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Review

Selectin glycoprotein ligands★

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Lectin selectins and their counter-receptors participate in discontinuous cell–cell interactions concurrent with leukocyte tethering and rolling on endothelium, which, in consequence, leads to leukocyte penetration to lymphatic organs and generation of inflammation sites. Counter-receptors are glycoproteins in which carbohydrate units, the direct selectin ligands, are built into the polypeptide framework. In this review, the distribution, structure and function of the main ligands and counter-receptors for P-, L- and E-selectins known so far, have been discussed. The common biosynthetic pathway of sialyl-Lewis x and sulpho-sialyl-Lewis x determinants of selectin ligands has been described.

Adhesion receptors mediate the cell–cell interactions, cell adhesion to extracellular matrix and cell migration over this matrix. Cell–cell adhesion is either "continuous" (in coherent tissues) or "discontinuous". The latter takes place in physiological lymphocyte recirculation between the blood vascular system and lymph nodes. It is also one of the initial processes during leukocyte recruitment into inflammation sites [1]. Lymphocytes leaving blood enter lymph nodes due to adhesion to high endothelial venules (HEV), which allows their extravasation. Lymphocytes are directed from lymph nodes back to blood *via*

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Abbreviations: ESL-1, E-selectin ligand 1; GlyCAM, glycosylation-dependent cell adhesion molecule; HEV, high endothelial venules; Le(a), Lewis(a) determinant; Le^x, Lewis(x) determinant; mAb, monoclonal antibody; MAdCAM, mucosal addressin cell adhesion molecule; PMN, polymorphonuclear leukocytes; PSGL-1, P-selectin glycoprotein ligand 1; sLe(a), sialyl-Lewis(a) determinant; sLe^x, sialyl-Lewis(x) determinant.

lymphatic vessels and thoracic duct. Apart from lymph nodes HEV are found in chronic inflammation sites, participating in lymphocyte extravasation. Selectins and their counter-receptors, present on the surface of leukocytes and endothelial cells, mediate adhesion.

SELECTINS

Selectins, one of the first adhesion receptors, mediate early transient cell–cell interactions, which occur during tethering and leukocyte rolling along HEV in lymph nodes and most of inflamated tissues. The selectin-mediated leukocyte attachment may stimulate expression of active integrins on the leukocyte surface, which in turn mediate interactions with the endothelial adhesins and contribute to strengthening of adhesion which becomes sufficient for leukocytes to overcome the endothelial barrier, called diapedesis.

The selectin family consists of three adhesion molecules: P(platelet)-selectin (CD62P), L(leukocyte)-selectin (CD62L) and E(endothelium)-selectin (CD62E). All selectins are glycoproteins which contain 30% or more of carbohydrates. Initially selectins were identified on lymphocytes as adhesion receptors for counter-receptors (glycoproteins or glycolipids) expressed on HEV of peripheral lymph nodes [2]. However later it has been demonstrated that the lymphocyte selectin is present also on neutrophils, monocytes and eosinophils. They mediate leukocyte rolling in post capillary venules of inflamed tissues stimulating accumulation of leukocytes in inflammation sites [3, 4]. The selectin mediated lymphocyte interactions with HEV are tissue specific. Lymphocyte subsets are able to recognize HEV in peripheral lymph nodes, mucosal lymphoid tissues, inflamed joint synovial fluid or skin with chronic inflammation [5]. However, the interactions are not species-specific, as lymphocyte selectins of one species may recognize lymph node HEV ligands of another animal species [6–9]. The endothelial selectins can recognize and bind counter-receptors on the leukocyte surface.

The selectins have a similar domain structure that includes an N-terminal Ca^{2+}-dependent lectin domain (CRD, carbohydrate recognition domain), a single epidermal growth factor-like (EGF-like) domain and variable numbers of short consensus repeats homologous to the domains found in complement binding proteins: two in L-selectin, six in E-selectin and nine in P-selectin [10, 11].

L-Selectin is constitutively expressed on the surface of all classes of leukocytes, where it is localized in microvilli. Leukocyte activation by inflammatory cytokines causes shedding of L-selectins from the cell surface by proteolysis [12]. P-Selectin is stored in granules of resting platelets or Weibel-Palade bodies of resting endothelial cells [13]. On stimulation of cells by different agonists, like thrombin or histamine, P-selectins are translocated onto the membrane surface within 10 min after stimulation [14]. E-Selectin is a protein transcriptively induced on cell surface of endothelial cells by inflammatory cytokines (e.g., IL-1, TNFβ) or lipopolysaccharide.

Since changes in the adhesive receptors on the endothelium surface were found to be related to atheromatosis determination of soluble E-selectin, a specific marker for endothelial activation or damage, can have diagnostical or prophylactical significances including children at high risk for early atherosclerosis (at least one parent after myocardial infarction). An increased level of soluble E-selectin in serum was detected only in the children who had mutation in the epidermal growth factor domain of the E-selectin gene (Żak *et al.*, unpublished data).

GENERAL LIGAND CHARACTERISTICS

Carbohydrates involved in cell–cell interactions mediated by selectins are directly bound by the lectin domain of those receptors. In this system, in contrast to a typical receptor–

ligand pair connection there exists a functional triad network [15]. This triad is composed of a receptor, a ligand and a carrier. A ligand carbohydrate together with a carrier protein forms a counter-receptor. A proper orientation of oligosaccharide chains and generation of "clustered saccharide patches" depends upon the protein component [6]. Moreover, the protein contains information for posttranslational modifications of counter-receptor, including proper glycosylation and sulphation of oligosaccharide chains or tyrosine residues [16]. Oligosaccharides of proper structure assembled spatially on the protein ligand and selectin is the same, and different selectins take part at different stages of the inflammatory reaction. Selective removal or ligand blockade by monoclonal antibodies (mAb) on cell surface makes impossible interaction with selectin. This might serve as an additional criterion of a true selectin-mediated interaction. Besides, a ligand should be selectively recognized by a specific selectin with relatively high affinity [18].

Studies on monoclonal antibodies to selectin ligands are frequently used for determination of various carbohydrate epitopes and definition of their function. Table 1 presents mAbs

Table 1. Specificity of antibodies against selectin ligands

Antibody	Antigenic carbohydrate structure	Structural specificity	References
HECA-452	CLA	NeuAcα2,3Galβ1,4(Fucα1,3)GlcNAc-R + NeuAcα2,3Galβ1,4(Fucα1,4)GlcNAc-R	[19–21]
	6-sulpho sLe^x	NeuAcα2,3Galβ1,4(Fucα1,3)(SO_4-6)GlcNAc-R	[22]
2F3	SLe^x	NeuAcα2,3Galβ1,4(Fucα1,3)GlcNAcβ1,3Galβ1-R	[23
	6-sulpho sLe^x	NeuAcα2,3Galβ1,4(Fucα1,3)(SO_4-6)GlcNAc-R	[22]
2H5	SLe^x	NeuAcα2,3Galβ1,4(Fucα1,3)GlcNAcβ1,3Galβ1-R	[24]
	6-sulpho sLe^x	NeuAcα2,3Galβ1,4(Fucα1,3)(SO_4-6)GlcNAc-R	[22]
CSLEX 1	SLe^x	NeuAcα2,3Galβ1,4(Fucα1,3)GlcNAcβ1,3Galβ1-R	[25]
FH6	SLe^x or s-diLe^x	NeuAcα2,3Galβ1,4(Fucα1,3)GlcNAcβ1,3Galβ1,4(Fucα1,3)GlcNAcβ1,3Galβ1-R	[26, 27]
AM-3	SLe^x	NeuAcα2,3Galβ1,4(Fucα1,3)GlcNAcβ1,3Galβ1-R	[28]
SNH3, SNH4	S-diLe^x	NeuAcα2,3Galβ1,4(Fucα1,3)GlcNAcβ1,3Galβ1,4(+/-Fucα1,3)GlcNAcβ1,3Galβ1-R	[29, 30]
	The related structures		
VIM2	CDw65	NeuAcα2,3Galβ1,4GlcNAcβ1,3Galβ1,4(Fucα1,3)GlcNAcβ1,3Galβ1-R	[31, 32]
N19-9	sLe(a) type 1	NeuAcα2,3Galβ1,3(Fucα1,4)GlcNAcβ1,3Galβ1-R	[33]
	Glycoproteins	Antigenic glycoproteins	
MECA-79	PNAd	Peripheral node addressin. The isolated complex of MECA-79 reactive proteins	[34, 35]
MECA-367	MAd	Mucosal vascular addressin (in Peyer`s patches, appendix)	[36]
QBEnd 10	CD34	Peripheral node addressin $CD34^+$	[37]
PL1, PL2	PGSL-1	Protein - dependent epitopes in PSGL-1	[38]

carrier work as a functional selectin counter-receptor. Moreover, the polypeptide chain of counter-receptor may contribute to additional protein–protein interactions.

Ligands interacting with selectin under specified conditions *in vitro*, must fulfill additional criteria to be active under physiological conditions *in vivo* [6]. Circulating blood cells expressing the potential ligand must be present in physiological conditions in the same place and at the same time when selectin is present on the endothelium [6, 16, 17]. Not in every tissue and every species expression of a recognizing the most common selectin ligand sialyl-Lewis(x) (sLe^x) and related antigen determinants. Some of the antibodies were elicited by immunization of mice with tumor cell-derived glycolipids containing sLe^x determinant [25–29]. While some react also with the respective protein-linked carbohydrate antigens present on HEV or on leukocyte surface [16, 39]. Recently, it has been proved [22] that five monoclonal antibodies: HECA-452, 2F3, 2H5, CSLEX and FH6 (Table 1), which react strongly with the purified sLe^x antigen, recognize also sLe^x on granulocytes and

monocytes, whereas only three of them (HECA-452, 2F3 and 2H5) recognize this antigen on HEV. Additional sulphation of the antigens makes unable their recognition by FH6 and CSLEX1. This has been confirmed by Japanese authors [22] who reported that synthetic 6-sulpho-sLe^x and 6,6′-bis-sulpho-sLe^x determinants are not recognizable by CSLEX1.

The common feature of most of the ligands recognized by all selectins is *N*-acetyllactosamine backbone of the type II (Galβ1,4GlcNAc) rather than type I (Galβ1,3GlcNAc), with sialic acid (NeuAc) residue in α2–3 glycoside linkage and fucose (Fuc) residue in either α1–3 or α1–4 linkage. Selectin-dependent cell adhesion requires both sialic acid and fucose residues in specific position and linkage.

The sLe^x ligand of selectins is common whereas sLe(a) is less frequent [21]. Although, as shown using mAb N19-9 [40], the latter determinants are not found in physiological conditions neither on vascular cells [6] nor on the surface human polymorphonuclear leukocytes (PMN) [40], they can be generated under pathological conditions e.g. in carcinomas [6, 41].

The sLe^x determinant is species-specific. It is expressed only on human PMN and is not expressed on neutrophiles of mammals including rodents and non-human primates [40]. Interestingly, rat selectins recognize and bind sLe^x *in vitro* though this structure is absent from their PMN *in vivo* [40]. *In vitro*, sLe^x tetrasaccharides only imitate natural rat ligands. All selectins recognize sLe^x [42] however, this common sialylated and fucosylated tetrasaccharide (Fig. 1) may be sufficient only in the E-selectin interactions. The other selectins prefer the ligands modified by additional sulphation, e.g. L-selectin binds sulpho-sLe^x [22, 35, 43] (Fig.1) whereas P-selectin, apart from sLe^x, requires at least one additional sulphated tyrosine residue of the carrier polypeptide chain (Fig. 1) [38]. Such ligand modifications increase their binding affinity with appropriate selectins.

Importance of α1-3 fucose residues in the ligand–selectin interactions has been repeatedly proven. Recent studies have pointed to

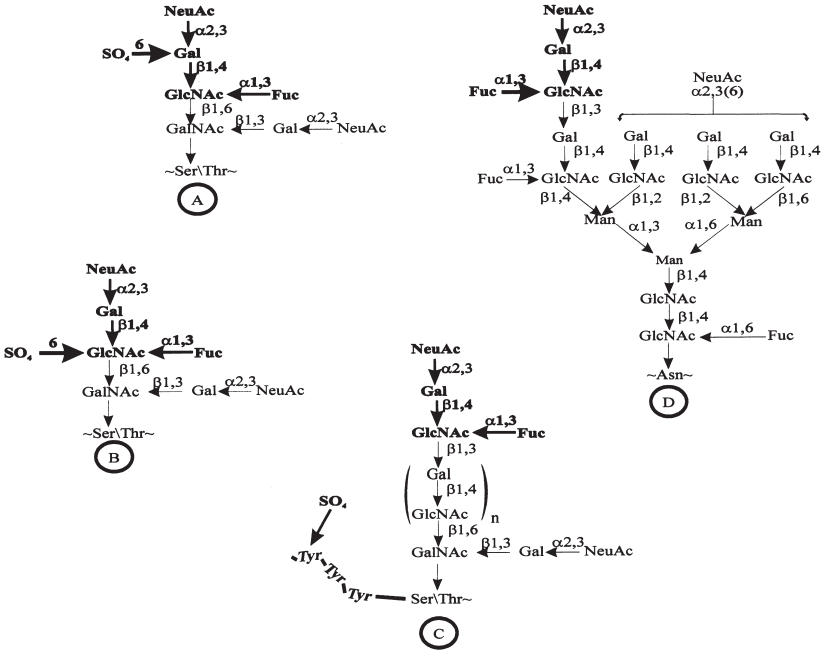

Figure 1. Common selectin ligands.

A, O-glycan, core-2 based 6′-sulpho-sLe^x L-selectin ligand; B, O-glycan, core-2 based 6-sulpho sLe^x L-selectin ligand; C, O-glycan, core-2 based sLe^x and sulphotyrosine of polypeptide P-selectin ligand; D, tetraantennary N-glycan terminated with sLe^x determinant an E-selectin ligand [43, 107]. sLe^x and sulpho-sLe^x determinants are in bold print.

the significance of leukocyte α-1,3-fucosyltransferases (Fuc-TIV, Fuc-TVII) in the construction of selectin ligands. Expression of these enzymes appears to be a key regulatory step in ligand biosynthesis [44]. Contribution of these enzymes to biosynthesis of selectin ligands has been recently confirmed through generation of mice genetically deficient in Fuc-TVII which exhibits profound defects in the ability to construct functional ligands for all three selectins [45–47]. Moreover, in the human congenital disease called leukocyte adhesion deficiency type II (LAD II) [48], a fucosylation defect results in decreased expression of all fucosylated *N*-acetyllactosamines and abolishes lymphocyte–HEV interactions, both *in vivo* and *in vitro*. However, this limitation might be incomplete and some leukocytes of those patients traffick normally probably because of the presence of alternative nonfucosylated ligands [6]. It appears that recognition by all selectins of only mutual oligosaccharides would be biologically impractical. That is probably why selectins can recognize also other carbohydrate ligands lacking fucose residues and completely different from sLe^x, such as glycosaminoglycans which contain many sulphate and hexuronic acid residues [7, 49–53], with binding sites that are identical with the Ca^{2+}-dependent sLe^x binding sites on selectins.

Various monovalent carbohydrate ligands are characterized by low selectin binding affinity, and their role in supporting leukocyte rolling is not clear [54] and high binding affinity of multiple carbohydrate ligands results from the presence of polypeptide carrier [6, 17, 54, 55].

Mechanisms underlying the phenomenon of conversion of multiple monovalent oligosaccharides with low binding affinity into selectin ligands showing high affinity binding, have been discussed by Varki [6]. According to this author the main mechanism consists in generation of a "clustered saccharide patch" by the multiple oligosaccharides attached to a polypeptide carrier. Selectin, recognizing a "clustered saccharide patch", binds a ligand very efficiently. Varki [6] suggests two possible ways of "clustered saccharide patch" generation (Fig. 2). One would depend on generation of (Fig. 2A), specific conformation of a single oligosaccharide chain under influence of neighbouring oligosaccharides densely distributed on polypeptide or a suitable conformation could be due to interactions of polypeptide chain with neighbouring oligosaccharides. The second possible generation of the binding site (Fig. 2B) is created only by specific single sugars and groups of multiple oligosaccharides (a "discontiguous saccharide patch"). In this case sugars interact with the lectin domain of selectin form "discontiguous saccharide epitope" *via*, for example, a hydroxyl group of one sugar chain, an acetyl group of another one and a carboxyl or sulphate group from a third oligosaccharide [6]. It is possible that a long glycosaminoglycan chain

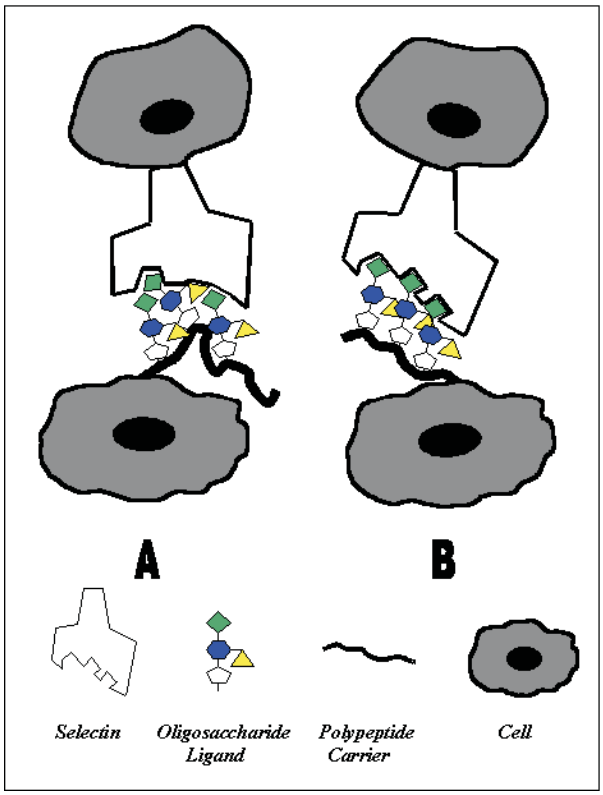

Figure 2. Generation of a "clustered saccharide patch", recognized by selectins, modified after [6].

Two mechanisms (A, B) proposed.

can imitate different oligosaccharides (e.g. sLe^x) and serves as a carrier of many carboxyl, hydroxyl or sulphate groups for selectin. In both types of generation of the patches, the polypeptide chain does not contribute directly to selectin recognition, though it may be important in rendering oligosaccharide chains accessible to interact with the lectin domain of selectin.

GLYCOPROTEIN COUNTER-RECEPTORS FOR SELECTINS

L-Selectin counter-receptors

Glycoproteins that are L-selectin counter-receptors occurring on high endothelial venules of peripheral lymph nodes and mucosal lymphatic tissue are "address signals" for L-selectin of lymphocytes. These glycoproteins have been generally called "vascular addressins" [5, 34, 36, 56]. Vascular addressins of peripheral lymph nodes contain epitopes recognized by mAb MECA-79 (Table 1), both *in vitro* and *in vivo*. Peyer's patches and mucosa lack these epitopes. The mAb MECA-79 cross-reacts with human and mice addressins of peripheral lymph nodes and is absolutely specific for HEV [57].

Specific mucosal HEV addressins [36] are necessary for homing and support of lymphocyte extravasation in Peyer's patches and mucosae. These addressins contain epitopes recognized by monoclonal antibodies MECA-367 (Table 1), which are not found in peripheral lymph nodes.

Peripheral nodes addressins are complexes of sulphated, fucosylated and sialylated glycoproteins containing different sLe^x-related determinants [22] precipitated by mAb MECA-79 *in vitro* [58, 59]. Recognition and binding of vascular addressins by L-selectin on HEV of peripheral lymph nodes and thus by MECA-79 antibodies depends on addressin sulphation [35]. Imai *et al.* [59] identified two sulphated glycoproteins (sgp) of 50 kDa and 90 kDa in peripheral lymph nodes, which were called sgp 50 and sgp 90, respectively. The polypeptide chain of cloned sgp 50 which appeared to consist of 132 amino acids, has two regions rich in O-glycosylated Ser/Thr residues and contains one N-glycosylated Asn residue. Sgp 50 lacks the transmembrane region of the peptide [60] and is a main secretory molecule, called the glycosylation-dependent cell adhesion molecule 1 (GlyCAM-1) [61, 62], a glycoprotein of mucin-type. Most O-glycans are branched with long β1,6-bound core-2 branch ended usually with a 6′-sulpho-sLe^x ligand (Fig. 1A). The main carbohydrate structure of a ligand is bound by lectin domain of L-selectin [63–65]. It has been demonstrated in *in vitro* studies [66], that 6′-sulpho-sLe^x is a more suitable L-selectin ligand than sLe^x. However, neither the L- nor E-selectin-mediated adhesion is supported by 6-sulpho-sLe^x (Fig. 1B) [66].

The secretory function of GlyCAM-1 is not thoroughly understood though its antiadhesive role or its ability to activate β_2-integrins on leukocyte surface and in consequence of GlyCAM-1 binding with L-selectin, has been suggested [67].

Another sulphated glycoprotein, sgp 90, which is the L-selectin counter-receptor, is a CD34 antigen glycoform [37, 68]. Sgp 90 is found on HEV of peripheral lymph nodes and other tissues [69], and also on the surface of human hematopoetic stem cells [37, 70]. Peripheral node addressin CD34 is a GlyCAM-1-like glycoprotein. Its polypeptide chain consists of a N-terminal mucin-like domain of 145 amino acids containing 35% Ser/Thr residues, a globular domain of 66 amino acids and a COOH-terminal transmembrane domain [70]. The mucin-like domain of CD34 carries carbohydrates responsible for binding to L-selectin; this binding is abolished by O-sialoglycoprotease which splits off the O-glycosylated domain [71]. The CD34 molecule consists of 60% of carbohydrates. The structure of oligosaccharides of CD34 has not been completely elucidated it is known that it

contains sialylated, fucosylated and sulphated O-glycans similar to those found in GlyCAM-1 [6, 17]. CD34 can be recognized by L-selectin due to its high affinity and relatively high specificity, but provided it is properly glycosylated and sulphated.

However, CD34 sialomucin in HEV of human tonsils represents only 50% of the entire L-selectin binding activity, as tested by leukocyte tethering and rolling *in vitro* [72]. Moreover, normal lymphocyte homing to lymph nodes, rolling on HEV, and leukocyte recruitment to inflammation sites have been observed in mice with the genetic defect of CD34 gene [73]. Thus, it may be suggested that some other alternative counter-receptors for L-selectin exist on lymph nodes and in inflammation HEV sites. Glycoprotein 200 (gp 200) might be a candidate for this role. Its expression on HEV of lymph nodes is increased 5–10-fold in mice with the genetic defect of either a single GlyCAM-1 gene or genes coding for both GlyCAM-1 and CD34 [16, 17].

The mucosal addressin cell adhesion molecule 1 (MAdCAM-1) is another mucin-like glycoprotein functioning as a counter-receptor for L-selectin. It is found in HEV of Peyer's patches and mesenteric lymph nodes and is responsible for homing and lymphocyte rolling [74, 75]. This glycoprotein functions at the same time as a counter-receptor for $\alpha_4\beta_7$-integrin [76].

It has been recently proposed by Clark *et al.* [39] that there are also glycoprotease-resistant O-glycoproteins different from MECA-79 antigen (Table 1) occurring on human HEV, but carrying sLe^x-like ligands which sufficiently ensure tethering and rolling of lymphocytes on HEV.

It is interesting that glycoprotein ligands for L-selectin are found not only on HEV; their expression has been discovered recently on the surface of granulocytes, monocytes, myeloid and lymphoid cell lines, but not on T lymphocytes of peripheral blood [77]. These ligands on the surface of leukocytes have either mucin or non-mucin-like structures which show different susceptibility to O-sialylglycoprotease. The ligand is Ca^{2+}-dependent, neuraminidase sensitive and structurally different from the ligands for L-selectin described previously on HEV [77]. L-Selectin ligand expressed on leukocytes may promote leukocyte–leukocyte interactions and leukocyte rolling on other leukocytes already adherent to activated endothelium *in vivo*, contributing to leukocyte penetration into inflammation sites [77]. On the other hand, leukocyte–leukocyte interactions may be maintained due to L-selectin binding of P-selectin glycoprotein ligand (PSGL-1), found on leukocyte surface [78].

In addition, one should mention a diverse molecule class exemplified by heparan sulphate proteoglycans with an L-selectin counter-receptor nature. Up to now, it has been proved that heparan sulphate proteoglycan of aortic active endothelium mediates the monocyte adhesion by binding of L-selectin [53]. Polyanionic heparan sulphate reacts with the lectin domain of L-selectin in a Ca^{2+}-dependent manner. The binding site in the lectin domain of L-selectin for this glycosaminoglycan corresponds to the binding site for sLe^x ligand of sialomucin.

P-Selectin counter-receptor

P-Selectin glycoprotein ligand-1 (PSGL-1) is a high affinity counter-receptor of P-selectin discovered by Moore *et al.* [79]. It is found on the surface of all types of leukocytes, including T cells, although not always in the active form [38, 80, 81]. Its distribution on the neutrophil surface is confined to microvilli which may considerably contribute to the adhesive function [80]. PSGL-1 is a membrane protein with a Ser/Thr/Pro-rich extracellular domain, transmembrane region and a cytoplasmatic tail [82]. A signal sequence and consensus tyrosine sulphation motif (Q-ATE-**YEYLDY**DFLPET) are located on the N-terminus of the extracellular domain consisting of three tyrosine residues; at least one tyrosine sulphate located within this motif is

required for high affinity binding both to P-selectin [83–85] and L-selectin [86]. The distal part of the polypeptide consists of 15 decameric sequence repeats A-T/M-E-A-Q-T-T-X-P/L-A/T, where X can be either P, A, Q, E or R [82]. Threonine/serine residues, present within and outside the decameric repeats are potential O-glycosylation sites. Numerous sialylated O-glycans containing sLe^x determinants (Fig. 1C) are required for P-selectin recognition [87, 88]. Thus PSGL-1 is also a mucin-type glycoprotein. Its extracellular domain contains also three potential N-glycosylation sites, but N-glycans are not required for P-selectin recognition [87]. The single cysteine residue of extracellular domain localized next to the transmembrane region promotes dimerization of PSGL-1 required for recognition of P-selectin [89]. On leukocytes, mature PSGL-1 occurs as a homodimer containing two identical 120 kDa units [79, 82] with a disulphide bond. PSGL-1 subunit structure is unique among selectin counter-receptors.

Monoclonal antibodies (Table 1) recognizing epitopes on N-terminus of PSGL-1 polypeptide are able to block completely rolling of neutrophils on P-selectin, both *in vitro* [38], and *in vivo* [90]. Moreover, truncation of the peptide (ten amino acids) from N-terminus of PSGL-1 abolishes binding of this counter-receptor to P-selectin [91]. A similar effect is caused by selective removal of extracellular domain of PSGL-1 from the leukocyte surface by O-sialylglycoprotease [92] or site-directed mutagenesis causing replacement of tyrosine residues (usually sulphated) for tryptophan [83].

It should be underlined that the cooperation between O-glycans carrying sLe^x and sulphotyrosine polypeptide in counter-receptor contributes to high affinity of the P-selectin binding. This feature is specific for PSGL-1.

E-Selectin counter-receptors

Leukocyte counter-receptors for E-selectin function during leukocyte recruitment to acute and chronic inflammation sites. They are expressed on diverse leukocyte subsets participating in inflammatory reaction, among others on neutrophils, monocytes [93, 94], α/β memory T-lymphocytes [54], γ/δ T-cells [95], natural killer cells [96] and activated B-cells [97]. Synthesis of E-selectin ligands, for example on α/β T-cells, occurs during their transition from naive to memory state, after antigen-induced proliferation [98]. It has also been suggested that synthesis of E-selectin ligand is controlled by the tissue microenvironment. The main and direct functional regulator of E-selectin ligand synthesis in human T-lymphoblasts appeared to be fucosyltransferase VII (99).

E-Selectin recognizes sLe^x and sLe(a) structures on glycoproteins detected by monoclonal antibody HECA-452 (Table 1) on different leukocyte subsets [21]. The HECA-452 antigen expressed on memory T-cells subpopulation has been called the cutaneous lymphocyte antigen (CLA) [20, 98]. This antigen is found on almost all T-cells, normal and skin infiltrating neoplasmatic ones, both circulating in blood and localized in inflammation sites beyond the skin [100, 101].

The glycoprotein E-selectin ligand of 250 kDa [102] has been identified on bovine γ/δ T-cells, whereas the150 kDa glycoprotein has been found on mice neutrophils and myeloid line cells [93]. The glycoprotein was cloned and called E-selectin ligand-1 (ESL-1) [103]. Its amino-acid sequence is in 94% identical to that of the previously described chicken cysteine-rich fibroblast growth factor receptor (CFR) [104], except for a 70-amino acid domain at the N-terminus. A similar protein, called MG 160, has been identified in the membrane of Golgi apparatus, showing more than 90% homology with ESL-1 [105]. Mature ESL-1 is transmembrane polypeptide containing 1148 amino acids [103]. Its polypeptide chain consists of a 1114 amino acid extracellular domain, a 21 amino acid transmembrane region and a 13 amino acid cytoplasmatic tail. Five putative N-glyco-

sylation sites are located on the extracellular domain [103]. ESL-1 carries only N-glycans [93, 94, 103]. E-Selectin recognizes only glycosylated ESL-1 forms of myeloid cells [103]. N-Glycans responsible for high affinity binding of E-selectin are terminated with sLe^x structures [106]. Three unusual N-glycan structures have been proposed [107], all tetraantennary N-glycans. One arm (β1,4) is 3-s-di-Le^x acetyllactosaminoglycan bound to a common pentasaccharide core structure [107] (Fig. 1D). These N-glycans constitute less than 3% of total carbohydrates bound to neutrophil surface protein and monocyte cell lines, which bind E-selectin with relatively high affinity [107]. Interestingly, N-glycans bind E-selectin entirely without any contribution of polypeptide backbone [107]. It should be also mentioned that L-selectin may occur as a functional E-selectin counter-receptor due to carrying sLe^x determinants [108]. Another proposed E-selectin counter-receptor is P-selectin glycoprotein ligand-1 [82, 109].

BIOSYNTHESIS OF sLEWIS(x) AND SULPHO-sLEWIS(x) DETERMINANT

Details of biosynthesis of selectin oligosaccharide ligands attached to a protein carrier are unknown. Presumably this process follows the known biosynthetic pathways of glycoprotein-linked oligosaccharide chains. Hitherto studies on biosynthesis of selectin oligosaccharide ligands were focused on the reactions modifying the basic *N*-acetyllactosamine or sialyl-*N*-acetyllactosamine units. This pathway is composed of an ordered series of reactions catalyzed by, at least, three types of enzymes: α1,3-fucosyltransferase(s) (Fuc-T), α2,3-sialyltransferase and sulphotransferase(s) (Fig. 3).

Fucosyltransferases catalyze the transfer of fucose from guanosine diphosphatefucose (GDP-Fuc) to the *N*-acetylglucosamine residue in the sialylated or non-sialylated precursor structures. Two families of fucosyltransferases have been described: α-1,2-fucosyltransferases and α-1,3-fucosyltransferases. These fucosyltransferases encoded by seven specific genes are designated in Genome Data Base as FUT 1 to FUT 7 i.e. in the order in which they were described. FUT 1 and FUT 2 genes encode α-1,2-fucosyltransferase H [110] and α-1,2-fucosyltransferase Se [111], respectively, which synthesize the H blood group antigen. The five different α-1,3-fucosyltransferases are encoded by five homologous human genes (FUT 3 to FUT 7). The enzymes have been described as Fuc-T III [112], Fuc-T IV [113, 114], Fuc-T V [115, 116], Fuc-T VI [117–119] and Fuc-T VII [120, 121]. They show characteristic substrate specificities, and varying cation, temperature, and pH requirements. Moreover, these fucosyltransferases exhibit different tissue-specific expression patterns. Fuc-T III, named also the Lewis enzyme, is the unique α-1,3/1,4-fucosyltransferase which uses preferentially type I precursors (Galβ1,3GlcNAc-) [112]. Fuc-T IV is the myeloid type of the enzyme present in myeloid cells and brain tissue which acts preferentially on type II precursors (Galβ1,4GlcNAc-), but poorly on sialylated precursors [32, 45, 46, 113, 114]. Fuc-T V, whose tissue expression pattern is still unknown, uses sialylated or non-sialylated type II precursors [16, 115, 116]. Fuc-T VI is an enzyme (of the plasma type) responsible for the expression of the plasma α-1,3-fucosyltransferase activity [117–119], probably originating from hepatocytes [122]. Fuc-T VII uses sialylated type II precursor to synthesize the sLe^x antigen expressed on leukocytes [45–47, 99, 120, 121].

Weston *et al.* [115] have previously demonstrated that Fuc-T V efficiently fucosylates both *N*-acetyllactosamine (Fig. 3) and α2,3-sialyllactosamine (Fig. 3II) substrates leading to generation of Le^x or sLe^x determinants, respectively. This enzyme is not expressed in leukocytes [16, 115, 116].

There are two isoenzymes of α1,3-fucosyltransferase in leukocytes. One of them, α1,3-fucosyltransferase IV (Fuc-T IV), known

as ELFT (ELAM-1 ligand fucosyltransferase) is the myeloid type enzyme [32, 45, 46, 113]. This isoenzyme is known to contribute mainly to the synthesis of Le^x [32, 45] (Fig. 3), whereas the second isoenzyme, α1,3-fucosyltransferase VII (Fuc-T VII) synthesizes only of transferring sialic acid residue preferably to type I and less efficiently to type II precursors, thus providing appropriate intermediates for the synthesis of sLe(a) and sLe^x determinants [124, 125]. The third α-2,3-sialyltransferase uses oligosaccharide substrates

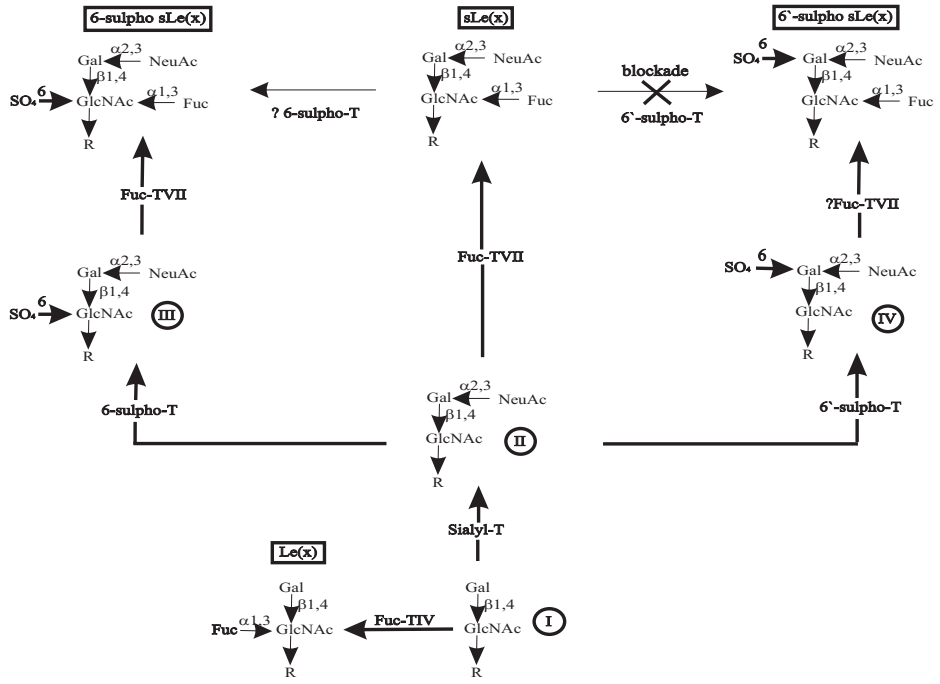

Figure 3. Biosynthesis of sLe^x, (sLe(a) and sulpho-sLe^x determinants of selectin ligands [43, 45, 131].

sLe^x and is distributed primarily on leukocytes and endothelial cells [45–47, 99, 120, 121]. In biosynthesis of sLe^x determinant, the action of Fuc-T VII on *N*-acetyllactosamine must be preceded by that of the specific sialyltransferase (Fig. 3).

Sialyltransferases catalyse the transfer of sialic acid from cytidine monophosphatesialic acid (CMP-NeuAc) to carbohydrate groups in the acceptor substrates of glycoproteins and glycolipids. Three α-2,3-sialyltransferase cDNAs have been cloned. The human Galβ(1,3/1,4)GlcNAc-α-2,3-sialyltransferase contributes primarily to the synthesis of sLe^x determinant because of its higher substrate preference for Galβ1,4GlcNAc (type II precursor) than for Galβ1,3GlcNAc (type I precursor). Expression of this sialyltransferase was detectable in granulocytic and monocytic cell lines [123]. Another human Galβ(1,3/1,4)GlcNAc-α-2,3-sialyltransferase is capable with terminal galactose in the Galβ1,3-GalNAc- and Galβ1,4GlcNAc- sequences but not with the Galβ1,3GlcNAc- sequence. This enzyme generates the terminal sequences NeuAcα2,3Galβ1,3GalNAc-R and NeuAcα2,3-Galβ1,4GlcNAc-R [126, 127].

To date, it has not been determined which of the enzymes, if not all of them, plays a main role in the synthesis of sLe^x determinants, which are E- and P-selectin ligands in leukocytes. It is unknown, which of isoforms, if not all, synthesizes the precursor for the two sulpho-sLe^x determinants (Fig. 3), carried by counter-receptor for L-selectins on HEV. In the course of sLe^x synthesis the sulphation of precursor occurs after sialylation but before fucosylation (Fig. 3) [128–131].

Considerable activity of GlcNAc-6-O-sulphotransferase is found in lymph nodes, thymus and spleen. The enzyme participates in the assembly of NeuAcα2,3Galβ1,4GlcNAc(6-SO_4)

(Fig. 3III) and 6-sulpho-Le^x [132]. Scudder *et al.* [129] proved that substitution of C6 of GlcNAc with sulphate (Fig. 3II) did not block fucosylation of C3 of the GlcNAc-6-SO_4 residue and the recombinant α1,3-fucosyltransferase generates sulpho-sLe^x structures containing sulphate at the C6 position of GlcNAc (Fig. 3). It has been shown that mouse Fuc-TVII generates both sLe^x and 6-sulpho-sLe^x determinants (Fig. 3), but not 6′-sulpho-sLe^x, from their non-fucosylated precursors [45, 133–135].

The spleen sulphotransferase recently characterized by Spiro & Bhoyroo [131] participates in the assembly of NeuAcα2,3Gal(6-SO_4)β1,4GlcNAc (Fig. 3IV) and 6′-sulpho-sLe^x, and transfers sulphate to C6 of galactose in the sialylated precursor (NeuAcα2,3Galβ1,4GlcNAc) (Fig. 3). Tetrasaccharide sLe^x appeared to be an inactive substrate for 6′-sulphotransferase, and does not contribute to generation of 6′-sulpho-sLe^x determinant (Fig. 3). This discovery is consistent with the fact that fucosylation follows sulphate addition in biosynthesis of L-selectin sulphated ligands. Strangely enough, the activity of this sulphotransferase is higher in spleen than in the lymph nodes and thymus [131]. Still, it should be clarified which α1,3-fucosyltransferase catalyzes the assembly of 6′-sulpho-sLe^x.

Finally, a modification of O-glycans catalyzed by a core-2 β1,6-*N*-acetylglucosaminyltransferase leading to oligosaccharide chain branching is also important [88, 109]. This modification is required for high affinity binding of a ligand to selectin.

SUMMARY

Common ligands recognized by all selectins are terminal sialylated and fucosylated *N*-acetyllactosamine residues which form sLe^x determinants. Sulphated ligands are preferable for both L- and P-selectins. Oligosaccharides which contain sulpho-sLe^x determinants are specific ligands of L-selectin. P-Selectin, besides a common sLe^x determinant, needs sulphation of at least one tyrosine residue in the polypetide chain for effective recognition. The "discontiguous saccharide epitope" generated by multiple oligosaccharide chains bound to the polypeptide carrier shows higher affinity for selectin than that generated by a single oligosaccharide chain. Endothelial counter-receptors for L-selectin are tissue specific sialomucins: GlyCAM-1, CD34, gp200 and MAdCAM-1. However, GlyCAM-1 is a secretory glycoprotein, so that its other functions, such as antiadhesive, have also been suggested. The P-selectin neutrophil glycoprotein ligand (PSGL-1) of high binding affinity containing sulphotyrosine residues in the polypeptide chain forms a main P-selectin counter-receptor. L- and P-selectins may also bind, with relatively high affinity, structurally diverse molecules, e.g. heparan sulphated glycosaminoglycans. The glycosaminoglycan and sLe^x binding sites in selectin are closely related and binding of both types of ligand is Ca^{2+}-dependent. Leukocyte ligand of E-selectin (ESL-1) is a glycoprotein counter-receptor which contains only N-glycans "ornamented" with sLe^x determinants. The amino-acid sequence of ESL-1 shows high homology to those of chicken fibroblast growth factor receptor and protein of Golgi membrane apparatus. Among reactions occurring during the biosynthesis of selectin ligands, sulphation and fucosylation are of essential importance. These reactions show a highly coordinated order. Generally, the expression of selectin ligands is determined by the genetically regulated expression of respective transferases in the cell.

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