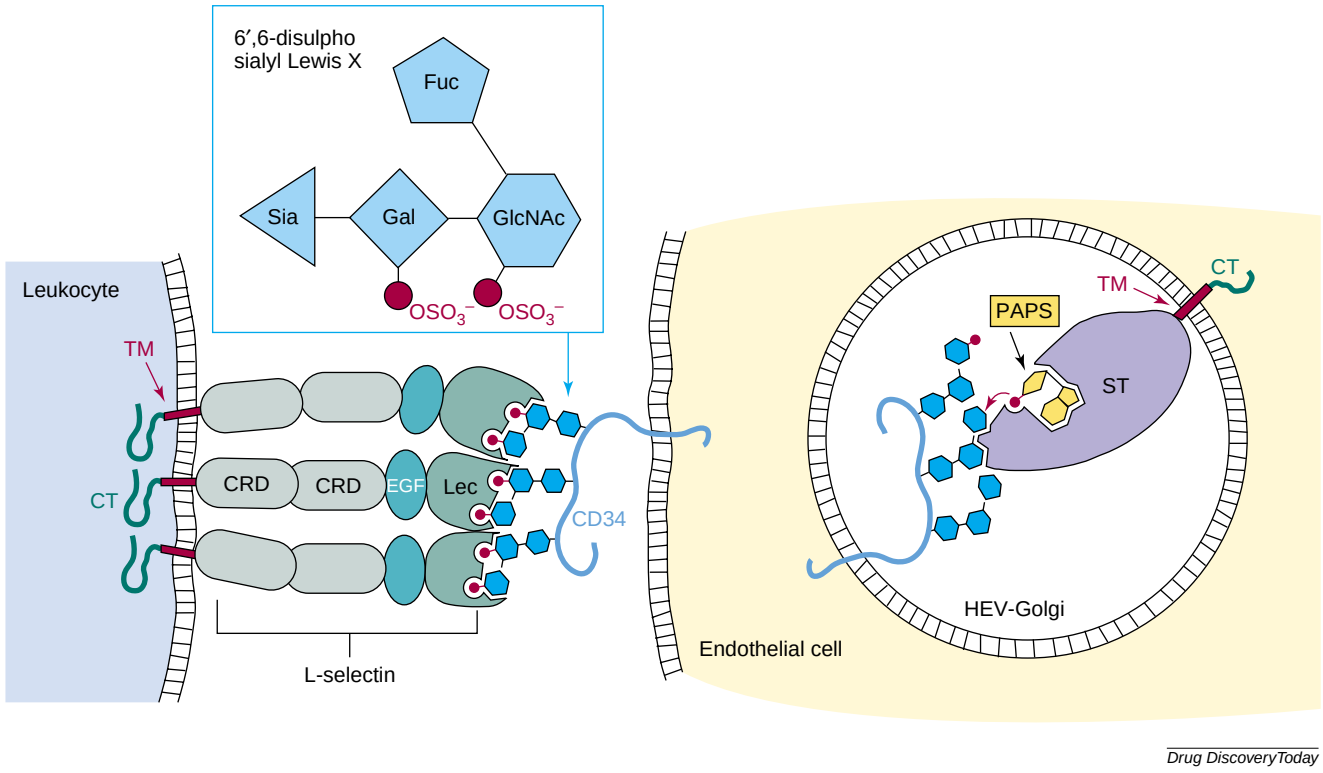

Figure 2. Modulation of L-selectin-mediated cell adhesion by inhibition of L-selectin ligand sulfotransferase (GST-3). This sulfotransferase (ST), as most members of its class, is embedded in the membrane of the Golgi compartment by virtue of its transmembrane domain (TM). It possesses a short N-terminal cytoplasmic tail (CT), and a large luminal domain that contains the catalytic activity. The enzyme transfers sulfate from phosphoadenosine 5′-phosphosulfate (PAPS) onto *N*-acetylglucosamine (GlcNAc), which is presented to the enzyme by a nascent endothelial mucin such as CD34. This sulfation, in the context of the mature sialyl Lewis X tetrasaccharide capping structure (sLe^x), is essential for productive adhesion and leukocyte extravasation. Another sulfation at the galactose within sLe^x contributes to the presumed high-affinity recognition determinant 6′,6-sulfo sialyl Lewis X, depicted in the boxed inset. Specific inhibition of GST-3 is thus expected to impair L-selectin-mediated leukocyte migration. L-selectin on the leukocyte is depicted as domain structure[76] comprised of its N-terminal ligand-binding lectin domain (LEC) followed by an epidermal growth factor-like domain (EGF), two complement consensus repeats (CRD), a TM and a CT. Abbreviations: Sia, sialic acid; Fuc, fucose; Gal, galactose.

invariably utilize 3′-phosphoadenosine 5′-phosphosulfate (PAPS) as an activated-sulfate donor. In mammalian cells, PAPS is synthesised from ATP and inorganic sulfate by PAPS synthetase[33], which is competitively inhibited by chlorate[34]. The sulfotransferase then transfers the 5′-sulfate from PAPS onto a hydroxyl (or less commonly an amino group) contained in a carbohydrate moiety of the acceptor glycoprotein or glycolipid (Fig. 3). The mechanism of sulfonyl transfer is unknown at present; however, a recent study[35] has suggested an in-line sulfonyl-transfer mechanism similar to the mechanism of phosphoryl transfer ascribed to many kinases[36]. Because sulfotransferases functionally and mechanistically resemble kinases, lessons from the discovery of kinase inhibitors can be applied to this class of enzymes (Fig. 3). In cell-free systems, carbohydrate sulfotransferases exhibit K_m values for PAPS within the range of 1–10 μM (Ref. 37; S. Bhakta, I. Polsky and S. Hemmerich, unpublished).

Heparin sulfotransferase

Heparan sulfate is a glucosaminoglycan (GAG) that is linked by xylose to serine residues of a core protein such as perlecan, syndecan or glypican[38]. Heparan sulfate is synthesised initially as extended repeats of a disaccharide unit containing glucuronic acid in β(1–4) linkage to *N*-acetylglucosamine ([GlcAβ(1–4)GlcNAcα(1–4)]*n*). These GAG chains undergo a series of ordered modification reactions, catalysed by at least four sulfotransferases of the heparin sulfotransferase family and one epimerase. The ensuing sulfations largely determine the biophysical properties of the mature proteoglycan and sometimes contribute to specific recognition epitopes recognized by endogenous and viral receptors. Thus, heparin, a particularly complex GAG synthesised exclusively by mast cells, contains a defined hexasaccharide epitope, which binds to antithrombin and imparts a conformational change that reveals its high-affinity binding site for thrombin, leading to inhibition of

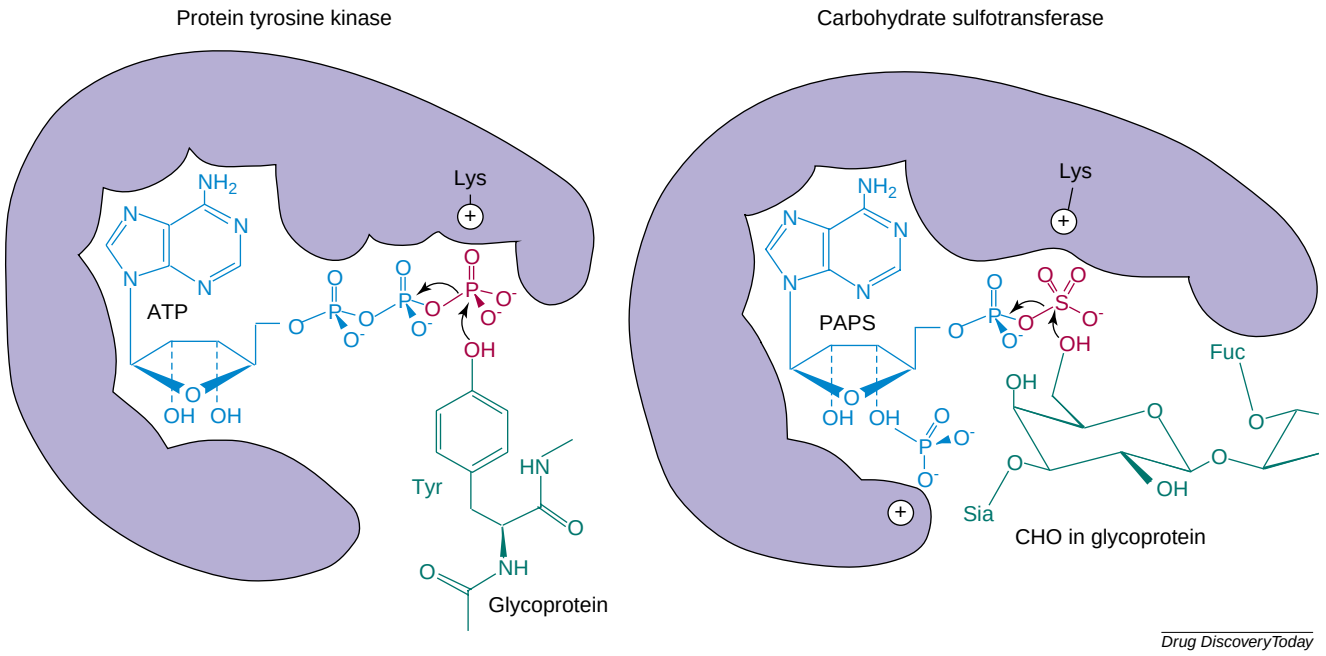

Figure 3. Carbohydrate sulfotransferases are analogous to kinases. Both classes of enzymes use very similar nucleotide donors of activated sulfate or phosphate, employ similar enzymatic mechanisms and feature similar structural elements in their active sites. Lessons from kinase inhibitor discovery can be applied to this new class of targets. Lys denotes a critical lysine residue found in the active sites of both types of enzymes. Abbreviations: PAPS, 3'-phosphoadenosine 5'-phosphosulfate; CHO, carbohydrate; Fuc, fucose; Sia, sialic acid.

this enzyme and therefore stopping the blood-clotting process[39]. The affinity of this antithrombin binding site in heparin depends crucially on 3-*O*-sulfated *N*-sulfoglucosamine within the hexasaccharide epitope[38]. The 3-*O*-sulfation has been shown to be the final and rate-limiting step in biosynthesis of the antithrombin-binding epitope of heparin and is catalysed by a particular member of the heparin sulfotransferase family termed 3-OST-1 (Refs 23,40). This enzyme is a somewhat peculiar carbohydrate sulfotransferase in that it lacks an obvious transmembrane domain but is nevertheless retained in the Golgi compartment. Growth-factor binding to heparan sulfate proteoglycan is also dependent on particular sulfated epitopes within the GAGs[41]. Furthermore, several viruses use specific sites on heparan sulfate proteoglycans for binding to cells and/or viral entry. Shukla and colleagues[24] have recently shown that 3-*O*-sulfation of specific *N*-acetylglucosamine residues in heparan sulfate chains, catalysed exclusively by another heparin sulfotransferase, 3-OST-3, is required for heparan-sulfate-dependent herpes simplex virus type 1 entry.

GSTs

The GSTs are a recently discovered class of carbohydrate sulfotransferases, which all catalyse sulfation at the 6-hydroxyl group of Gal, GalNAc or N-GlcNAc (Ref. 27). To date, seven members of this family have been described in human and six in mouse[42]. Although most of these enzymes are broadly expressed and have been shown to function in sulfation of non-heparan sulfate proteoglycans, two members of the GST family, GST-3 and GST-4, have been implicated in lymphocyte migration and inflammation, both by virtue of their restricted expression pattern and the results of reconstitution experiments described later.

Sulfation in lymphocyte migration and inflammation

Lymphocytes enter peripheral lymphoid organs, such as lymph nodes, via the blood, by interaction with a specialized post-capillary venule known as a high endothelial venule (HEV)[43]. Extravasation of lymphocytes through HEVs occurs as a cascade of events, the first of which involves tethering and rolling of the lymphocyte (Fig. 4) along the HEV[44]. This is followed by chemokine-mediated activation of β_2-integrins on the lymphocytes, leading to their arrest and subsequent transmigration into the lymph node parenchyma. L-selectin, the peripheral lymph-node homing receptor, is a lectin-like cell adhesion molecule and has been shown to mediate tethering and rolling of lymphocytes along HEVs. L-selectin interacts with HEV-associated ligands collectively known as the peripheral node vascular addressin (PNAd). PNAd was initially identified using the monoclonal antibody MECA-79 (Ref. 45).

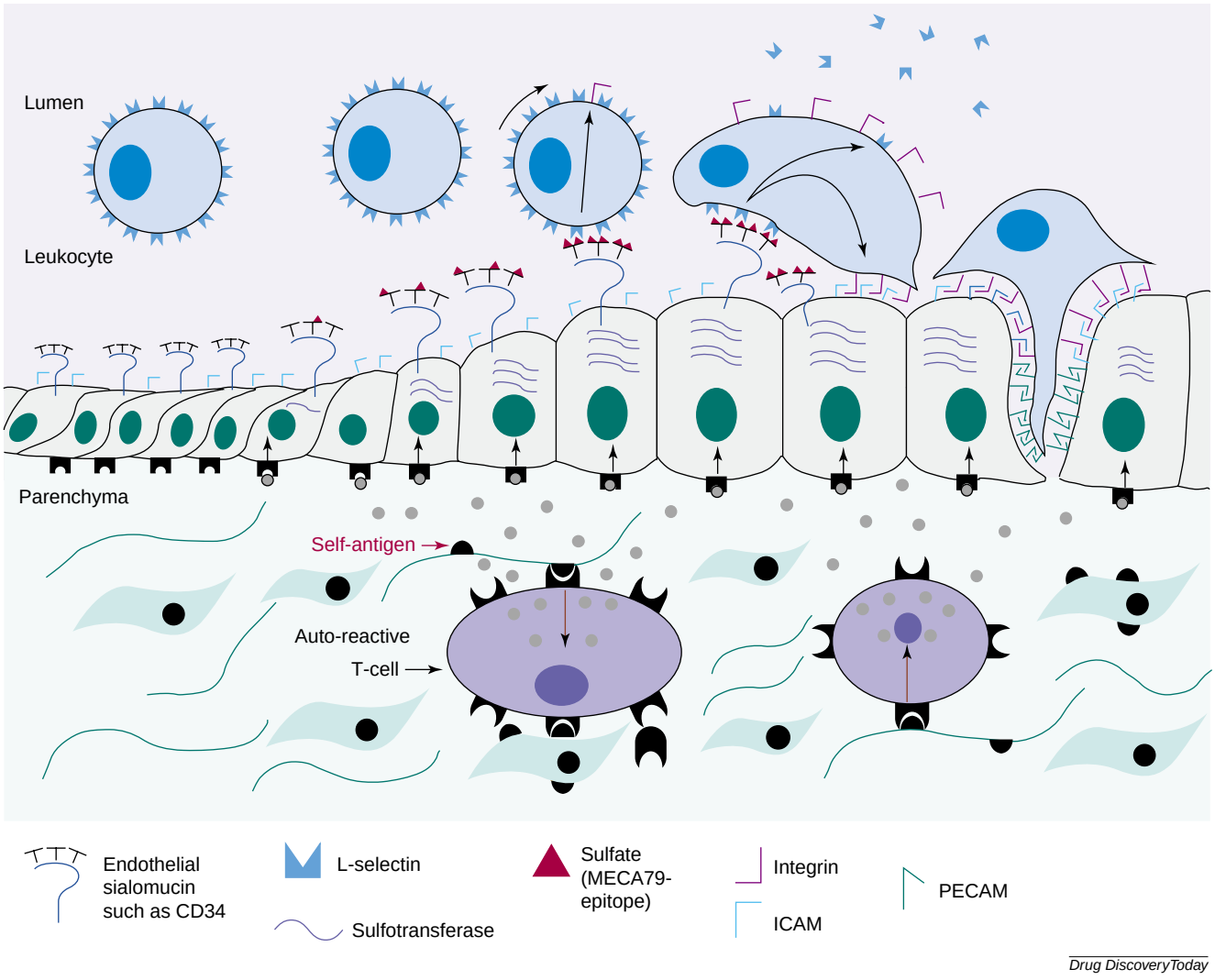

Figure 4. Pharmacological rationale for inhibition of L-selectin ligand sulfotransferase in chronic inflammation. At the onset of organ-specific autoimmune disease, small clonal populations of autoreactive T cells in the tissue site, somehow, perhaps through the action of soluble factors, induce in adjacent flat venular endothelium a phenotypical change to high endothelial venules (HEV). In the course of this change, an HEV-specific sulfotransferase (GST-3 or a similar enzyme) is induced, which in turn sulfates O-linked carbohydrate on endothelial sialomucins such as CD34. This modification renders these otherwise non-adhesive cell surface molecules adhesive for L-selectin, which is expressed constitutively on blood-borne lymphocytes. These naïve lymphocytes are then recruited from the bloodstream to roll along the endothelium. During this process, chemokine-mediated activation of lymphocyte integrins leads to arrest of the lymphocyte followed by transendothelial migration, which is also mediated by integrins and their counter-receptors, such as intercellular adhesion molecule-1 (ICAM-1), as well as homotypic adhesion molecules such as platelet endothelial cell adhesion molecule-1 (PECAM-1). Following arrival in the parenchymal tissue, these non-clonal naïve lymphocytes can effect tissue destruction through secretion of lytic enzymes and inflammatory mediators. This overall process greatly amplifies the initially limited autoimmune response of the self-reactive T cells. Inhibition of the HEV sulfotransferase could block this amplification mechanism and thus limit disease progression.

This antibody binds to HEVs and blocks the adhesion of lymphocytes to HEVs in peripheral nodes, but not in Peyer's patches. MECA-79 antigens are also induced in a number of human inflammatory diseases and experimental inflammation models. The former include rheumatoid arthritis and various types of skin inflammation[46], cardiac allograft rejection[47] and bronchial asthma[48]. Examples of the latter include a transgenic mouse model of pancreatic inflammation[49] and thymic hyperplasia in the AKR/J mouse, a model of aberrant lymphocyte migration[50]. The occurrence of the MECA-79 epitope is currently accepted as a predictor for L-selectin ligand activity. Studies have shown that the MECA-79 epitope represents a carbohydrate-based post-translational modification that is presented on HEVs by several endothelial glycoproteins[51,52]. In addition, the MECA-79 epitope is sulfated, and sulfation

is crucial for ligand recognition by either L-selectin or the antibody[53]. In an extensive biochemical analysis of a mouse peripheral node HEV ligand, GlyCAM-1 (Ref. 54), we found that the major capping groups presented by this ligand are 6-sulfo Lewis X and 6′-sulfo Lewis X, that is, the sialyl Lewis X tetrasaccharide [Siaα2-3Galβ1-4(Fucα1-3)GlcNAc] bearing a sulfate moiety at the 6-hydroxyl group of either Gal or GlcNAc[55,56] (Fig. 2). The 6-sulfo-Lewis X determinant has also been found on human HEVs[57].

Carbohydrate sulfotransferases: functional in L-selectin ligand biosynthesis

The discovery of the 6-sulfo-Lewis X determinant prompted a search for the enzymes that catalyse the respective sulfations in biosynthesis of this epitope. Based on the regiochemistry of the sulfations in these moieties, the enzymes of the GST family were obvious candidates. Of the seven human enzymes, only GST-3, also called high endothelial cell GlcNAc 6-*O*-sulfotransferase (HEC-GlcNAc6ST) or L-selectin ligand sulfotransferase (LSST), is expressed specifically at high levels in HEVs[58]. The same enzyme is induced in HEV-like vessels in thymic hyperplasia in the AKR/J mouse[59]. Reconstitution experiments demonstrated that GST-3 was able to generate 6-sulfo sialyl Lewis X and functional L-selectin ligands in transfected cells[58,59]. The identity of the sulfotransferase responsible for sulfation of galactose in biosynthesis of the 6′-sulfo sialyl Lewis X epitope is unclear as yet. However, the widely expressed enzyme GST-1, also called keratan sulfate Gal 6-*O*-sulfotransferase (KSGal6ST), has been shown *in vitro* to generate L-selectin ligand activity and synergize with GST-3 in the generation of high affinity ligands[58]. Another pair of highly related GlcNAc 6-*O*-sulfotransferases, GST-4α and GST-4β, are expressed predominantly in intestinal tissues[60] and their genes are clustered with the GST-3 gene on human chromosome 16q23.1–23.2 (Ref. 42). Therefore, GST-4α and GST-4β might be involved in biosynthesis of L-selectin ligands in gut-associated lymphoid tissues.

GST-3 as a target for anti-inflammatory drug discovery

Following their discovery in the late 1980s, the three selectins, E-, P-, and L-selectin, became prime targets for anti-inflammatory drug discovery[61]. L-selectin in particular was an attractive target because its inhibition could be beneficial on two levels:

- as shown in the L-selectin-deficient mouse, compromised L-selectin function manifests itself as blunted responses in short-term inflammation models such as thioglycollate peritonitis, delayed type hypersensitivity or septic shock[62], while leaving humoral and cellular immune responses mostly intact[63]; and
- inhibition of L-selectin could effectively control the recruitment of naïve lymphocytes to inflamed lesions in settings of chronic inflammatory autoimmune diseases (Fig. 4).

So far, potent competitive inhibitors of selectin function have remained elusive. The reasons underlying the poor chemical tractability of the selectins are probably a combination of the factors previously mentioned, such as extended and flat ligand-receptor interfaces[11], avidity due to receptor oligomerization and clustering[12,13], and entropic penalties associated with inhibition of cell adhesion by small molecules (Fig. 1). The only small-molecule selectin antagonist currently in clinical trial (TBC1269, shown in Fig. 5a, Texas Biotechnology, Houston, TX, USA), although derived from a glycomimetic approach modeling the sialyl Lewis X tetrasaccharide, apparently does not act as a competitive inhibitor because it does not affect selectin-mediated leukocyte rolling *in vivo*[64]. As mentioned previously, the carbohydrate sulfotransferase GST-3 might be involved in the biosynthesis of L-selectin ligands in peripheral lymph node HEVs. This finding provides a novel approach for modulation of L-selectin-mediated cell adhesion (Fig. 2). Inhibition of GST-3 in the Golgi compartment is expected to compromise the crucial sulfation modifications that are imparted by the enzyme on the nascent L-selectin ligands during the process of its vesicular transport to the cell surface. As a result, the under-sulfated ligand, upon arrival at the plasma membrane, will be unable to support adhesion to L-selectin. This approach is expected to circumvent the difficulties faced with direct inhibition of L-selectin itself.

Chemical tractability of carbohydrate sulfotransferase

As illustrated in Fig. 3, sulfotransferases bear analogies to protein kinases. Thus, the sulfate donor PAPS is chemically very similar to ATP, and both classes of enzyme employ similar mechanisms[35,65]. Most small-molecule protein-kinase inhibitors competitively bind to ATP-binding sites and high degrees of selectivity can be achieved for small-molecule inhibitors despite considerable sequence and structural similarities among related kinases[66]. By analogy, carbohydrate sulfotransferases should be subject to inhibition by small molecules that competitively bind to the PAPS-binding sites in these enzymes. The degree of selectivity for a particular carbohydrate sulfotransferase, such as GST-3, that can be obtained with small-molecule inhibitors targeted to the PAPS-binding site, remains to be determined. However, given the possibility of functional redundancy between related isozymes (such as GST-2 and GST-3), inhibitors with overlapping specificities could be advantageous.

Theoretically, inhibitors could also be directed at the substrate-binding sites in the sulfotransferases of interest. However, because these substrate-binding sites are tailored for interaction with carbohydrates, involving multiple polar bonds and often water bridges, small molecules that effectively inhibit these interactions are deemed less feasible. Yet, a drug-like moiety exhibiting low affinity to the substrate-binding domain, linked appropriately to a moiety with high affinity to the PAPS-binding site could synergize to result in a potent and more specific inhibitor of the targeted carbohydrate sulfotransferase.

Some of the cytosolic sulfotransferases that bear little, if any, sequence homology to the Golgi sulfotransferases, are effectively inhibited by polyphenols such as quercitin[67,68], in addition to commonly used drugs such as cyclizine and ibuprofen[69]. Despite their apparent lack of sequence homology, crystallographic studies have revealed high structural similarity between the PAPS-binding sites in estrogen sulfotransferase, a prototypic cytosolic sulfotransferase, and the heparin deacetylase *N*-sulfotransferase-1 (NST-1; Ref. 31), one of the first carbohydrate sulfotransferases to be discovered. Bertozzi and colleagues have screened a panel of kinase inhibitors against the rhizobial GlcNAc 6-*O*-sulfotransferase (NodH) and identified three purine structures with IC_{50} values of between 20 and 40 μM[70] (Fig. 5b).

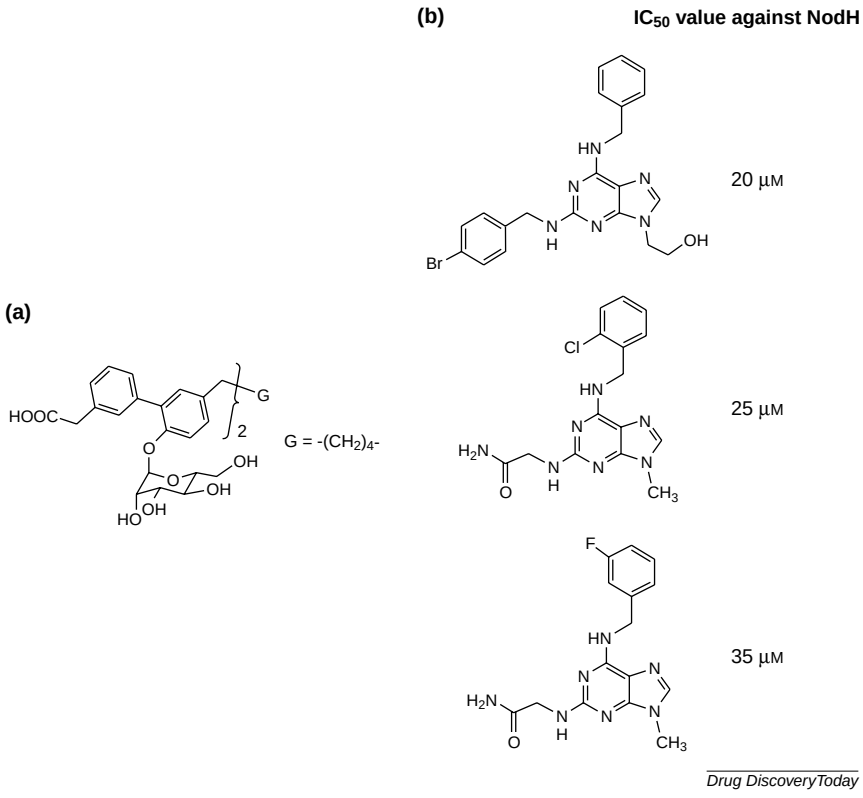

Figure 5. **(a)** Structure of the pan-selectin inhibitor TBC1269[77]. **(b)** Structure of three purines identified in a screen for inhibitors of the rhizobial *N*-acetylglucosamine 6-*O*-sulfotransferase NodH[70].

Because the carbohydrate sulfotransferases are Golgi-resident enzymes, an inhibitor must be able to traverse at least two membranes, the plasma membrane and the highly organized membrane of the Golgi vesicle, to be useful as a drug. This imposes certain restrictions on the biophysical properties of the drug candidate such as the need for a high degree of lipophilicity and lack of charge. Charged molecules could be delivered to the target using pro-drug approaches. In addition, because of the highly permeable nature of putative sulfotransferase inhibitor drugs, most proteins residing in the cytosol, and possibly the cell nucleus, will be exposed to such a compound. This carries a much higher risk of toxicity compared with a direct inhibitor of cell adhesion that targets an extracellular receptor and can be designed to be plasma-membrane impermeable. Thus, the conceptual advantage of sulfotransferase inhibition over direct cell adhesion as discussed previously (Fig. 1) is to some degree balanced by the disadvantage inherent in the subcellular localization of the enzyme target versus the adhesion receptor. The prevailing approach will only be determined in future studies.

Activity assays for carbohydrate sulfotransferases

Carbohydrate sulfotransferases can be readily expressed in soluble form in mammalian or insect cells[29,30,71]. A number of different homogenous-phase carbohydrate sulfotransferase assays have been reported. Appropriate simple benzyl glycosides in addition to neoglycolipids can serve as acceptor molecules in sulfotransferase-catalysed transfer of ^{35}S from ^{35}S-labelled PAPS[30,71,72]. Several carbohydrate sulfotransferases can directly sulfate GAGs, which are subsequently digested by appropriate hydrolases and the resultant sulfated oligosaccharides can be resolved by HPLC[29]. Furthermore, Wong and colleagues have recently reported a regenerative assay, in which the sulfotransferase reaction is coupled to synthesis of PAPS from 3′-phosphoadenosine-5′-phosphate (PAP), via reverse catalysis by recombinant

rat aryl sulfotransferase IV, using *p*-nitrophenylsulfate as a sulfate donor and indicator of enzyme turnover[73]. Carbohydrate sulfotransferase activity in intact cells is commonly estimated by measuring the product of the targeted sulfotransferase, that is, the appropriately sulfated glycoprotein substrate, after it has been transported to the plasma membrane or secreted into the extracellular medium. As already discussed, many compounds that can inhibit carbohydrate sulfotransferases in cell-free assays are expected to lack cell-based activity because access to the Golgi vesicles is restricted to either sufficiently permeant molecules or molecules that can utilize one of the active transport systems that connect the cytosol and the Golgi lumen.

Crystallography

The sulfotransferase domain of NST-1 has recently been expressed in *Escherichia coli* and crystallized as a binary complex with PAPS[31]. The three-dimensional structure of its PAPS-binding site exhibits striking structural similarity to the equivalent site in the cytosolic estrogen sulfotransferase. Both enzymes contain a lysine that interacts with the 5′-phosphosulfate moiety of PAPS and is necessary for enzymatic activity, in analogy to the lysine that is required in the ATP-binding site of most kinases (Fig. 3). The GSTs have so far eluded crystallization because of the low quantities of active recombinant enzyme yielded by various expression systems. Shinkai *et al.*[74] have recently reported a system for high-level expression of recombinant soluble fucosyltransferase VII, another Golgi-associated enzyme with type II topology. This methodology could be adaptable to yield high-level expression of the GSTs.

Conclusions

Carbohydrate sulfotransferases are emerging as a novel class of enzymes crucially involved in biological pathways linked to cell–cell, cell–matrix, or cell–virus adhesion. These enzymes show many similarities to the kinases and pose similar opportunities for small-molecule inhibitor discovery. As enzymes, these targets should be chemically more tractable than the adhesion molecules that function in the targeted pathway. However, their subcellular localization to the Golgi lumen presents two major barriers for access by small molecules: the plasma membrane and the highly organized Golgi vesicle membrane. Primary and cell-based assays are readily available; these targets could yield novel therapeutic agents for inflammation, viral infection and tumour metastasis.

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