# The role of selectins in inflammation and disease

## Klaus Ley

Cardiovascular Research Center and Departments of Biomedical Engineering, Molecular Physiology and Biological Physics, MR5 Building, Room 1013, University of Virginia, PO Box 801394, Charlottesville, VA 22908–1394, USA

**Selectins are carbohydrate-binding molecules that bind to fucosylated and sialylated glycoprotein ligands, and are found on endothelial cells, leukocytes and platelets. They are involved in trafficking of cells of the innate immune system, T lymphocytes and platelets. An absence of selectins or selectin ligands has serious consequences in mice or humans, leading to recurrent bacterial infections and persistent disease. Selectins are involved in constitutive lymphocyte homing, and in chronic and acute inflammation processes, including post-ischemic inflammation in muscle, kidney and heart, skin inflammation, atherosclerosis, glomerulonephritis and lupus erythematosus. Selectin-neutralizing monoclonal antibodies, recombinant soluble P-selectin glycoprotein ligand 1 and small-molecule inhibitors of selectins have been tested in clinical trials on patients with multiple trauma, cardiac indications and pediatric asthma, respectively. Anti-selectin antibodies have also been successfully used in preclinical models to deliver imaging contrast agents and therapeutics to sites of inflammation. Further improvements in the efficiency, availability, specificity and pharmacokinetics of selectin inhibitors, and specialized application routes and schedules, hold promise for therapeutic indications.**

The selectins are a family of three type-I cell-surface glycoproteins: E-, L- and P-selectin [1]. L-selectin is expressed on all granulocytes and monocytes and on most lymphocytes. P-selectin is stored in α-granules of platelets and in Weibel–Palade bodies of endothelial cells, and is translocated to the cell surface of activated endothelial cells and platelets. E-selectin is not expressed under baseline conditions, except in skin microvessels [2], but is rapidly induced by inflammatory cytokines.

Selectins show a significant degree of sequence homology among themselves (except in the transmembrane and cytoplasmic domains) and between species (Fig. 1). Analysis of this homology has revealed that the lectin domain, which binds sugars, is most conserved, suggesting that the three selectins bind similar sugar structures. Interestingly, the cytoplasmic and transmembrane domains are highly conserved between species, but not conserved across the selectins. These parts of the selectin molecules are responsible for their targeting to different compartments: P-selectin to secretory granules, E-selectin to the plasma membrane, and L-selectin to the tips of microfolds on leukocytes.

## Selectin ligands

There are many candidate ligands for selectins, but only P-selectin glycoprotein ligand 1 (PSGL-1) has been extensively characterized at the molecular, cellular and functional level (Fig. 2). Knockout mice lacking the gene encoding PSGL-1 [3,4] show delayed neutrophil recruitment and moderate neutrophilia (threefold elevation), similar to that of P-selectin knockout mice [5,6]. In addition to being responsible for ~90% of P-selectin binding, PSGL-1 is also the most important L-selectin ligand in inflammatory settings, where it is presented by already adherent leukocytes and by leukocyte fragments [7]. PSGL-1 can also bind to E-selectin, but is not the major E-selectin ligand, which remains to be discovered.

L-selectin ligands have been identified in high endothelial venules of secondary lymphatic organs and are collectively known as peripheral node addressins (PNAd) (for review see [8]). Among them, podocalyxin [9] is most likely to serve a physiologically relevant function, as a ligand for L-selectin in lymphocyte homing to lymph nodes.

## Inflammation

In most organs, leukocyte recruitment proceeds in a cascade-like fashion from capture to rolling to a systematic decrease of rolling velocity to firm adhesion and transmigration [10]. The selectins participate in the capture, rolling and slow-rolling steps [11]. In addition, there is evidence that selectin engagement can trigger signaling events in the leukocyte through L-selectin [12] and PSGL-1 [13], and in endothelial cells through E-selectin [14].

Effective neutrophil recruitment requires selectins, as demonstrated in mice that lack selectins [15,16] or selectin ligands [17], and in patients suffering from a rare disease called leukocyte adhesion deficiency type II (LAD-II) [18]. These individuals have a mutation in the gene encoding a fucose transporter [19], and cannot effectively incorporate fucose into selectin ligands. As a result, leukocytes cannot bind E-, L- or P-selectin, and patients suffer from bacterial infections of the mucosal membranes and skin. In some, the effect can be overcome by oral administration of fucose [20], and in other patients, the management includes antibiotic treatment.

**(a)**

Lectin domain    EGF domain    2–9 CR domains    Cytoplasmic domain

Plasma membrane

**(b)** Approximate amino acid identity

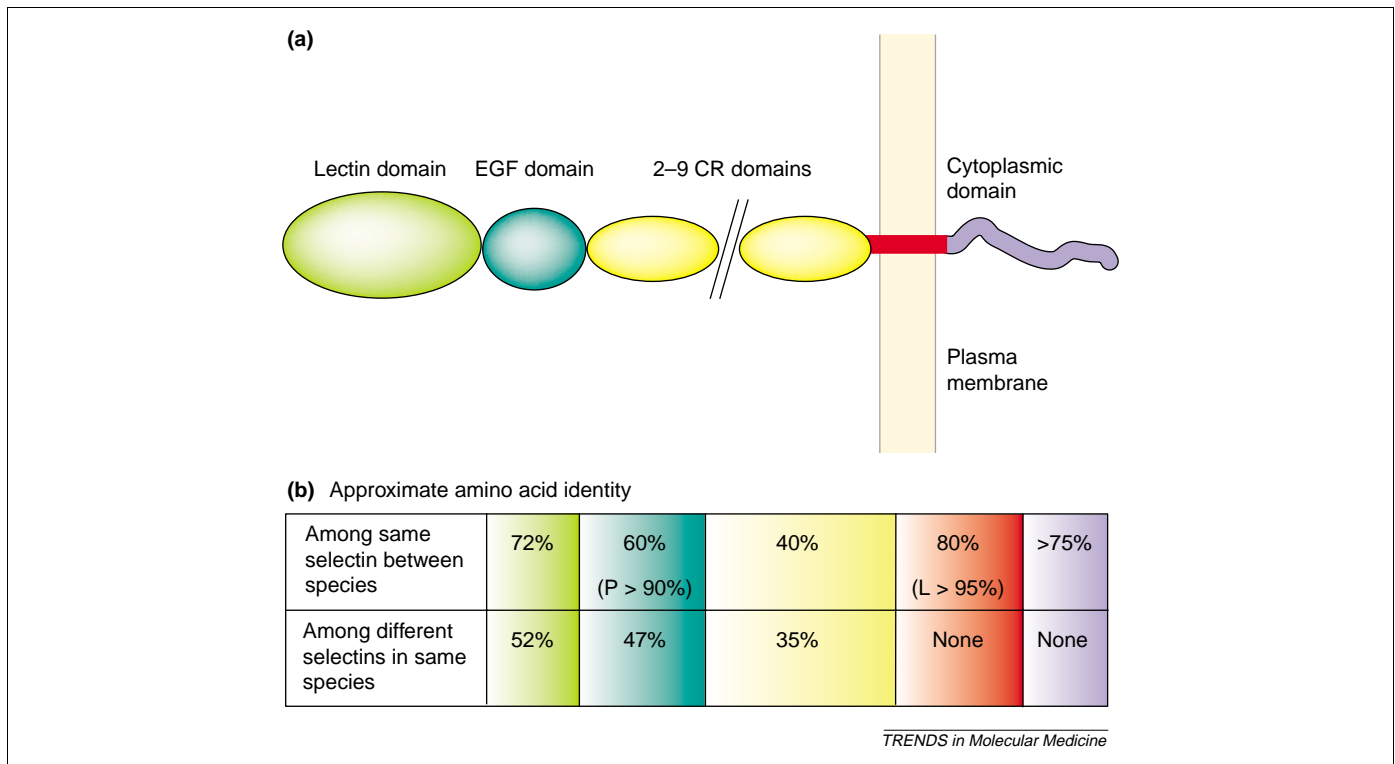| | | | | | |
|---|---|---|---|---|---|
| Among same selectin between species | 72% | 60% (P > 90%) | 40% | 80% (L > 95%) | >75% |
| Among different selectins in same species | 52% | 47% | 35% | None | None |

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**Fig. 1**. Selectin structure. (a) Selectins are composed of an N-terminal lectin domain (lime green), an epidermal growth factor (EGF) domain (dark green), two (L-selectin), six (E-selectin) or nine (P-selectin) consensus repeats with homology to complement regulatory (CR) proteins (yellow), a transmembrane domain (red) and a cytoplasmic domain (purple). (b) Amino acid sequence identity within each domain, among different species (human, mouse and cow) (top row) and among different selectins in the same species (bottom row). Adapted, with permission, from [1].

The phenotype of LAD-II patients is partially recapitulated in mice lacking fucosyl transferase (FT) VII [21], the main enzyme responsible for fucosylation of selectin ligands, and in mice lacking both FTVII and FTIV [22]. Under vivarium conditions, these mice have only a marginally increased susceptibility to becoming overtly ill, a phenotype shared by mice lacking all three selectins [23]. However, mice lacking only P- and E-selectin develop severe skin disease [24,25], which appears to be dependent upon an inappropriate immune response to the increased bacterial load, because the skin disease is not observed in immunodeficient mice [26]. Among mice with single selectin mutations, P-selectin-deficient mice show a 2−4 hour delay in neutrophil infiltration in many models [6] and have subtle defects in hemostasis [27]. E-selectin-deficient mice display no overt inflammatory defect, but do show subtle changes in neutrophil rolling and adhesion [28].

In many disease models, mice lacking P-selectin, E-selectin or both are significantly protected from neutrophil-dependent injury, for example following ischemia−reperfusion [29,30]. Interestingly, this protection is not evident in lung models. In some models, mice lacking L-selectin show a significant and consistent reduction in inflammation [31], probably resulting from decreased recruitment of inflammatory cells owing to the loss of the interaction between PSGL-1 on already adherent cells and L-selectin on incoming leukocytes [7]. A relatively common polymorphism in the gene encoding E-selectin, which alters the serine residue at position 128 to an arginine, is associated with increased incidence of restenosis after balloon angioplasty [32]. Eosinophils are important in type-2 inflammation, such as asthma, and blockade or elimination of P- or E-selectin has been shown to reduce eosinophil recruitment in models of such conditions [33].

Unfortunately, owing to the severe consequences of an absence of selectins or selectin ligands, long-term selectin blockade might not be a practical therapy for chronic inflammatory diseases. However, it is possible that partial inhibition of selectin function might allow host defense to continue successfully. In fact, in animal models, leukocyte rolling must be inhibited by >90% to cause a significant defect in neutrophil recruitment [34]. Hence, transient blockade of selectin function could be a beneficial intervention in a well-controlled clinical setting. Examples could include treatment of the ischemia−reperfusion injury associated with organ transplantation [35], and the prevention of restenosis after arterial injury induced by balloon angioplasty or stent placement [36].

### Selectin-dependent monocyte functions
Monocytes express functional PSGL-1 and use selectins to leave the vascular system. In models of atherosclerosis, blocking of P-selectin reduces monocyte rolling and adhesion to the arterial endothelium [37]; P-selectin-deficient mice show a reduction in the size of atherosclerotic lesions [38] and decreased neointima formation after arterial injury [39]. The role of E-selectin is less significant, and mice lacking this molecule show only a modest reduction in atherosclerotic lesion size. However, mice lacking both E- and P-selectin have smaller lesions
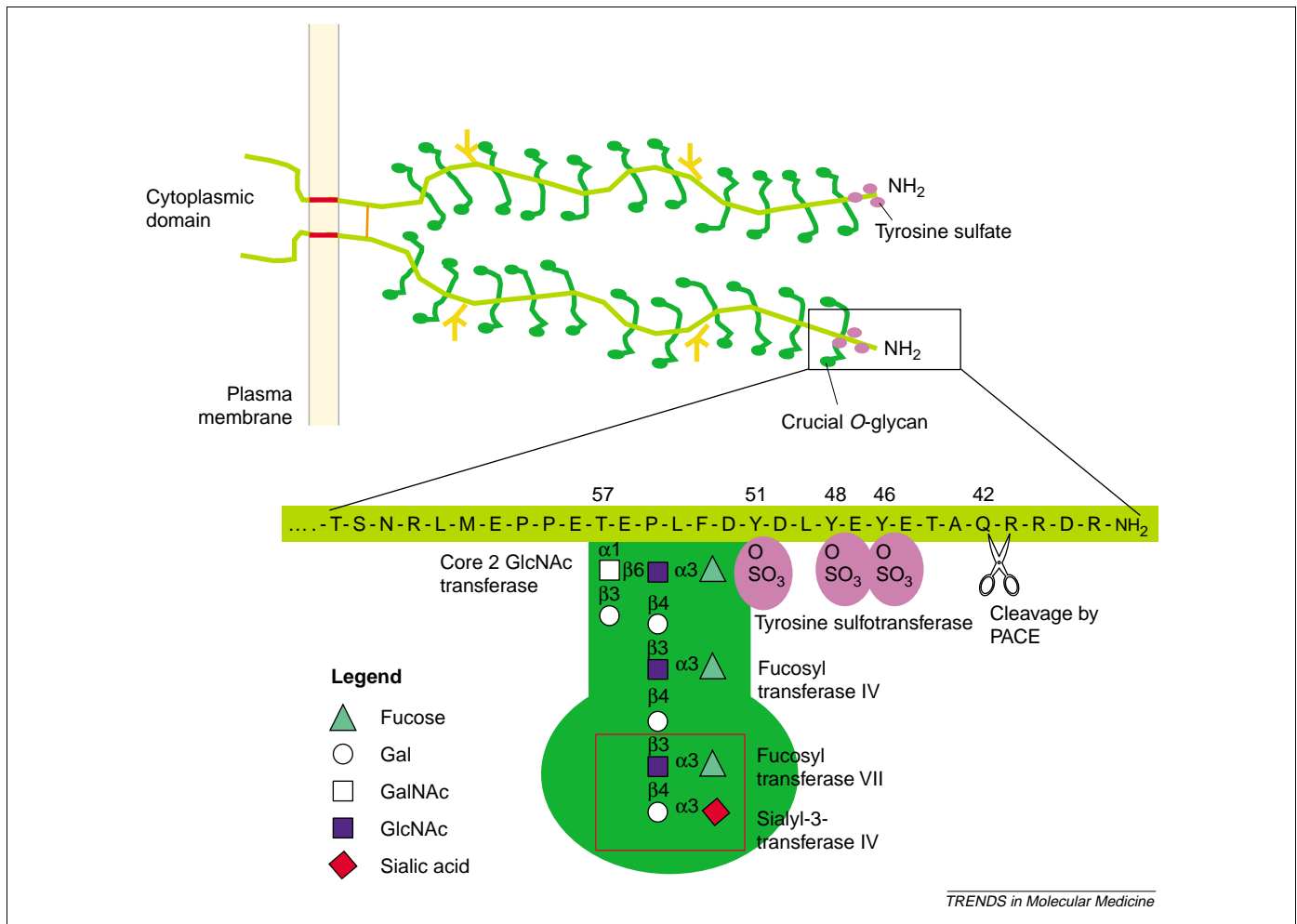
**Fig. 2**. Structure of the human P-selectin glycoprotein ligand 1 (PSGL-1) homodimer. N-terminal tyrosine sulfates (purple) are followed by a long, extended glycoprotein backbone (lime green) with many *O*-linked carbohydrates (dark green) and some *N*-linked carbohydrates (yellow). A functionally important (crucial) *O*-glycan is indicated. A stabilizing disulfide bond (S–S) (vertical orange line) is located near the plasma membrane, and the transmembrane domain (red) and the short cytoplasmic tail are also shown. Below, the amino acid sequence from residue 38 onwards is shown with tyrosine sulfates (purple) and the crucial *O*-glycan at T57 (dark green) indicated. Typical carbohydrate side-chains are shown, with linkage types indicated by Greek letters and responsible enzymes indicated next to the respective linkage. The sialyl Lewis[x] (minimal selectin recognition) motif is highlighted by a red box. Mature PSGL-1 is cleaved by PACE, a furin-like protease, at Q42. Abbreviation: Gal, galactosamine; GalNAc, *N*-acetylgalactosamine; GlcNAc, *N*-acetylglucosamine.

than mice lacking either selectin alone. Unfortunately, E- and P-selectin-deficient mice show spontaneous disease, which complicates interpretation of the results. A role for L-selectin in monocyte adhesion has been demonstrated *in vitro*, but no function for this molecule has so far been suggested in *in vivo* models of atherosclerosis. Although monocyte recruitment is significant in many types of inflammation, the role of selectins has not been investigated systematically.

### Selectin-dependent platelet functions

Activated platelets express P-selectin, which binds PSGL-1 on leukocytes and monocytes (Fig. 3). This interaction is responsible for the recruitment of inflammatory leukocytes to thrombi [40], where they are thought to help organize and resolve the thrombus, although the role of selectins has not been formally tested in models of this process. An additional function of platelet P-selectin is in the recruitment of monocyte-derived microparticles, which are a rich source of the blood-clotting element 'tissue factor', to the forming thrombus [41] (Fig. 3). Furthermore, platelet P-selectin is required for efficient interaction with monocytes and endothelial cells, in the context of atherosclerotic lesions. Activated platelets deposit proinflammatory chemokines on the surface of endothelial cells and monocytes and accelerate atherosclerosis [42]; blockade or elimination of platelet P-selectin function reduces atherosclerosis in mouse models. It appears likely that some of the beneficial preventative effects of drugs such as aspirin and clopidogrel are mediated by a reduction in platelet–leukocyte and platelet–endothelial interactions.

**Table 1**. L-selectin-dependent interactions

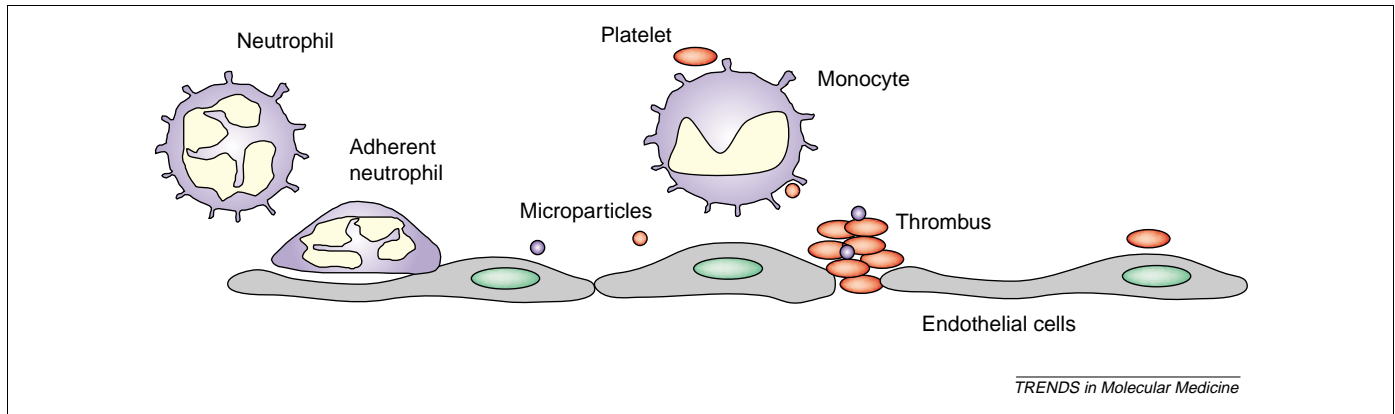| L-selectin-expressing cell | Interacting cell | L-selectin ligand | Function |
|---|---|---|---|
| T lymphocyte | High endothelial venule | Peripheral node addressins | T-cell homing |
| Leukocyte | Leukocyte | P-selectin glycoprotein ligand 1 (PSGL-1) | Amplified inflammation |
| Leukocyte | Leukocyte-derived microparticle | PSGL-1 | Amplified inflammation |

**Fig. 3**. Leukocyte, platelet and endothelial interactions. Via the binding of selectins to their ligands, neutrophils and monocytes can interact with adherent neutrophils, the endothelium, platelets (red ellipses) and platelet-derived microparticles (small red circles). Thrombi attract monocyte- and neutrophil-derived microparticles (small purple circles). Complete lists of selectin-mediated interactions are given in Tables 1–3.

## Selectin functions in the adaptive immune system

Mice lacking L-selectin show a severe defect in the homing of naive T lymphocytes to peripheral lymph nodes, a moderate defect in homing to mesenteric lymph nodes, and a subtle defect in homing to Peyer's patches; cells expressing L-selectin cannot roll on high endothelial venules in lymph nodes, which express L-selectin ligands that are different from PSGL-1 [8,43]. Central memory T cells also express L-selectin, and their homing to peripheral lymph nodes might also be impaired in L-selectin-deficient mice. L-selectin is normally shed from the cell surface following cell activation, by a process that requires the cell-surface protease TACE (tumor-necrosis-factor-α-converting enzyme) [44]. This shedding of L-selectin might regulate the inflammatory response [45], although L-selectin could also have functions in leukocyte migration [46].

All T cells express the gene encoding PSGL-1, but the molecule is not functional on most of these cells, because FTVII and core 2 *N*-acetylglucosamine (GlcNAc) transferase are not expressed and hence PSGL-1 has no selectin-binding activity. A subset of T cells express cutaneous lymphocyte antigen and use constitutively expressed E-selectin to gain access to the skin compartment [47]. T cells polarized in the direction of type 1 [T helper 1 (Th1) and T cytotoxic 1 (Tc1)] express FTVII and core 2 GlcNAc transferase [48,49], thus acquiring the ability to bind P- and E-selectin. A selectin-dependent homing defect of Th1 cells to skin and some other organs has been described [50], but it is unclear whether selectin blockade would result in protection in disease models. Mice lacking P- and E-selectin show a severe plasmacytosis in their

peripheral lymph nodes, but this might be secondary to the defects in the innate immune system in these mice.

## Organ specificity

Intravital microscopic, cell homing and disease studies all suggest that selectin recruitment differs among organs. For example, neutrophil recruitment to the liver and lung is largely selectin-independent [51,52], although exceptions exist in some lung models. In general terms, based on the pathologies seen in mice and humans, P- and E-selectin are important in neutrophil recruitment to skin and mucosal membranes, and to kidney, skeletal muscle and heart, but not to liver or to lung after ischemia−reperfusion. P- and E-selectin are also responsible for neutrophil homeostasis, and their elimination results in elevated neutrophil counts through an inter-leukin-17 (IL-17)- and granulocyte-colony-stimulating factor (G-CSF)-dependent mechanism [53].

L-selectin-deficient mice show a 90% reduction in naive T cells in lymph nodes and display many other, more subtle, inflammatory and immune defects [31,54]. It is not clear whether the blocking of L-selectin would be beneficial in any known pathology, but significant effects might be expected in autoimmune diseases. The organ specificity of selectin requirement is puzzling and highlights our incomplete understanding of the leukocyte adhesion cascade. The general paradigm of an adhesion cascade [10] holds for skeletal muscle, heart, kidney and skin, but certainly not for all organs.

In a few disease models, exacerbation of disease was induced by the absence or blockade of selectins. For example, P-selectin-deficient mice have an increased

**Table 2. P-selectin-dependent interactions**

| P-selectin-expressing cell | Interacting cell | P-selectin ligand | Function |
|---|---|---|---|
| Endothelial cell | Neutrophil, eosinophil | P-selectin glycoprotein ligand 1 (PSGL-1) | Inflammation |
| Endothelial cell | Monocyte | PSGL-1 | Atherosclerosis, inflammation |
| Endothelial cell | T helper 1, T cytotoxic 1 cell | PSGL-1 | Immune response |
| Endothelial cell | Platelet | Unknown | Unknown |
| Platelet | Monocyte, neutrophil | PSGL-1 | Amplified inflammation |
| Platelet | Endothelial cell | Unknown | Accelerated atherosclerosis |
| Platelet microparticle | Monocyte, neutrophil | PSGL-1 | Unknown |
| Platelet | Monocyte microparticle | PSGL-1 | Enhanced coagulation |

**Table 3. E-selectin-dependent interactions**

| E-selectin expressing cell | Interacting cell | E-selectin ligand | Function |
| --- | --- | --- | --- |
| Endothelial cell | Neutrophil, monocyte | Unknown and P-selectin glycoprotein ligand 1 (PSGL-1) | Inflammation |
| Endothelial cell | Cutaneous-lymphocyte-antigen-positive lymphocyte | Unknown and PSGL-1 | Homing to skin |

tendency to develop glomerulonephritis [55], and also show accelerated symptoms in a model of collagen-induced arthritis [56]. The mechanisms by which these diseases are exacerbated are not understood, but could be secondary to a lack of particular regulatory T cells.

### Use of selectins for targeted delivery and diagnostics
P- and E-selectins are expressed at reasonably high density on the luminal plasma membrane of vascular endothelial cells at sites of inflammation, and are therefore, in principle, suitable targets for the delivery of drugs, plasmids for gene therapy, and imaging contrast agents. Indeed, P-selectin has successfully been targeted by ultrasound contrast agents [57], enabling the selective and specific imaging of inflamed kidney and heart muscle. Antibody conjugates have also been used to deliver drugs to inflamed endothelium, and proof-of-concept experiments have shown feasibility in an *in vitro* system [58]. Although these selectin-based targeting approaches cannot yet be used for clinical diagnostic or therapeutic purposes, the results to date are promising.

The selectins are also found as soluble molecules in serum or plasma. Plasma P- and E-selectin levels might be risk factors for vascular disease [59] and Crohn's disease, but the diagnostic value of soluble P-selectin has yet to be validated in large clinical trials.

### Therapeutic use of selectin inhibitors
Four classes of selectin inhibitors have been developed and tested in preclinical models and some clinical trials. The first developed were carbohydrate-based selectin inhibitors of the sialyl Lewis[x] type, which inhibit all three selectins at high concentrations. However, their unfavorable pharmacokinetics, low affinity and relatively high production costs made them unsuitable for further development.

Second, antibodies to selectins have been developed and humanized, including antibodies that block more than one selectin. For example, Protein Design Laboratories has produced a humanized version of an anti-L-selectin antibody (DREG-55) and is conducting a phase-II, multicenter, double-blind, placebo-controlled trial, designed to enroll up to 84 subjects who have sustained multiple trauma with injuries involving two or more organ systems (www.pdl.com). The same antibody is also being tested in psoriasis patients. Selectin-inhibiting antibodies have been effective in preclinical models of ischemia–reperfusion, but their clinical efficacy has not yet been demonstrated.

Third, a recombinant truncated form of a PSGL-1–immunoglobulin fusion protein has shown promise as a selectin inhibitor [60] (mainly aimed at P- and L-selectin) in many models, and has entered clinical trials. Although this molecule shows good affinity and pharmacokinetics, it must be produced in mammalian cells that are co-transfected with fucosyl transferase and core 2 GlcNAc transferase, which makes production of even the moderate amounts needed for clinical trials very expensive. Wyeth conducted clinical trials with this molecule but these were recently discontinued (www.wyeth.com).

Fourth, a few small-molecule inhibitors of selectins, known as glycomimetics, have been developed, notably for E-selectin [61]. However, there are few preclinical disease models that are E-selectin-dependent [30]. Another small-molecule inhibitor of selectin function is TBC-1269 (Bimosiamose), which is being developed by Texas Biotechnology as an inhaled formulation for pediatric asthma (www.tbc.com). A double-blind, placebo-controlled phase-IIa study was started in August 2002.

### Conclusions
Selectins are crucial for the innate immune response, as demonstrated in selectin-deficient and selectin-ligand-deficient patients and in mouse models. Disease treatment using selectin inhibition shows most promise in ischemia–reperfusion-type situations and in skin diseases. However, chronic selectin inhibition will probably produce unfavorable consequences by suppressing the innate immune system. The effects of transient selectin inhibition in well-controlled clinical settings, such as organ transplantation, or balloon angioplasty with or without stent placement, have not yet been sufficiently explored.

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