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L-selectin in inflammation, infection and immunity

Nikolai A. Raffler[1], Jesús Rivera-Nieves[2], Klaus Ley[1,3,*]

[1]Department of Biomedical Engineering, University of Virginia Health System, Charlottesville, VA 22908-1394, USA
[2]Digestive Health Center of Excellence, University of Virginia Health System, Charlottesville, VA 22908-1394, USA
[3]Robert M. Berne Cardiovascular Research Center, University of Virginia Health System, P.O. Box 801394, Charlottesville, VA 22908-1394, USA

L-selectin (CD62L) is an adhesion molecule expressed on most leukocytes. This article describes recent findings of L-selectin's role in the recruitment of T cells to sites of inflammation and its contribution to the acquisition of immunologic memory. We discuss the regulation of L-selectin expression during and after the activation of T cells and its physiological function during the course of inflammation. Different disease models and current approaches for drug development are reviewed.

Introduction

The ability of inflammatory cells to respond to pathogens is crucial for maintaining health in multicellular organisms. In mammals, lymphocytes must leave the circulation and move to secondary lymphoid organs, such as lymph nodes, where antigens are presented. This immune surveillance allows the lymphocytes to find their cognate antigen. After antigen encounter, guided delivery of immune cells to sites of inflammation orchestrates host defense. Adhesion molecules control both constitutive and inflammatory leukocyte trafficking. The selectins, especially L-selectin, play a pivotal role in the initial tethering of leukocytes to the endothelium and to other leukocytes. L-selectin is responsible for (i) constitutive lymphocyte trafficking to lymph nodes and Peyer's patches and (ii) directing lymphocytes and neutrophils to sites of inflammation. Blocking L-selectin directly influences inflammatory processes. Upon T cell activation L-selectin is shed from the leukocyte surface. L-selectin on lymphocytes regulates their circulating and homing properties. Several T cell subsets with different L-selectin expression profiles have been described within the memory T cell compartment. In addition, subsets of T cells ($CD62L^+$ or $CD62L^-$) have been associated with various diseases. However, the transcriptional and translational mechanisms regulating the expression of L-selectin in lymphocytes are not well understood.

Selectins and ligands

The three selectins, L-(leukocyte, CD62L), E-(endothelial, CD62E) and P-selectin (platelet, endothelial, CD62P) are characterized by a very similar modular structure and their ability to bind carbohydrate ligands [1]. L-selectin expression on the leukocyte surface facilitates interactions that allow leukocytes to leave the bloodstream, make random contacts and tether to activated endothelial cells where they start rolling and subsequently firmly adhere. Transmigration through the endothelial cell layer allows leukocytes to encounter their target antigen in inflamed tissues. L-selectin is exclusively expressed on leukocytes (including all myeloid cells, naïve T cells and some activated T cells). Its endothelial ligands include glycosylation-dependent cell adhesion mole-

*Corresponding author: K. Ley (klausley@virginia.edu)

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cule-1 (GlyCAM-1), CD34, sialylated glycoprotein of 200 kD (Sgp200) and podocalyxin-like protein (PCLP), collectively known as peripheral-node addressin (PNAd) adhesion molecules. Mucosal addressin cell adhesion molecule-1 (MAdCAM-1) isolated from young mice has been reported to bind L-selectin but the *in vivo* relevance of this observation is unclear [2]. During inflammation, L-selectin mediates leukocyte–leukocyte interactions (secondary capture) using leukocyte P-selectin glycoprotein ligand-1 (PSGL-1, CD162) [3]. For an overview of L-selectin ligands see Ref. [4].

T cell activation

Naïve T cells express high surface levels of L-selectin. A variety of stimuli (T cell receptor engagement, CD3 cross-linking, protein kinase C activation) influence the surface expression of L-selectin *in vivo* and *in vitro*. L-selectin is regulated at transcriptional, translational and proteolytic levels. During the activation of T cells, factors determining surface L-selectin include mRNA expression, mRNA stability, transcription rate and shedding rate [5]. Murine $CD4^+$ and $CD8^+$ T cells stimulated with anti-CD3 antibody show an initial loss of surface L-selectin due to shedding but the T cells re-express surface L-selectin up to threefold over the level of naïve cells after 48 h. By seven days, all cells become L-selectin$^{low/negative}$ [5]. Hence, the *in vitro* activation profile can be separated into three distinct phases: (i) early phase dominated by shedding, (ii) middle phase, where increased mRNA stability leads to higher L-selectin message levels and therefore increased surface protein and (iii) late phase, where the transcription rate is reduced and the cells remain in a L-selectin^{low} state (Fig. 1) [5]. Most T cells entering a memory state are L-selectin^{low}. However, it is not known how effector cells regain L-selectin expression and become L-selectin^{high} central memory cells [6]. It has been discussed that naïve T cells might be able to bypass the effector state and directly develop into memory cells (dashed arrows in Fig. 1) [7].

Memory acquisition

After naïve T cells become activated by exposure to an antigen they develop an immune response. Activated cells rapidly divide and differentiate into L-selectin^{low} effector cells, which home to inflammatory sites and induce pathogen clearance. After successful elimination of the antigen, most T cells undergo apoptosis but some of the cells develop into memory cells. These long-lived, antigen-specific memory cells reside in a quiescent state but exhibit a qualitatively and quantitatively different response upon antigen rechallenge. They can be subdivided into three major classes: (i) effector memory cells (T_{EM}, 'activated'), (ii) central memory cells (T_{CM}, 'resting') [6] and (iii) intermediate memory cells (T_{IM}) [8]. These subsets can be categorized by the following surface markers: T_{EM} are $CD62L^-$/CC chemokine receptor-7 $(CCR7)^-$, T_{CM} are $CD62L^+/CCR7^+$ and T_{IM} are $CD62L^-/CCR7^+$. Because CD62L is required for homing to lymphatic organs, and the receptor pair CCR7/CC chemokine ligand-21 (CCL21) controls T cell influx into lymph nodes, these coexpressing subsets are crucial for immune control. In an adoptive transfer model, it has been shown that out of a heterogeneous pool of memory T cells expressing high and low levels of L-selectin those which were CD62L^{low} resembled a more effector-like activation, whereas the CD62L^{high} subset showed characteristics of a central memory phenotype [9]. These data suggest that re-expression of L-selectin allows memory cells (T_{CM}) to home to peripheral lymph nodes and Peyer's patches, where

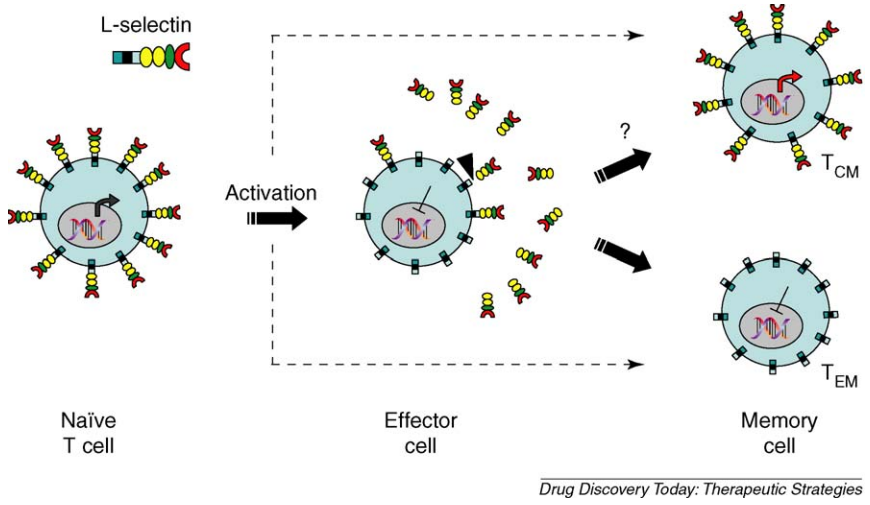

Figure 1. L-selectin on T cells. When L-selectin^{high} naïve T cells get activated upon antigen encounter and shed L-selectin, they become effector cells with downregulated L-selectin expression. These cells further develop into memory cells after the disease has been cleared. The memory cells are either L-selectin^{low} (T_{EM}) or re-express L-selectin (T_{CM}), although the mechanism is still unclear. It has been discussed that T cells can directly become memory cells [7] (dashed arrows).

they reside to rapidly respond to re-stimulation. Recently, it was shown that the T_{CM} subpopulation was more effective in the recall of a memory response, indicating that the recall response is subject to change over time [10]. Nevertheless, it is still unclear which pathway of differentiation leads to the generation of subsets bearing different L-selectin levels. Three models are currently discussed. Antigen stimulation might create T_{CM} which develop into T_{EM} [6], or vice versa [11]. Furthermore, repeated antigen exposure can influence memory development [8,12].

Acute inflammation

Acute inflammation is the immediate response to tissue injury (physical, chemical, microbiologic, among others). However, when the acute phase cannot be resolved (persistent injury or infection, prolonged toxin exposure, autoimmunity, among others), infiltrating leukocytes can cause tissue destruction or fibrosis, and a chronic disease develops. L-selectin is an important player in these inflammatory processes [13].

L-selectin-deficient mice ($CD62L^{-/-}$) provide a valuable tool to study the role of CD62L in inflammatory settings. When compared to L-selectin-sufficient mice, they show (i) lower numbers of lymphocytes, (ii) increased memory cell counts, (iii) altered homing profiles of sub-populations of lymphocytes and (iv) impaired T cell proliferation and cytokine production [14–17]. Loss of L-selectin on the cell surface results in a trafficking defect [14]. Results from these knockout studies also demonstrate that L-selectin is a mediator of leukocyte recruitment to sites of inflammation [18]. In venules of the inflamed cremaster muscle of mice, L-selectin participates in leukocyte capturing and rolling, and controls leukocyte influx [19,20].

Bacterial infections, their initial clearance and the associated evolving long-term immunity are processes an organism might encounter repeatedly. In *Leishmania major* infection, $CD4^+$ T_{CM} cells are required for the clearance of pathogen and the resolution of disease after re-infection. L-$selectin^+$ $CD4^+$ T cells adoptively transferred from immune mice proliferated more vigorously upon re-stimulation as compared to $CD4^+$ T cells from naïve mice [21]. When *Leishmania*-experienced T cells were isolated from immune mice and stimulated with soluble leishmanial antigen (SLA) the responding cells could be separated into two distinct populations. The first one was $CD62L^{low}$ and produced interferon γ (IFN-γ) and IL-2, whereas the second was $CD62L^{high}$ and secreted mainly IL-2 [21]. When $CD62L^{low}$ and $CD62L^{high}$ cells were directly purified from immune mice and stimulated with SLA, both populations proliferated but the interferon γ production was entirely limited to the $CD62L^{low}$ cells, which was confirmed *in vivo*. These data suggest that immunity is regulated by (i) effector T cells ($CD62L^{low}$, IFN-γ^{pos}) which require persistent antigen presence and (ii) T_{CM} cells ($CD62L^{high}$, IFN-γ^{neg}) which do not need the persistent antigen for maintenance but can proliferate and migrate upon re-stimulation and develop an effector phenotype. Taken together, these data suggest that presence of L-selectin is crucial for the clearance of acute inflammation. However, under certain circumstances excessive leukocyte infiltration can increase the severity of an injury.

Inflammatory bowel disease (IBD)

L-selectin also regulates migration of leukocytes into chronically inflamed tissues. For chronic diseases such as diabetes or Crohn's disease (CD), L-selectin was identified as an important adhesion molecule.

In the SAMP1/Yit mouse model of small intestinal inflammatory bowel disease, over 55% of the gut-homing β_7-$integrin^+$ cells in the mesenteric lymph nodes (MLN) coexpressed L-selectin. In splenocytes, however, $CD62L^+$ cells decreased from 85 to 30% as the disease progressed between 4 and 40 weeks of age [22]. To explore the significance of the presence of $CD62L^+CD4^+$ T cells, they were analyzed in detail. The β_7-$integrin^+/CD62L^{high}$ population coexpressed α_4-integrin but not α_E-integrin. About 10% were not of naïve phenotype, as they were $CD69^+$, $CD45RB^{low}$, $CD44^{high}$ or $CD25^+$. Within the MLN $CD4^+$ population, the $CD62L^-$ cells were the main producers of IFN-γ after anti-CD3 stimulation, whereas $CD62L^+$ cells produced mainly tumor necrosis factor α (TNF-α). This cytokine plays a pivotal role in the pathogenesis of chronic small intestinal IBD, as its blockade resolves IBD in mice and results in a clinical response in 70% of patients with Crohn's disease [23]. To determine which subset of T cells induces chronic ileitis, the three populations ($CD4^+/CD62L^{unfractioned}$, $CD4^+/CD62L^+$ and $CD4^+/CD62L^-$) were collected, sorted and separately adoptively transferred into severe combined immunodeficiency (SCID) mice. All three $CD4^+$ populations induced ileitis in SCID mice, but for the $CD4^+/CD62L^-$ subset the severity of the inflammation was significantly reduced. By contrast, $CD4^+/CD62L^+$ and $CD4^+/CD62L^{unfractioned}$ cells resulted in high inflammatory scores, indicating that the $CD62L^+$ cells were mainly responsible for the induction of ileitis. In another study, it was found that L-selectin and $\alpha_4\beta_7$ integrin, but not intercellular adhesion molecule-1 (ICAM-1), were responsible for the trafficking of lymphocytes into the gut-associated lymphoid tissue of mice [24]. Furthermore, others have identified $CD4^+/CD62L^+$ T cells as mediators of experimental colitis [25–27]. It appears that the integrin expression profiles, together with the homing capacity of L-selectin are responsible for disease control. Whereas in acute disease models $CD62L^+$ cells seem to be beneficial, they are mainly responsible for onset and development of diseases in chronic models.

Cancer

To identify the role of L-selectin in cancer, the antitumoral properties of $CD4^+$ T-helper-1 (T_{H_1}) and T_{H_2} cells have been

investigated [28]. $CD4^+/CD62L^-$ tumor-draining lymph node T cells have been collected and either cultured without manipulation under T_{H_1} conditions or after IFN-γ-blockade and IL-4 administration to develop a T_{H_2} phenotype. The activated and expanded cells were then used to treat murine pulmonary metastases and it was found that the $CD4^+/CD62L^-$ derived T_{H_1} type cells exhibited strong antitumor efficacy, whereas the T_{H_2} and $CD8^+/CD62L^+$ cells performed very poorly. This is in accordance with another study, where tumor-specific $CD62L^{low}$ cells with a T_{H_1} cytokine profile showed antitumor activity [29]. Taken together, this suggests that the $CD62L^-$ population might provide a useful tool for adoptive immunotransfer therapy.

Drug development

Several different strategies have been employed to modulate L-selectin interactions (Table 1) [30–32]. Multiple points of intervention are discussed and investigated (Fig. 2). One method is direct blockade of the interaction between L-selectin and its ligands (e.g. by monoclonal antibodies, small molecules or aptamers). Soluble protein ligands and oligosaccharides as ligand analogs provide valuable tools to interfere with L-selectin binding. Furthermore, strategies targeting upstream or downstream processes such as blocking of enzymes responsible for essential post-translational modifications of L-selectin ligands (e.g. sulfotransferases, sialyltransferases, fucosyltransferases, core2GlcNAc-transferases) are also promising.

Monoclonal antibodies

Antibodies directed against CD62L can inhibit the recruitment of lymphocytes, monocytes and neutrophils to inflammatory sites [33–35]. Use of antibodies has therefore been a major strategy for the development of drugs. SMART™ anti-L-selectin antibody (aselizumab, a humanized mAb from the IgG_4 subclass, Protein Design Labs (http://www.pdl.com) and Scil Technology (http://www.scil.com)) and EL-246 (recognizes E- and L-selectin, Ligocyte (http://www.ligocyte.com)) are products currently studied in clinical trials, but several others have been developed. Administration of anti-L-selectin mAb LAM1-3 (Cell Genesys (http://www.cellgenesys.com)) showed protective effects during ischemia and reperfusion experiments [36–39]. In addition, the effects of severe lung injury were attenuated using EL-246 [40,41]. Further, it has recently been shown that EL-246 effectively blocks neutrophil infiltration after heart transplantation, suggesting a positive role for postreperfusion injury [42]. However, EL-246 was not able to reduce lung injury in baboons [43]. The humanized DREG-200 antibody against L-selectin was effective in attenuating myocardial necrosis after ischemia and reperfusion in a feline model [44]. The Phase II trial for aselizumab in trauma patients, however, resulted in no sig-

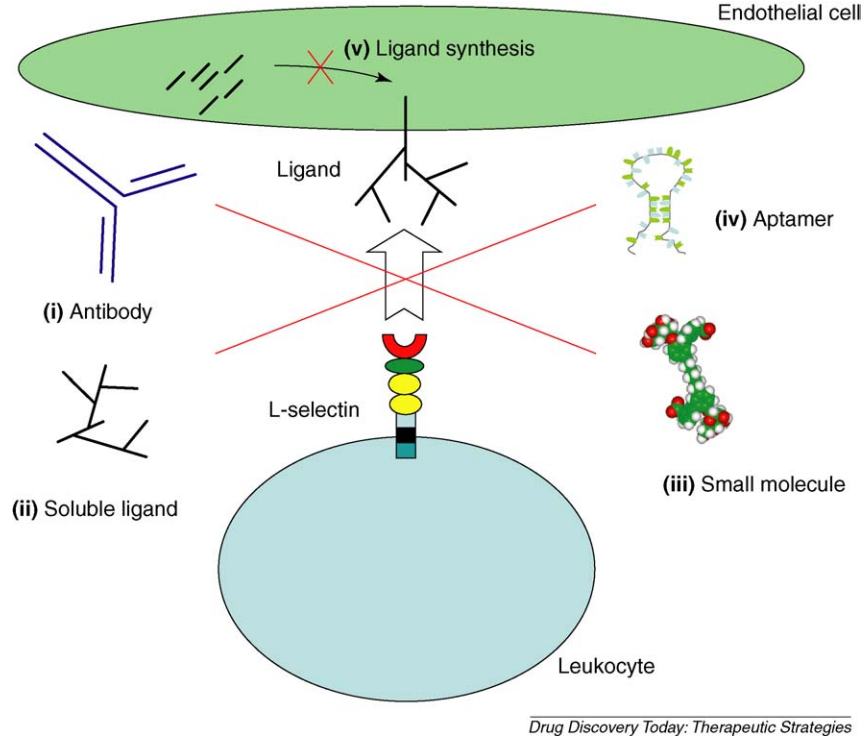

Figure 2. L-selectin inhibitors. Different strategies have been followed to develop L-selectin inhibitors: (i) monoclonal antibodies directed against L-selectin, (ii) soluble ligands to saturate L-selectin epitopes, (iii) small molecules, (iv) aptamers that block L-selectin and (v) blockade of enzymes that synthesize L-selectin binding partners.

Table 1. Therapeutic strategies of L-selectin inhibition (adapted from Refs [13,31,32,69,70])

Strategy	Examples	Properties	Indication	Latest developments	Developed by	Refs
Carbohydrates	Fucoidin	Sulphated polysaccharide, toxic, purification from natural sources		Failed		[53,54]
	Sialyl Lewis X (sLe^X)	Tetrasaccharide, low affinity, low specificity		Failed		[49]
	Cylexin (CY-1503)	Complex oligosaccharide, sLe^X analog, low bioavailability, low stability	Ischemia and reperfusion injury, stroke, transplantation	Failed (human safety studies finished, but no clinical efficacy)	Cytel (n/a)[a], Epimmune (http://www.epimmune.com)	[50–52,71,72]
Proteins	TS-1 (rPSGL-Ig)	Recombinant soluble PSGL-1 fused to IgG_1	Ischemia and reperfusion injury, myocardial infarction	Disappointing results in myocardial infarction; re-established and in Phase II trials [73]	Wyeth (http://www.wyeth.com), Thios (http://www.thios.com)	[55–57,74]
Small molecules	Bimosiamose (TBC-1269)	Pan-selectin antagonist	Asthma, psoriasis (topical), dermatitis (atopical)	Phase IIa [75]	Revotar (http://www.revotar.com), Texas Biotech (http://www.tbc.com), Encysive (http://www.encysive.com)	[61–65,76]
	BMS-190394	Selectin inhibitor, sulfatide analog	Inflammation		Bristol-Myers Squibb (http://www.bms.com)	[58]
	OC 229648	Carbohydrate-free inhibitor		Pre-clinical	Ontogen Kanebo (http://www.kanebo.com)	[60,61]
Sulfotransferase inhibitors			Asthma, inflammatory bowel disease, chronic obstructive pulmonary disorder, psoriasis	No results reported	Thios (http://www.thios.com)	
Antibodies	SMART™ anti-L-selectin mAb (aselizumab)	Humanized mAb of IgG_4 subclass	Trauma	Phase II completed, but no significant effect [77]	Protein Design Labs (http://www.pdl.com), Scil Technology (http://www.scil.com)	
	EL-246	Recognizes L- and E-selectin	Chronic obstructive pulmonary disease, cardiovascular inflammation	Pre-clinical	Ligocyte (http://www.ligocyte.com)	[41,40,42,43]
	LAM1-3	Function blocking L-selectin mAb			Cell Genesys (http://www.cellgenesys.com), Abgenix (http://www.abgenix.com)	[38,37,39,36]
	DREG-55, DREG-200	Humanized mAb of IgG_1 subclass		Phase Ib, but no results reported	Protein Design Labs (http://www.pdl.com)	[44,78]
Aptamers	RNA aptamer, DNA aptamer	Oligonucleotide, weak stability, rapid degradation		No efficacy in animal disease models	Gilead Sciences (http://www.gilead.com), Archemix (http://www.archemix.com)	[46,47]

[a] n/a: not available.

nificant differences compared to the placebo group [45]. In summary, inflammatory processes can be abated by the administration of antibodies against L-selectin but resounding clinical success was yet to be achieved.

Aptamers

During the mid-1990s, efforts were made to select inhibitors from random nucleic acid sequence space using systematic evolution of ligands by exponential enrichment (SELEX). NeXstar Pharmaceuticals, now part of Gilead Sciences (http://www.gilead.com), produced specific aptamers against L-selectin [46,47]. However, nucleic acids are difficult to synthesize in large quantities and are generally subject to fast degradation.

Inhibitors of ligand modification

Sulfoadhesin is an oligosaccharide structure (sulfated and *N*-acetylated) present on endothelial cells and is recognized by L-selectin on the surface of leukocytes. Thios Pharmaceuticals Inc. (http://www.thios.com) was developing a humanized antibody against the sulfoadhesin epitope and a small molecule inhibitor that can block the sulfotransferase GST-3, which is responsible for the addition of sulfate groups to sulfoadhesin [48]. There is currently no clinical evidence for the effectiveness of this strategy.

Ligand mimetics and analogs

Sugar moieties are the immediate binding partners of selectins. Therefore, carbohydrate mimetics (glycomimetics) should interrupt the docking between L-selectin and its ligands. The chemically synthesized tetrasaccharide sLe^X is capable of attenuating reperfusion injury [49]. An analog of sLe^X from former Cytel, now Epimmune (http://www.epimmune.com), CY-1503, was tested in ischemia and reperfusion models and showed significantly reduced injury [50–52]. However, not all studies have confirmed this protective effect of CY-1503. The complex oligosaccharide fucoidin can block neutrophil infiltration into inflamed rabbit lungs [53] but fucoidin also has strong anticoagulant effects [54] and an unfavorable toxicity profile. All sugar-based inhibitors require complex manufacturing procedures and are often metabolized rather quickly, resulting in reduced availability and low efficacy.

Another ligand mimetic strategy uses a recombinant soluble form of the L-selectin ligand PSGL-1, rPSGL-1-Ig, now termed TS-1 [55], licensed by Wyeth (http://www.wyeth.com). This protein inhibits rolling of all three selectins *in vivo* and exerts remarkable cardioprotective effects at very low doses in myocardial ischemia and reperfusion experiments [56,57].

Small molecule inhibitors

Small molecules capable of blocking L-selectin interactions have also shown promising results. For example, the low molecular weight reagent BMS-190394 (Bristol-Myers Squibb (http://www.bms.com), a selectin inhibitor for E-, P- and L-selectin, has shown anti-inflammatory activity in an infection model in rats [58]. A novel, carbohydrate-free compound (OC 229648, Ontogen Kanebo (http://www.kanebo.com)) was active in an inflammatory mouse model but lacked efficacy when administered in airway disease in sheep [59,60]. Bimosiamose (TBC-1269), a pan-selectin antagonist in development by Revotar (http://www.revotar.com), was effective in the treatment of liver ischemia and reperfusion injuries in rats but was unable to attenuate asthmatic responses when administered intravenously during Phase II trials [61–63]. TBC-1269 inhibited murine leukocyte rolling in flow chamber experiments, thus suggesting anti-inflammatory activity of the molecule [64]. In kidney allograft rejection, TBC-1269 showed anti-inflammatory activity by reducing the expression of chemokines and cytokines, as well as PSGL-1 [65]. Recent data demonstrated that inhaled bimosiamose significantly attenuated the maximum late allergic response in patients with asthma [66]. This proof-of-concept study is one of the first to show clinical efficacy of a small molecule selectin antagonist in inflammatory airway disease.

Conclusions

L-selectin plays an important role in leukocyte adhesion, where it mediates initial capturing and tethering. During T cell activation, L-selectin is shed from the surface, resulting in L-selectin^{low} effector cells. It is unclear which cell population proliferates after activation and what drives T cells to become long-term memory cells after the disease has been cleared.

Under certain circumstances, it might be necessary to block leukocyte adhesion to prevent massive infiltration and tissue damage. L-selectin is a relevant player in acute diseases such as bacterial infections as well as in chronic inflammatory processes such as Crohn's disease. Therefore, L-selectin represents a valuable and attractive target for therapeutical interventions. Multiple strategies have been employed to find and develop inhibitory drugs (Table 1). These studies suggest that neutralization of L-selectin-mediated interactions is capable of attenuating leukocyte infiltration into inflammatory sites and reducing the progression of inflammation. It has been questioned, however, if blockage of selectins is biologically relevant [67]. Short half-life in the circulation, low bioavailability or insufficient binding affinity (high IC_{50}) are problems of some of the drugs developed so far. As selectin interactions are redundant [68], the efficacy of inhibitors directed to only one of the selectins can be low. However, strategies targeting multiple selectins, such as EL-246 antibody (recognizing E- and L-selectin) or the small molecule inhibitor bimosiamose (targets all selectins) have proven to be highly efficient. Although clinically viable strategies have yet to emerge, it is evident that L-selectin is an important and promising target molecule.

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