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Selectins: critical mediators of leukocyte recruitment

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Selectins are multi-functional adhesion molecules that mediate the initial interactions between circulating leukocytes and the endothelium. First identified over a decade ago, selectins have provided insight into areas as diverse as normal lymphocyte homing, leukocyte recruitment during inflammatory responses, carbohydrate ligand biosynthesis and adhesion-mediated signalling. This review will examine the selectins and their ligands with a focus on recent findings using knockout technology as well as the emerging role of selectins as signalling molecules.

Key words: selectin / leukocyte / ligands / rolling / signalling

Introduction

Leukocyte recruitment from the blood stream into tissues is a central step in the inflammatory process. Leukocyte tethering and rolling, activation and firm adhesion comprise the classic paradigm of inflammatory cell recruitment.[1] Specific families of adhesion molecules mediate each step in this cascade. Tethering and rolling are the first steps in this process and are predominately mediated by the selectin family of adhesion molecules.[1,2]

The selectins are calcium-dependent, type I transmembrane glycoproteins that bind to sialylated carbohydrate moieties present on target proteins. Three selectins have been identified: P-, E- and L-selectin. All three selectin genes are closely linked in a gene cluster on chromosome 1[3]. Structurally, the selectins are very similar to each other (Figure 1). Each has a lectin-like domain at the NH_2-terminus, which is central to the binding properties of the protein. This is followed by an EGF-like domain and various numbers of consensus repeat (CR) domains. The selectins are anchored in the membrane by a single transmembrane domain and contain a small cytoplasmic tail. The major structural difference between the selectins lies in the number of CR domains. In humans, P-selectin is the longest member of the family with nine CRs; E-selectin has six and L-selectin contains only two. Detailed descriptions of the selectins and their expression profiles have been previously reviewed,[1,2] thus only a brief description is provided here.

P-selectin (CD62P, GMP-140, PADGEM, LECAM-3) is a 140 kDa protein originally purified from platelets and then later found to also be expressed in endothelial cells.[4,5] In both cell types, P-selectin is constitutively expressed in secretory granules; α-granules in platelets and Weibel-Palade bodies in endothelial cells[4–6]. Upon appropriate stimulation (Table 1), Weibel-Palade bodies fuse with the plasma membrane, causing surface expression of P-selectin within minutes. For this reason, P-selectin is often the selectin involved in mediating early leukocyte recruitment during inflammatory responses.[7,8] P-selectin can also be transcriptionally regulated. IL-4, IL-13 and oncostatin M all increase P-selectin mRNA synthesis and protein production in human endothelial cells.[9–11] In contrast, TNF-α, lipopolysaccharide (LPS) and IL-1 do not increase P-selectin mRNA synthesis in human cells. This is likely due to a lack of a traditional NF-κB binding site within the human P-selectin promoter.[12] Of note, the murine P-selectin promoter does contain a traditional NF-κB binding site and P-selectin expression is regulated by mediators such as TNF-α and LPS in murine cells.[12,13] Once expressed on the surface of endothelial cells, P-selectin is rapidly internalized by endocytosis.[14]

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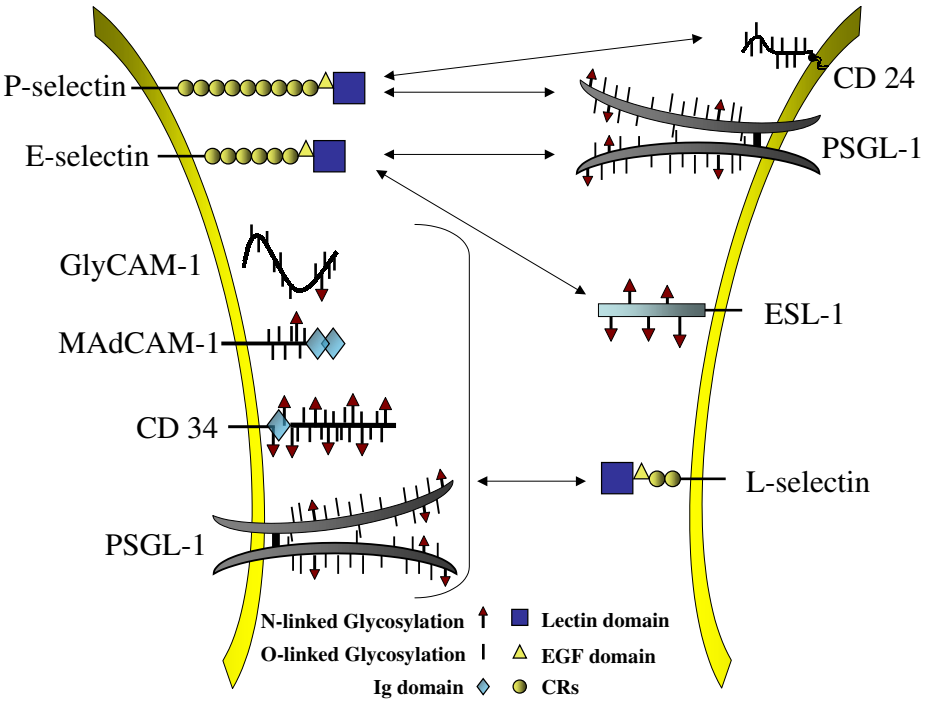

Figure 1. The selectins and their ligands. The generic expression of the selectins and their ligands on targets cells is shown here. Of note, interactions between L-selectin and PSGL-1 occur between L-selectin expressed on one leukocyte and PSGL-1 expressed on another.

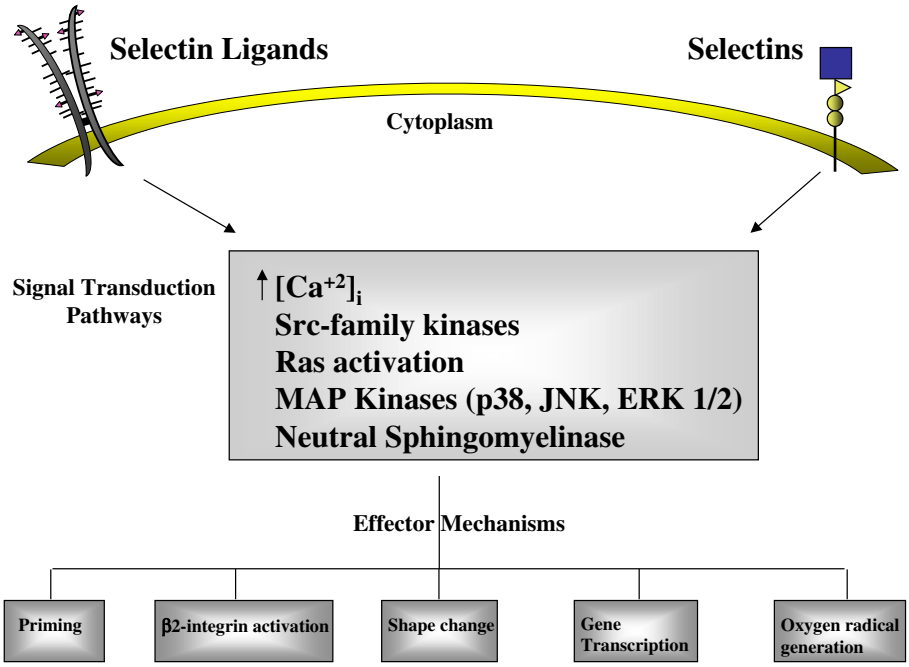

Figure 2. Signalling cascades initiated by ligation of either the selectins or their ligands. Binding of selectins to their ligands is associated with the activation of multiple signalling pathways and downstream effector functions. The specific pathways linking the selectins and their ligands to downstream functional events are currently being investigated by several groups.

Table 1. E-selectin and P-selectin surface expression by HUVEC in response to various stimuli

Selectin	Stimulus	Peak in surface expression
E-selectin	IL-1	4 h [15]
	LPS	4–8 h [82]
	Oncostatin M	4 h [9]
	TNF-α	4 h [15]
P-selectin	Histamine	3–5 min [14]
	IL-4	24 h [10]
	IL-13	24–48 h [11]
	Oncostatin M	15 min [9]
		24 h [10]
	Thrombin	30 min [14]

E-selectin (CD62E, ELAM-1, LECAM-2) is also expressed by endothelial cells. Unlike P-selectin, E-selectin is not constitutively present in endothelial cells. Instead, E-selectin expression is transcriptionally regulated by mediators such as TNF-α and IL-1 (Table 1). Following stimulation with these cytokines, peak expression of E-selectin occurs in 4 h and then declines within 24 h.[15] Transcriptional regulation of E-selectin is dependent on several transcription factors including NF-κB and AP-1. Once expressed on the cell surface, E-selectin is slowly internalized, and directed to lysosomes for degradation.[16]

L-selectin (CD62L, LAM-1, LECAM-1, gp90^{MEL}, DREG) is expressed by most leukocytes and was first identified as a lymphocyte homing receptor.[17] L-selectin is unique in that it is the only selectin involved in mediating lymphocyte recruitment into lymphatic tissue. Although L-selectin is constitutively expressed by leukocytes, it is rapidly shed from the surface upon cellular activation.[18] In some models of inflammation, L-selectin serves as an important mediator of neutrophil recruitment in later stages of an inflammatory response.[8]

Ligands

Following the original identification of the selectins, tremendous effort was devoted to the discovery and characterization of their ligands. Early on it was established that all three selectins bind to the carbohydrate sialyl Lewis^x (sLe^x) with low affinity; however, identifying high affinity glycoprotein ligands that are functional *in vivo* has proven to be more difficult.[19] One of the best characterized selectin ligands is P-selectin glycoprotein ligand-1 (PSGL-1), which is expressed primarily on myeloid, lymphoid and dendritic cells.[20] To function as a ligand for P-selectin, PSGL-1 must be modified with fucosylated and sialylated O-linked oligosaccharides.[21] Additionally, one or more *N*-terminal tyrosine residues of PSGL-1 must be sulfated.[22] A role for interactions between P-selectin and PSGL-1 in leukocyte recruitment has been well established both *in vitro* and *in vivo*. *In vitro* inhibition of PSGL-1 completely eliminates neutrophil rolling on P-selectin,[23] while genetic deletion of PSGL-1 attenuates P-selectin-mediated rolling of leukocytes *in vivo*.[24]

Another molecule that functions as a P-selectin ligand is CD24. CD24 is a small glycoprotein that is highly decorated with O-linked oligosaccharides and associates with the plasma membrane via a glycosylphosphatidylinositol anchor (GPI)[2] (Figure 1). For cells that express both CD24 and PSGL-1, such as neutrophils, PSGL-1 appears to play the dominant role in interactions with P-selectin.[23] In cells that express CD24 in the absence of PSGL-1, however, CD24 can mediate rolling on P-selectin.[25] CD24-positive, PSGL-1-negative breast carcinoma cells roll on P-selectin in a CD24-dependent fashion, and also exhibit P-selectin-dependent rolling on inflamed endothelium *in vivo*, providing evidence that CD24 functions as a physiologically relevant P-selectin ligand.[25]

E-selectin ligand-1 (ESL-1) was originally identified as an E-selectin-binding protein in the mouse neutrophilic cell line 32Dc13.[26] The peptide backbone of ESL-1 is expressed in a variety of cell types, although it has been detected as an E-selectin-binding glycoform only in myeloid cells.[27] Antibodies against ESL-1 partially inhibit adhesion of 32Dcl3 cells to purified E-selectin, suggesting that it has biological function.[27]

Two other ligands proposed for E-selectin are PSGL-1 and L-selectin. E-selectin can bind to both of these molecules;[28–30] however, it is unclear whether or not these are functionally important interactions. Both PSGL-1 and L-selectin antibodies attenuate neutrophil rolling on E-selectin;[29,31] however, in one study this was due to inhibition of leukocyte–leukocyte interactions, suggesting that neutrophils do not use PSGL-1 and L-selectin for direct tethering on E-selectin.[31] In contrast, it has also been reported that direct interactions between E-selectin and L-selectin are inhibited by L-selectin antibodies under flow conditions.[29] Results from a PSGL-1 knockout mouse suggest that PSGL-1 is not required

for leukocyte rolling on E-selectin;[24] however, a recent report supports a role for PSGL-1 in T_H1 cell rolling on E-selectin *in vivo*.[32] To date, a satisfactory E-selectin tethering ligand has yet to be identified.

Four L-selectin ligands have been identified from the high endothelial venules (HEV) of lymph nodes (LN): GlyCAM-1, MAdCAM-1, CD34 and Sgp200. Glycosylation-dependent cell adhesion molecule (GlyCAM-1) is postulated to be a physiologically relevant L-selectin ligand based on the observation that Jurkat cells and peripheral blood leukocytes roll on GlyCAM-1 *in vitro*.[33] GlyCAM-1, however, is a secreted protein that is not found on the surface of HEV,[34] making it therefore unlikely to mediate rolling *in vivo*. Recent evidence suggests that GlyCAM-1 may instead induce signalling responses in leukocytes through L-selectin.[35,36]

Mucosal addressin cell adhesion molecule (MAdCAM-1) is a cell adhesion molecule with a dual role in supporting lymphocyte tethering and rolling through both L-selectin[37] and the $\alpha_4\beta_7$ integrin.[38] Appropriate posttranslational modification of MAdCAM-1 is necessary to support L-selectin-mediated rolling, as is indicated by the observation that lymphocytes can only role on MAdCAM-1 isolated from sources that express the MECA-79 epitope.[37] *In vivo,* a functional role for MAdCAM-1 in the tethering and rolling of lymphocytes on the HEV of the Peyer's patch has been demonstrated.[39]

Less is known about the roles of CD34 and Sgp200 as L-selectin ligands. CD34 from mouse peripheral LN binds L-selectin,[40] and CD34 from human tonsils supports L-selectin-dependent lymphocyte rolling.[41] Sgp200 is a poorly characterized molecule that has not yet been cloned. Evidence that Sgp200 functions as an L-selectin ligand comes from the observation that it can be precipitate from mouse LN lysates using an L-selectin-IgG chimera.[42]

As is the case for both P- and E-selectin, L-selectin can also utilize PSGL-1 as a ligand. L-selectin binds directly to PSGL-1 from a number of cell types, including neutrophils and monocytes.[43] Interactions between L-selectin and PSGL-1 have been shown to support neutrophil on neutrophil rolling,[44] which leads to the formation of secondary tethers, thereby amplifying leukocyte recruitment at sites of inflammation.

Although many glycoproteins that can function as selectin ligands have been identified, there is still much work to be done in this area. One of the greatest challenges in selectin biology is determining which of the selectin–ligand pairs that have been identified using *in vitro* methods are actually functional *in vivo*.[19] Selectin knockout mice, as well as mice deficient for selectin ligands, should serve as invaluable tools in addressing this important question.

Selectin knockouts

Extensive research has been invested in determining the exact role each selectin member contributes to an inflammatory response. Mice that are genetically deficient in P-selectin,[7] E-selectin[45] or L-selectin[46] have been generated. Among the single knockout mice, L-selectin-deficient mice have the most severe phenotype. Lymphocytes from these mice fail to bind to HEV, have smaller and reduced cellularity in peripheral LN as well as impaired recruitment in several models of inflammation.[46,47] P-selectin-deficient mice have mild neutrophilia[7] and exhibit protection in models of ischemia-reperfusion, lung transplantation and atherosclerosis.[48–50] These mice also display impaired recruitment in response to an inflammatory stimuli; however, this is only a delay, as recruitment is recovered at later time points.[7] In contrast, E-selectin-deficient mice display no impairments in leukocyte recruitment in models of inflammation and contact hypersensitivity.[45] E-selectin, however, has been shown to play an important role in mediating host defense against *Streptococcus pneumoniae,* as these mice have higher rates of mortality and bacteremia than wild type mice and mice lacking P-selectin.[51]

Although E-selectin appears to have no dominant role in mediating recruitment in models of inflammation, its role in combination with P-selectin is quite different. Mice double deficient in both P- and E-selectin ($P/E^{-/-}$) show a much more severe phenotype than any of the single selectin knockouts.[52,53] These mice have extreme leukocytosis,[52,54] elevated IL-3 and GM-CSF levels, splenomegaly, develop spontaneous skin infections and were the first selectin knockout mice to exhibit altered hematopoiesis.[52] $P/E^{-/-}$ mice also have a reduced delayed-type hypersensitivity response to oxazolone,[55] impaired wound healing[56] and exhibit protective effects in atherosclerosis.[57] In response to TNF-α-stimulation, $P/E^{-/-}$ mice exhibit a 46-fold reduction in rolling compared to wild type mice and a 20-fold decrease compared to P-selectin$^{-/-}$ mice.[52–54] In thioglycollate-induced peritonitis, neutrophil influx at 2 and 4 h in $P/E^{-/-}$

mice is similar to that seen in P-selectin$^{-/-}$ mice; however, at 8 h influx of neutrophils is significantly reduced compared to P-selectin$^{-/-}$ and wild-type littermates.[52] The co-dependence on both P-selectin and E-selectin in these models may reflect the ability of both P-selectin and E-selectin to be regulated by NF-κB in murine systems.

Double deficient mice lacking P- and L-selectin (P/L$^{-/-}$) and E- and L-selectin (E/L$^{-/-}$) have also been generated by both homologous recombination[54] and by bone marrow transplantation.[58] P/L$^{-/-}$ mice have leukocytosis and severely suppressed leukocyte rolling and adhesion in TNF-α-stimulated venules.[54] Interestingly, E/L$^{-/-}$ mice display no increase in the number of circulating leukocytes and in TNF-α-treated mice there is no change in the amount of rolling leukocytes in mesenteric venules.[54,58] These data suggest that P-selectin expression in E/L$^{-/-}$ mice is able to compensate for the loss of E- and L-selectin. In thioglycollate-induced peritonitis, there is an initial delay in neutrophil at 2 h in both P/L$^{-/-}$ and E/L$^{-/-}$ mice; however, recovery was seen in E/L$^{-/-}$ mice at later time points. These data reveal two important points. Firstly, mice deficient in P- and L-selectin demonstrate that E-selectin is inefficient at mediating capture and rolling of leukocytes as these mice have impaired leukocyte rolling and adhesion. Secondly, these data show the key role P-selectin plays in mediating rolling in an inflammatory situation since P/E$^{-/-}$ and P/L$^{-/-}$ mice have severely impaired rolling.

Although displaying a severe defect in leukocyte recruitment, mice deficient in any two selectins can still support leukocyte rolling and adhesion. For example, there is clearly an early defect in neutrophil emigration in *Streptococcus pneumoniae*-induced peritonitis in E/P$^{-/-}$ mice; however, at 24 h emigration was no different from wild type littermates.[53] To address this mice deficient in all three selectins (E/L/P$^{-/-}$) have also been generated.[54,58,59]

Triple deficient mice are viable and fertile. They have leukocytosis, but less severe than in E/P$^{-/-}$ mice, and they rarely develop severe mucocutaneous infections or pulmonary inflammation, as do E/P$^{-/-}$ mice.[54,59] In untreated and TNF-α-treated mice, there was a significant reduction in the amount of rolling and adherent leukocytes in the mesenteric venules that was beyond that of any of the double mutant mice.[54,58,59] The minimal rolling seen after 6 h of TNF-α treatment is completely blocked after a blocking α_4 antibody was administered.[58,59] In response to thioglycollate-induced peritonitis, neutrophil emigration is significantly reduced in triple selectin deficient mice at all time points examined.[59]

Forlow and Ley have recently gone beyond the triple selectin deficient mouse and have generated mice lacking all three selectins and ICAM-1 (E/P/L/I$^{-/-}$).[60] In response to thioglycollate, there is a severe reduction in rolling, firm adhesion and neutrophil recruitment into the peritoneal cavity. In venules of cremaster muscle treated with TNF-α, there is a significant reduction in the number of rolling leukocytes in E/P/L/I$^{-/-}$ mice; however, it is not significantly different from triple selectin knockout mice. Although rolling is significantly impaired, there are still substantial amounts of adhesion in the venules of E/P/L$^{-/-}$ and E/P/L/I$^{-/-}$ mice. Administration of an α_4-integrin antibody blocked residual rolling and most residual leukocyte adhesion in these animals, indicating that α_4-dependent mechanisms play a role in these systems.[60]

The extensive research in the selectin knockout field has provided valuable information on the role selectins play in leukocyte rolling and adhesion. Single selectin knockouts and antibody studies show that P-selectin is important in mediating early leukocyte recruitment.[7,8] Double knockouts have revealed the importance of P-selectin and have shown it to be the most versatile member of the selectin family. Triple selectin-deficient and E/P/L/I$^{-/-}$ mice have demonstrated that selectins are important in mediating rolling; however, selectin-independent mechanisms exist and removing additional adhesion molecules does not provide additive levels of inhibition.

Signalling

Selectins were originally identified as cell adhesion molecules; however, it has recently been shown that selectins can also act as signalling molecules (Figure 2). A role for L-selectin in signalling was first hypothesized based on observations of cellular responses to L-selectin activation. In neutrophils, activation of L-selectin by antibody cross-linking or sulfatides induces a variety of responses, including calcium flux,[61] upregulation of surface-expressed Mac-1[62,63] and activation of the respiratory burst.[62] L-selectin activation has also been shown to potentiate neutrophil responses to other stimuli, such as formyl-Met-Leu-Phe (fMLP)[62] and

interleukin-8 (IL-8).[63] These responses to L-selectin cross-linking are not restricted to neutrophils, since L-selectin activation also induces calcium flux in peripheral blood mononuclear cells (PBMC)[64] and functionally upregulates β_1^{36} and β_2^{35} integrins in naive, but not memory, T lymphocytes.

Following the discovery that L-selectin activation leads to functional changes in leukocytes, groups began to investigate the intracellular signalling events leading to these changes. In neutrophils, L-selectin cross-linking induces tyrosine phosphorylation[65] as well as activation of the mitogen-activated protein kinases (MAP kinases). Specifically, there is evidence for Erk 1 and 2 activation[63,65] and p38 activation,[63] with p38 activity required for upregulation of Mac-1 surface expression.[63] In Jurkat T cells, L-selectin stimulation activates the Src-family kinase $p56^{lck}$. This initiates a signalling cascade involving L-selectin phosphorylation, recruitment of signalling molecules Grb2/Sos, and activation of Ras, Erk 1 and 2, and Rac2. Functional activity of $p56^{lck}$, Ras, and Rac2 are all needed for superoxide generation.[66] L-selectin cross-linking also leads to $p56^{lck}$-dependent activation of JNK MAP kinase,[67] as well as $p56^{lck}$-independent activation of a neutral sphingomyelinase.[68]

In contrast to L-selectin, little is known about the roles of P- and E-selectin as signalling receptors. Cross-linking of P-selectin induces Ca^{2+} transients[69] in HUVEC, suggesting that P-selectin can transduce signals across the plasma membrane. The observation that P-selectin constitutively associates with $pp60^{src}$ in platelets further supports a signalling role for P-selectin.[70] E-selectin cross-linking in HUVEC induces Ca^{2+} transients, actin polymerization,[69] and tyrosine phosphorylation of E-selectin.[71] Phosphorylated E-selectin recruits the tyrosine phosphatase SHP2, leading to Erk 1 and 2 activation.[71]

In addition to functioning as signalling molecules, selectins can also induce signals in adherent cells via interactions with selectin ligands (Figure 2). Adhesion to activated platelets induces P-selectin-dependent responses such as superoxide generation[72] and β_2-integrin activation[73] in leukocytes. These results suggest that P-selectin induces signalling in adherent cells; however, they do not rule out the possibility that P-selectin merely serves to bring platelets and leukocytes into close proximity, thereby facilitating leukocyte responses to other platelet-derived molecules such as chemokines. Simplified systems that use recombinant P-selectin or cross-linking of P-selectin ligands in place of adhesion to platelets have been used to address this issue. Using these systems, it has been shown that P-selectin directly induces functional responses in neutrophils and monocytes, including gene expression[74] and cytokine production.[74,75] Additionally, adhesion to P-selectin potentiates responses to secondary stimuli such as PAF[76] and chemokines.[77] Taken together, these data strongly indicate that P-selectin directly induces functional responses in adherent cells.

The intracellular signalling pathways activated in leukocytes by adhesion to P-selectin have only recently been studied. In neutrophils, cross-linking of PSGL-1 induces tyrosine phosphorylation and activation of the Ras signalling pathway,[75] while adhesion to P-selectin-expressing CHO cells activates the Src-family kinases $p54^{lyn}$ and $p56^{hck}$[78]. Adhesion of T lymphocytes to P-selectin also leads to tyrosine phosphorylation of a number of proteins, including the focal adhesion kinase $pp125^{FAK}$ and paxillin.[79] Signalling in response to P-selectin therefore remains an important area for further study.

Leukocyte responses to adhesion to E-selectin are similar to those observed upon adhesion to P-selectin. Activation of the β_2-integrins in response to E-selectin binding has been reported for neutrophils adherent to IL-1β-stimulated HUVEC.[80] In monocytes, soluble E-selectin induces chemotaxis and tyrosine phosphorylation of a number of signalling proteins, including $pp60^{src}$, $p54^{lyn}$ and $p56^{hck}$, Erk 1 and 2, and p38.[81]

Over the last few years, our understanding of the signalling roles of the selectins has grown tremendously. The ability of selectins to function as bi-directional signalling molecules, thereby initiating signalling cascades in both rolling leukocytes and the endothelium, raises many exciting possibilities. Instead of merely functioning to physically capture leukocytes from the bloodstream, it is likely that tethering and rolling via the selectins also serves to induce functional changes in leukocytes and endothelial cells that may be important for generating and regulating an inflammatory response. In leukocytes, adhesion via the selectins has been shown to activate β_2-integrins, which may promote firm adhesion and recruitment into inflamed tissues. Adhesion also primes for responses to chemotactic stimuli, which may facilitate migration through the tissues, or enhance leukocyte effector functions once the site of inflammation has been reached. In endothelial cells, the consequences of selectin-mediated adhesion are less clear; however, they may include changes in intracellular junctions that promote leukocyte extravasation, or downregulation

of cell adhesion molecules, which would lead to a dampening of leukocyte recruitment. Additional research into the emerging field of selectin-mediated signalling will undoubtedly provide exciting insights into its importance in inflammation.

Conclusions

It has been over a decade since the initial identification of the selectins as a family of adhesion molecules. In that time, great advances in our understanding of these molecules have been made. Biochemical studies have lead to an understanding of the relationship between the structure and function of the selectins. Selectin ligands have been identified, and the requirements for selectin–ligand recognition have been extensively studied. Mice that are genetically deficient in one or more of the selectins have been generated, and have provided insight into the roles of the selectins in a variety of disease states. In spite of all of the new knowledge that has been acquired, much still remains for discovery. Perhaps the best example of this lies in the area of selectin-mediated signalling. This 'new frontier' in selectin science presents us with many new questions and promises to provide us with exciting new answers.

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