Selectins and other mammalian sialic acid-binding lectins

Ajit Varki

University of California, San Diego, La Jolla, California, USA

Several recently discovered mammalian cell adhesion proteins recognize and bind to sialic acid-containing ligands. Reports concerning the molecular specificities of these interactions have been intriguing but somewhat confusing, partly because of pitfalls in methodology or interpretation. Nevertheless, these protein–carbohydrate recognition phenomena are important in the normal biology of blood cells and in the pathophysiology of many diseases.

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Introduction

It has long been believed by glycobiologists that the specific types of sugar chains found on mammalian cell surfaces must be involved in specific cell–cell interactions. Until recently, such recognition was reported mainly between mammalian oligosaccharides and noxious agents such as viruses, bacteria and toxins. These interactions are usually not for the benefit of the mammalian organism.

Sialic acids are usually the outermost sugars on mammalian cell surfaces. As such, they can specifically or non-specifically repel cell–cell interactions because of their negative charge. In contrast, several recently discovered adhesion proteins specifically recognize and bind to sugar chains with sialic acids as critical components. Many excellent reviews have been published concerning the best studied group of sialic acid-binding lectins, the selectins, and are not referred to here because of bibliography size limits. This brief review focuses only upon some recent progress in this area. Particular attention is given to the sialyloligosaccharide ligands for the selectins, the pitfalls in studying them, and the multi-step nature of the cell–cell interactions in which selectins are involved. The reader is referred to the original literature cited for details about these and other related studies.

Structure and nomenclature

The cloning of three independently discovered adhesion proteins uncovered a common sequence motif (Fig. 1) [1–5,6•]. The amino-terminal lectin-like domains of each predicted that they would recognize specific carbohydrates, and this has indeed turned out to be the case. Given the convergent nature of this field, there are many different names for these proteins (Table 1). Recently, a consensus nomenclature was reached for the three homologous molecules, which are now called the selectins [7••].

The CD22β lectin [8•,9••] and Factor H of the alternate complement pathway [10] also recognize sialylated ligands, but bear no obvious homology to one another, nor to any of the selectins. With the other known sialic acid-binding lectins (the macrophage sialoadhesin [11•,12••], the ganglioside-binding protein [13••] and the placental lectin [14]), no primary sequence information is yet available. Each stand on their own at present, and bear names assigned by their discoverers. Some general features of each of these lectins are summarized in Table 1 and Fig. 2.

Selectins and their oligosaccharide ligands: biosynthesis and structure–function relationships

Because sialic acids themselves are widespread, the specific binding of these lectins must involve additional features of the underlying oligosaccharide structure. In the past two years, there have been a steady stream of papers concerning the oligosaccharide ligands recognized by the selectin family [15–46]. The declarative titles of some of these articles might lead one to believe that final answers have been found. However, upon further reading even an afficionado of oligosaccharide structure will admit to bewilderment at the plethora of information and opinions concerning this issue. For example, published opinions range from the suggestion that the simple tetrasaccharide sialyl-Lewis^x (see Fig. 3) is both necessary and sufficient for biologically relevant binding of the E- and P-selectins, to those that indicate requirements for much more complex recognition motifs. At least some of the confusion may arise from assuming that the well recognized principles of protein–protein interactions can be extrapolated directly to oligosaccharide-protein binding.

Abbreviations

CHO— Chinese hamster ovary; **I-CAM**— intracellular adhesion molecule.

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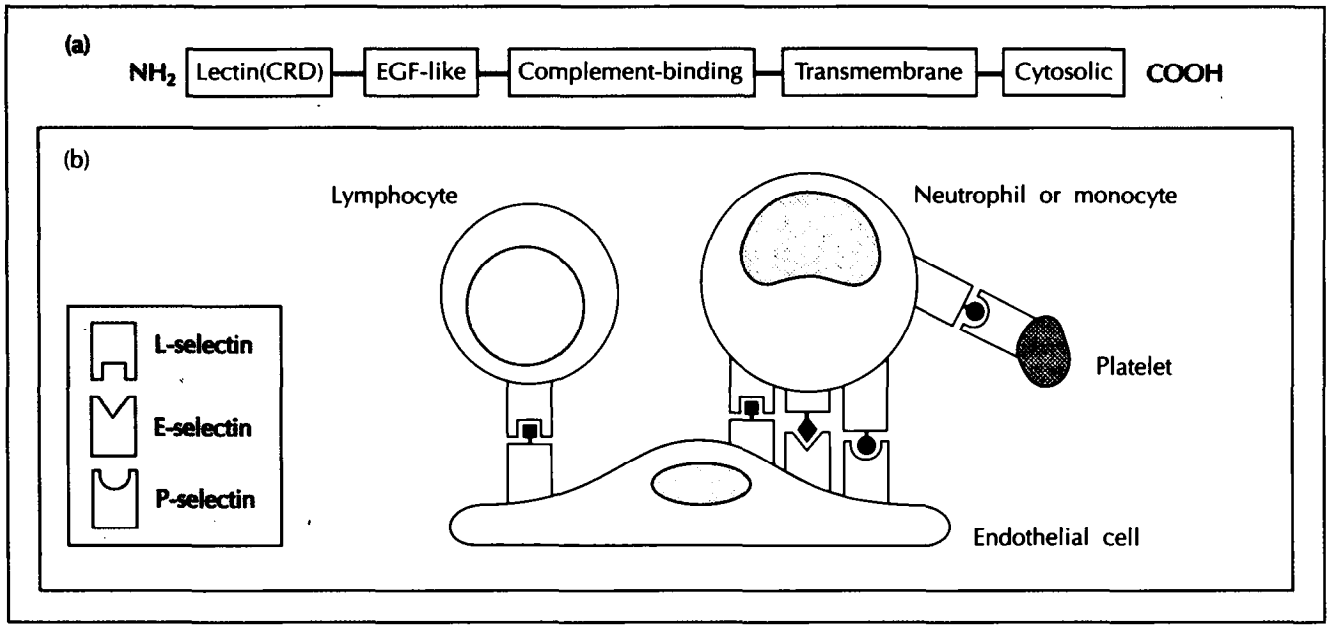

Fig. 1. The selectin family of cell adhesion proteins showing the common sequence motifs (a). The cell types that express the selectins under resting and/or stimulated states are also shown (b). The general nature of the oligosaccharides known to be involved in receptor-binding are indicated: ♦, sialylated fucosylated oligosaccharides (attached to ?); ♦, sialylated fucosylated oligosaccharides (attached to ?); ▮, sialylated (sulphated? fucosylated?) oligosaccharides (attached to ?). CRD, carbohydrate-recognition domain; EGF, epidermal growth factor.

Table 1. The sialic-acid-binding mammalian lectins.

Receptor (synonyms)	Location of the receptor	Location of the ligand	Structural features necessary for binding	Proposed functions
L-selectin (MEL-14 Ag, gp90MEL, LAM-1, Leu8, Ly22, TQ1, DREG.56)	Lymphocytes, neutrophils, monocytes, eosinophils	Endothelial cells (constitutive or stimulated expression)	Sialic acid(s), fucose residues?, sulfate esters?, phosphate esters?, (on a specific glycoprotein?)	Lymphocyte recirculation into lymph nodes. Neutrophil 'rolling' prior to extravasation?
E-selectin (ELAM-1)	Activated or chronically inflamed endothelial cells	Neutrophils, monocytes and certain lymphocytes	Sialylated fucosylated lactosamine chains including sialyl-Lewis^x and sialyl-Lewis^a (on glycolipids or glycoproteins?)	Neutrophil adhesion to activated endothelium. Entry of certain lymphocyte subsets into inflamed areas
P-selectin (GMP-140, PADGEM, CD62)	Platelets, endothelial cells (stored in granules)	Neutrophils, monocytes and certain lymphocytes	Sialylated fucosylated lactosamine chains, including sialyl-Lewis^x and sialyl-Lewis^a (?on a specific myeloid glycoprotein)	Neutrophil adhesion to activated platelets and endothelium. 'Rolling' of neutrophils prior to extravasation?
CD22β	B cells	Certain T cells, activated B cells	Sialic acids on CD45RO, sialic acids of CD-75, α2-6-linked sialic acids	Facilitates interactions between lymphocytes during response to antigens?
Sialoadhesin (SER, SAR)	Bone marrow and lymph node macrophages	Immature myeloid cells, lymphoid cells?	Sialic acids on O-linked oligosaccharides or gangliosides Siaα2-3Galβ1-3GalNAc1-R	Facilitates role of macrophages in supporting development of hematopoeitic cells?
H protein of the alternate complement pathway	Soluble in circulating plasma	'Non-activating' cell surfaces	Sialic acids on a variety of glycoconjugates (proper binding requires the glycerol-like side-chain of sialic acids?)	Facilitates continued access of the H protein to C3b on cell surfaces, preventing amplification
Ganglioside-binding protein	Membrane-associated in myelin sheaths?	Neuronal cells?	Sialic acids on neural gangliosides	Organization of myelin??
Human placental lectin	?Soluble in matrix	Placental cells?	Sialic acids on gangliosides or glycoproteins (preference for O-acetylated sialic acids)	Unknown

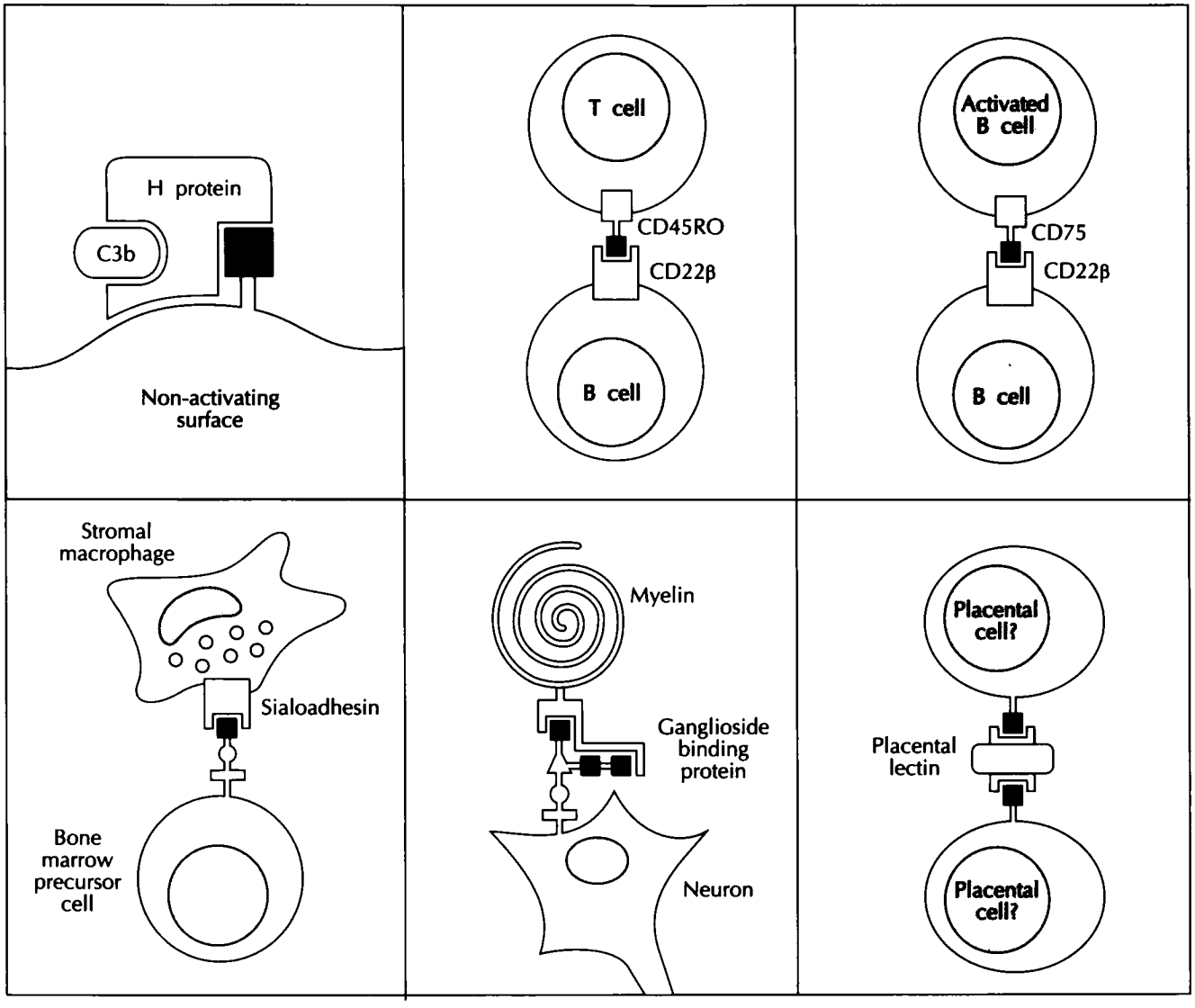

Fig. 2. Mammalian sialic acid-binding proteins other than the selectins. The cell types or tissues known to carry the receptors and the ligands are shown. Details of the minimal ligand structure recognized for each case are not shown. ■, sialic acid.

Lessons from the past about lectins

Past studies of plant and animal lectins have shown some distinctive features of protein–oligosaccharide interactions. First, defining the binding specificity of a lectin in terms of the simplest monosaccharide or oligosaccharide recognized is useful (e.g. M6P receptors recognize mannose-6-phosphate). However, the biologically relevant ligands tend to be much more complex (mannose-6-phosphate has an association constant in the micromolar range for the M6P receptors). Thus, the identification of sialylated fucosylated lactosaminoglycans (such as sialyl-Lewis^x) as ligands for the selectins may represent only a first step in the right direction.

Second, multivalency is the rule rather than the exception in lectin binding and can involve multiple branches of a single oligosaccharide, multiple oligosaccharides on a single macromolecule, or multiple oligosaccharides on multiple macromolecules. This gives rise to a spectrum of possible interactions, ranging from trivial to very high-affinity binding. Which of these is biologically relevant may well depend upon the situation under consideration.

Third, the precise conditions used to study the interactions (time, temperature, shear force, etc.) can greatly affect lectin-binding, and hence the interpretation of its significance.

Fourth, apparently unrelated oligosaccharides can inhibit the same lectin-like interactions because some aspects of their structure in free solution mimic one another.

What do saccharide inhibition studies tell us?

Although inhibition studies with defined oligosaccharides are very useful, they must be interpreted with caution. The plethora of saccharides that inhibit L-selectin binding are a case in point. On the one hand, they do reinforce the notion that carbohydrates are critical in the interaction. On the other hand, if interpreted liberally, they could lead to the conclusion that the natural ligand must contain fucose-3-sulfate, mannose-6-phosphate, α-linked sialic acids and ceramide-linked galactose-3-sulfate. Some of these may well turn out to be present in the native lig-

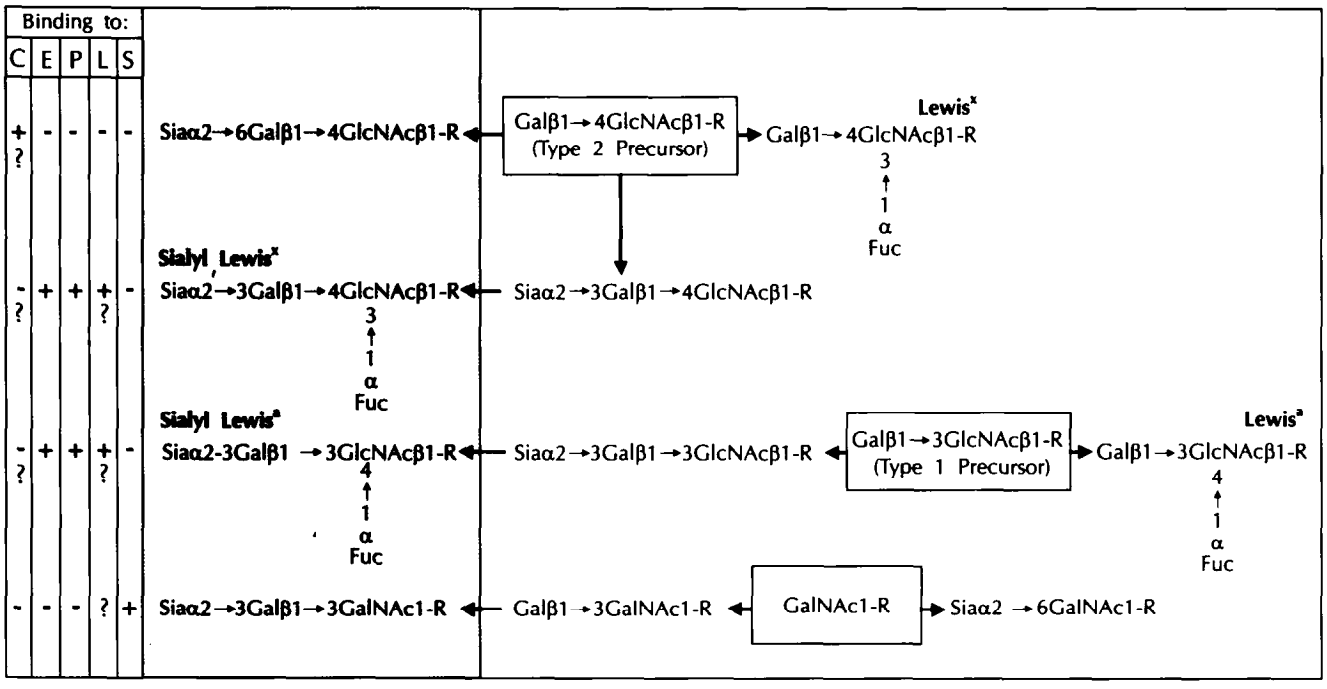

Fig. 3. Some sialyl-oligosaccharides recognized by the sialic acid-binding proteins. The minimal terminal sequences known to be recognized by various sialic-acid binding mammalian lectins are shown in the boxed area. C, CD22β; E, E-selectin; L, L-selectin; P, P-selectin; S, sialoadhesin. The immediate biosynthetic precursor structures of each are indicated, along with a few of the possible alternative biosynthetic pathways. Fuc, fucose; Gal, galactose; GalNAc, N-acetylgalactosamine; GlcNAC, N-acetylglucosamine; R, core sugar chain, Sia, sialic acid.

and, but others are probably acting as structural mimics in the inhibition experiments. However, recent evidence does suggest that sulfated oligosaccharides may be recognized by selectins with a calcium-independent binding site, separate from that which recognizes the sialylated ligands [47,48•]. If so, exploring the natural ligands for these receptors will be even more complicated.

Difficulties in using antibodies against carbohydrates

The exquisite specificities of monoclonal antibodies have been used successfully to study the importance of particular regions of polypeptides in cell–cell interactions. However, this approach is less useful with anti-carbohydrate antibodies. Most such antibodies are IgMs, and by sheer size are likely to block much more than just their cognate ligands. Further, the widespread distribution of the cognate oligosaccharide antigens on surface proteins and lipids can result in a very high surface density of the applied antibody. Thus, controls that result in a similar surface density of class-matched antibodies are required.

High densities can obscure ligand specificity

Purified receptors or ligands in immobilized form (e.g. on plastic surfaces) have allowed identification of many biologically important binding reactions. Likewise, transient transfection experiments can provide high levels of specific receptors expressed on COS cells. These two approaches have even been elegantly combined by overlaying COS cells overexpressing a selectin onto purified glycolipids on thin-layer chromatography plates. However, it is somewhat difficult to use data from such experiments to predict the precise nature of the biologically relevant ligand. This is because unnaturally high densities of the receptor and/or ligand are likely to be present. Thus, mutant or transfected Chinese hamster ovary (CHO) cells expressing high levels of sialylated fucosylated lactosaminoglycans bind quite well to plastic surfaces coated with the P-selectin, and also to COS cells over-expressing this receptor. This whole-cell binding is indistinguishable from that of neutrophils or HL-60 cells carrying the biologically relevant natural ligand for the selectin. However, direct binding studies indicate that the CHO cells simply have very high levels of low-affinity non-saturable binding sites, whereas the myeloid cells have high-affinity, saturable binding sites. In fact, the latter account for only a very small portion of the sialylated oligosaccharides on the myeloid cell surface, and may be present on a single glycoprotein (Moore KL, Stultz NL, Smith DL, Cummings RC, Varki A, McEver RP: *Blood* 1991 [abstract], **78**:108a). The challenge then is to discover the precise structure of such high-affinity ligand(s) for each selectin.

Inhibition studies with glycolipids

Inhibition of lectin-binding by pure glycolipids has also been used to define the nature of the cognate oligosaccharide ligands. Dramatic differences in the inhibitory capacity of various structures are seen, and apparent inhibition constants in the nanomolar range suggest that certain sugar sequences are both necessary and sufficient for biologically relevant binding. However, pure sialylated glycolipids (gangliosides) have very low critical micellar

concentrations and hence usually exist as micelles. This results in a high-density, functionally multivalent presentation of the oligosaccharide ligand. In the natural situation, however, gangliosides usually represent only a small percentage of the total cell-surface lipid, and thus could not easily present themselves at such high densities. On the other hand, it must be admitted that domain formation or clustering of gangliosides could occur in specialized areas of a cell surface.

Using glycosidases on intact cells

Treating cells with glycosidases is a useful way to explore the specificities of lectin-binding. However, many commercially available glycosidases contain traces of proteases and other glycosidases. This is not usually of concern when they are used to sequence pure oligosaccharides, because small amounts can be used for short periods of time and the consequences can be determined directly by chemical analysis. However, extensively removing a specific type of sugar from a complex cell-surface 'glycocalyx' is virtually impossible, even using large quantities of enzymes and/or extended incubation periods. This is like trying to eradicate a specific type of plant from the Amazon jungle using a squadron of helicopters with flame throwers. The mission is likely to be only partially successful and some collateral damage can be expected. Additionally, certain sialidase preparations (e.g. the α2-3-specific sialidase from New Castle Disease virus) consist of entire virions, and steric hindrance to enzyme action on cell surfaces is of even more concern. Furthermore, such preparations also have a hemagglutinating activity, which could result in spurious cell–cell adhesion. In spite of these problems, sialidases have played a useful role in exploring the binding of most of the lectins discussed here. One way to improve specificity is to carry out a control experiment in which a specific sialidase inhibitor is added. However, this requires careful titration of the concentrations of the enzyme and the inhibitor.

Generating specificity from common structures

Although sialylated, fucosylated lactosaminoglycans are found in abundance on the cells recognized by the selectins [49••,50•], they are also found on many other cell types and secreted proteins. How can such a relatively common structural motif be used to make specific ligands for the selectins? One explanation is that physical barriers limit their availability; e.g. the oligosaccharides found on many epithelial glycoproteins would not be encountered by the intra-vascularly expressed selectins, except in pathological circumstances such as cancer. However, it is more likely that specificity might be provided by defined spacing and/or presentations of the relevant oligosaccharides on specific protein carriers. It is also possible that portions of a relevant glycoprotein carrier might serve as direct contact points for enhancing the specificity of binding. Finally, it is possible that a common oligosaccharide structure could become more specialized (e.g. by sulfation, or modifications of the sialic acids).

Sialic acids: a diverse family

Most studies equate 'sialic acid' with the common sugar N-acetyl-neuraminic acid. The sialic acids are actually a diverse family of acidic sugars, which have in common only a nine-carbon backbone and an α-linkage. This diversity is largely ignored in studies of sialyloligosaccharides, primarily for technical reasons. However, there are clear examples where specific types of the sialic acids can mediate specific binding phenomena (see [51] for review). Thus, it is possible that the binding specificities of some of these sialic-binding proteins could be determined, modulated or diversified by changes in the type of sialic acid in the natural ligands.

Studies of cell–cell interactions *in vitro* and *in vivo*

Many studies of cell–cell interactions involving the selectins have been published recently [52–85] and cannot be described individually here. However, some general principles that emerge are discussed below.

Measuring adhesion: the importance of motion

Until recently, most assays for adhesion were performed in static systems. However, blood cells normally experience substantial shear forces in the vasculature. Under such conditions, the differences in on–off rates between lectin-like interactions and protein–protein interactions may be particularly important. Thus, although integrin-mediated adhesion plays a prominent role in static assays (particularly at physiological temperatures and even more so if the cells have been activated), the contributions of lectins are harder to detect under such conditions. Upon introducing a shear force (e.g. rocking, flow conditions), the multiple contributions of different adhesion molecules can be detected more specifically. However, in any such studies, interference with any one of the involved systems can result in complete loss of adhesion. Consider a rock-climber on a sheer cliff face, buffeted by strong winds. Each and every one of the climber's holds are critical and detachment of even one would be fatal.

Multiple steps and combinatorial possibilities

Although *in vitro* assays can be engineered to emphasize the contributions of single ligand–receptor pairs, the natural process by which a leukocyte passes through an endothelial barrier involves many interactive mechanisms. Current published data suggest that multiple sequential steps occur in leucocyte–endothelium interactions. Different combinations of these steps could account for the generation of specificity using a limited set of receptor–ligand and cytokine–receptor pairs. For example, current literature suggests that the following steps occur in acute neutrophil extravasation from the circulation: (i) activation of the endothelium and/or neutrophil by an exogenous agent; (ii) rapid expression of

the P-selectin on the activated endothelial surface; (iii) expression of an as yet unknown L-selectin ligand on the activated endothelium; (iv) an unexplained improvement in the binding affinity of the neutrophil L-selectin; (v) 'rolling' of the neutrophil on the endothelium, mediated by either or both of these selectin interactions; (vi) shedding of the L-selectin from the neutrophil surface; (vii) delivery of 'juxtacrine' signals between the endothelium and the neutrophil (e.g. platelet-activating factor, interleukin-8); (viii) an unexplained improvement in the binding affinity of the neutrophil integrins for endothelial intracellular adhesion molecule (I-CAM); (ix) arrest of the neutrophil on the endothelium; (x) recruitment of more integrin receptors to the neutrophil cell surface; (xi) new synthesis of E-selectin and I-CAM molecules by the endothelium, enhancing the binding of the neutrophils; and (xii) neutrophil spreading and invasion.

Which of these events actually occur in a given situation, in precisely what order, and how much they each contribute probably depends upon the inciting stimulus, the specific cell types involved and the overall setting.

Neutrophil extravasation can be harmful

In the normal animal, neutrophils constantly exit the circulation and destroy invading microorganisms. In active infections this process is accelerated and serves a vital role in the survival of the host. However, in many pathological states (including some induced by man-made manipulations), this process proceeds unchecked, resulting in unwanted tissue injury by the neutrophil. Because the selectins appear to be involved at an early phase of this extravasation, the possibility of blocking or attenuating these pathological processes has raised great excitement among biologists, synthetic chemists, pathologists, clinicians, biotechnologists and investors. In this regard, recent published studies in animal systems have been very promising [54•,66•,80••,81], providing strong grounds for such optimism.

Conclusions and future prospects

The excitement in the field of selectins is palpable, and with numerous academic laboratories and biotechnology companies jumping into the fray, progress will continue to be fast and furious. In fact, this review will no longer be 'current' by the time it is published. However, as with uncontrolled and excessively rapid DNA replication, the error rate is likely to continue to be high. Fortunately, the inevitable self-correcting mechanisms of science will come into play, and when the dust settles, the major issues should be resolved. Action in the following areas is likely to continue.

From the biologists point of view, it seems critical to first elucidate the exact nature of the natural ligand for these receptors. However, biotechnology companies will continue to generate synthetic ligands for the selectins, which could be used as soluble inhibitors in the whole animal. From the strictly pharmacotherapeutic point of view, what may be sufficient is a compound with favourable pharmacology that will effectively inhibit the interaction. How closely such a compound is related to the natural ligand is of interest, but is not immediately critical.

The role of the selectins in leucocyte traffic is clear in acute inflammatory situations, and evidence for their role in chronic inflammatory states has been forthcoming. Because the half-life of some cells (e.g. the neutrophil) is very short even under normal circumstances, the question arises of whether the same mechanisms are operational in regulating normal neutrophil traffic.

The mechanisms for temporal and spatial regulation of expression of these receptors await further exploration. In the case of their cognate oligosaccharide ligands, the regulation of the relevant glycosyltransferases and the protein scaffolds that carry the sugars requires investigation.

Because the cytoplasmic tails of the selectin molecules are highly conserved, they could be involved in delivering signals upon binding. If the selectin ligands indeed turn out to be carried by specific proteins, it is also possible that these molecules deliver signals to the cells carrying them.

With the limited number of receptor–ligands for blood cells and endothelia described so far, it is hard to explain the extent of specificity seen in leucocyte homing to specific tissues, under specific conditions. Various combinatorial possibilities between the multiple steps in leucocyte–endothelium interaction could be responsible for generating a range of distinct interaction sets. This may explain the many different types of leucocyte homing that can occur, using a few ligand–receptor pairs, and a few juxtacrine signals. Exploration of such combinatorial possibilities is likely to continue.

Of the receptors described in this review, some were initially identified as lectins, primarily on the basis of their amino acid sequence. It is safe to predict that more such 'reverse glycobiology' [86] will occur, and that the family of sialic acid-binding proteins will expand. If this happens, defining the fine specificities of the sialyloligosaccharide ligands for each receptor will become even more important.

References and recommended reading

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An attempt is made to reach a consensus regarding nomenclature of one of the lectins. The acronym LECAM is then extended to the other two homologous proteins, namely, LECAM-2 and LECAM-3. However, this has now been abandoned in favour of 'selectins' (see [7••]).

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Yet another of a classic group of papers that first reported a role for the sialyl-Lewis^x motif in recognition by the E-selectin (ELAM-1).

26. •• TRUE DD, SINGER MS, LASKY LA, ROSEN SD: **Requirement for Sialic Acid on the Endothelial Ligand of a Lymphocyte Homing Receptor.** *J Cell Biol* 1990, 111:2757–2764.

Closes the loop on the seminal observation made by the same group in 1985, that sialic acid must be important in the recognition of high endothelial venules of lymph nodes by circulating lymphocytes. In this instance, the requirement for sialic acid is demonstrated directly using a recombinant soluble form of the L-selectin.

27. • MOORE KL, VARKI A, MCEVER RP: **GMP-140 Binds to a Glycoprotein Receptor on Human Neutrophils: Evidence for a Lectin-like Interaction.** *J Cell Biol* 1991, 112:491–499.

Direct evidence for a small number of high-affinity binding sites for the P-selectin (GMP-140) on myeloid cells. This study also shows that sialic acid is required for binding and that the Lewis^x motif (see [19]) cannot itself be sufficient for recognition.

28. • KAMEYAMA A, ISHIDA H, KISO M, HASEGAWA A: **Total Synthesis of Sialyl Lewis X.** *Carbohydr Res* 1991, 209:c1–c4.

Another example of efforts by synthetic chemists to produce the minimum ligands for the selectins.

29. •• BERG EL, ROBINSON MK, MANSSON O, BUTCHER EC, MANGNANI JL: **A Carbohydrate Domain Common to Both Sialyl Le^a and Sialyl Le^x is Recognized by the Endothelial Cell Leukocyte Adhesion Molecule ELAM-1.** *J Biol Chem* 1991, 266:14869–14872.

A surprising and counter-intuitive observation that sialyl-Lewis^a (an isomer of sialyl-Lewis^x; see Fig. 2) is recognized equally well by the E-selectin. The result is rationalized by model building which indicates that the two isomers can present a similar face to the receptor. This may help to explain some disparate results concerning the recognition of different cell types by the E-selectin, and underscores the potential importance of sialyl Lewis^a antigen expression in malignant cells. It remains to be seen if this observation also applies to the other two selectins.

30. • POLLEY MJ, PHILLIPS ML, WAYNER E, NUDELMAN E, SINGHAL AK, HAKOMORI S, PAULSON JC: **CD62 and Endothelial Cell-Leukocyte Adhesion Molecule 1 (ELAM-1) Recognize the Same Carbohydrate Ligand, Sialyl-Lewis x.** *Proc Natl Acad Sci U S A* 1991, 88:6224–6228.

Demonstrates that the cognate ligands for two of the selectins have much in common and could be identical. However, the conclusions are based predominantly upon inhibition studies with multivalent glycolipids and anti-carbohydrate antibodies.

31. •• BERG EL, ROBINSON MK, WARNOCK RA, BUTCHER EC: **The Human Peripheral Lymph Node Vascular Addressin is a Ligand for LECAM-1, the Peripheral Lymph Node Homing Receptor.** *J Cell Biol* 1991, 114:343–349.

A family of glycoproteins recognized by the MECA-79 antibody molecules is purified. Although the quantities obtained are low, the material allows specific binding of lymphocytes, and the interaction is abolished by sialidase treatment.

32. •• IMAI Y, SINGER MS, FENNIE C, LASKY LA, ROSEN SD: **Identification of a Carbohydrate-based Endothelial Ligand for a Lymphocyte Homing Receptor.** *J Cell Biol* 1991, 113:1213–1222.

Isolation of sulfated, sialylated glycoproteins from metabolically labelled lymph nodes that fulfil all criteria for being the cognate ligands for the L-selectins. The challenge now is to decipher the specific sialylated oligo-saccharide sequences on the major 50 kD protein that are responsible for the specific binding.

33. • PICKER LJ, WARNOCK RA, BURNS AR, DOERSCHUK CM, BERG EL, BUTCHER EC: **The Neutrophil Selectin LECAM-1 Presents Carbohydrate Ligands to the Vascular Selectins ELAM-1 and GMP-140.** *Cell* 1991, 66:921–933.

It is suggested that the L-selectin on neutrophils selectively carries the primary oligosaccharide ligands for the P- and E-selectins. However, this cannot be reconciled with the fact that this molecule is rapidly lost from activated neutrophils, which continue to be bound quite well by the E- and P-selectins

34. PIGOTT R, NEEDHAM LA, EDWARDS RM, WALKER C, POWER C: **Structural and Functional Studies of the Endothelial Activation Antigen Endothelial Leukocyte Adhesion Molecule-1 Using a Panel of Monoclonal Antibodies.** *J Immunol* 1991, 147:130–135

35. •• LOWE JB, KUKOWSKA-LATALLO JF, NAIR RP, LARSEN RD, MARKS RM, MACHER BA, KELLY RJ, ERNST LK: **Molecular Cloning of a Human Fucosyltransferase Gene that Determines Expression of the Lewis x and VIM-2 Epitopes but not ELAM-1-dependent Cell Adhesion.** *J Biol Chem* 1991, 266:17467–17477.

Underscores the importance of different fucosyltransferases with differing substrate specificities in determining specific recognition by the selectins. Shows that the fucosyltransferase expressed by the ELFT gene (see [23]) could not have generated high-affinity recognition by the E-selectin, and that the VIM-2 structure (see [21]) is not a very good ligand.

36. •• ZHOU Q, MOORE KL, SMITH DF, VARKI A, MCEVER RP, CUMMINGS RD: **The Selectin GMP-140 Binds to Sialylated, Fucosylated Lactosaminoglycans on Both Myeloid and Nonmyeloid Cells.** *J Cell Biol* 1991, 115:557–564.

Further evidence that the non-sialylated Lewis^x determinant cannot be the specific ligand for P-selectin (GMP-140). Expression of sialylated, fucosylated lactosaminoglycans is sufficient to induce binding of the P-selectin. However, high-affinity binding is only seen with myeloid cells. This indicates that such sugar sequences are necessary, but not sufficient to mediate biologically relevant binding by the P-selectin.

37. • HUANG K, GEOFFROY JS, SINGER MS, ROSEN SD: **A Lymphocyte Homing Receptor (L-Selectin) Mediates the *In Vitro* Attachment of Lymphocytes to Myelinated Tracts of the Central Nervous System.** *J Clin Invest* 1991, 88:1778–1783.

An intriguing observation that cognate ligands for the L-selectin are not confined to the endothelium.

38. • SKACEL PO, EDWARDS AJ, HARRISON CT, WATKINS WM: **Enzymic Control of the Expression of the x Determinant (CD15) in Human Myeloid Cells During Maturation: the Regulatory Role of 6′-Sialyltransferase.** *Blood* 1991, 78:1452–1460.

Underscores the importance of branch points in the biosynthesis of oligosaccharides in determining the final product synthesized by a given cell.

39. TAKADA A, OHMORI K, TAKAHASHI N, TSUYUOKA K, YAGO A, ZENITA K, HASEGAWA A, KANNAGI R: **Adhesion of Human Cancer Cells to Vascular Endothelium Mediated by a Carbohydrate Antigen, Sialyl Lewis A.** *Biochem Biophys Res Commun* 1991, 179:713–719.

40. WATSON SR, IMAI Y, FENNIE C, GEOFFREY J, SINGER M, ROSEN SD, LASKY LA: **The Complement Binding-like Domains of the Murine Homing Receptor Facilitate Lectin Activity.** *J Cell Biol* 1991, 115:235–243.

41. • KANSAS GS, SPERTINI O, STOOLMAN LM, TEDDER TF: **Molecular Mapping of Functional Domains of the Leukocyte Receptor for Endothelium, LAM-1.** *J Cell Biol* 1991, 114:351–358.

Studies using truncated constructs and monoclonal antibodies suggest that other domains of the L-selectin may be important in determining binding. In some cases, it is difficult to distinguish non-specific effects on conformation of the protein caused by the truncations from specific changes in regions critical for the interactions (see also [40]).

42. • MONTGOMERY KF, OSBORN L, HESSION C, TIZARD R, GOFF D, VASSALLO C, TARR PI, BOMSZTYK K, LOBB R, HARLAN JM: **Activation of Endothelial-Leukocyte Adhesion Molecule 1 (ELAM-1) Gene Transcription.** *Proc Natl Acad Sci U S A* 1991, 88:6523–6527.

A first attempt to explore the critical issues regarding the regulation of selectin expression.

43. •• KUMAR R, POTVIN B, MUELLER WA, STANLEY P: **Cloning of a Human α (1,3)-Fucosyltransferase Gene that Encodes ELFT but does not Confer ELAM-1 Recognition on Chinese Hamster Ovary Cell Transfectants.** *J Biol Chem* 1991, 266:21777–21783.

A laboratory experienced in the biology of fucosyltransferases enters the fray. This study reaches similar conclusions to Lowe *et al.* [35••].

44. •• TYRRELL D, JAMES P, RAO N, FOXALL C, ABBAS S, DASGUPTA F, NASHED M, KASEGAWA A, KISO M, ASA D, KIDD J, BRANDLEY BK: **Structural Requirements for the Carbohydrate Ligand of E-Selectin.** *Proc Natl Acad Sci U S A* 1991, 88:10372–10376.

Careful studies aimed at delineating the minimal structural requirements for recognition by the E-selectin, including specific groups on individual monosaccharide components.

45. LEEUWENBERG JFM, TAN A, JEUNHOMME TMAA, PLOEGH HL, BUURMAN WA: **The Ligand Recognized by ELAM-1 on HL60 Cells is not Carried by N-linked Oligosaccharides.** *Eur J Immunol* 1991, 21:3057–3059.

46. • BERG EL, YOSHINO T, ROTT LS, ROBINSON MK, WARNOCK RA, KISHIMOTO TK, PICKER LJ, BUTCHER EC: **The Cutaneous Lym-**

phocyte Antigen is a Skin Lymphocyte Homing Receptor for the Vascular Lectin Endothelial Cell-leukocyte Adhesion Molecule 1. *J Exp Med* 1991, 174:1461–1466.
E-selectin-mediated recognition of a specific subset of lymphocytes, which do not appear to carry the sialyl-Lewis^x determinant (as determined by a monoclonal antibody). However, sialidase treatment generates the core Lewis^x determinant.

47. SKINNER MP, LUCAS CM, BURNS GF, CHESTERMAN CN, BERENDT MC: **GMP-140 Binding to Neutrophils is Inhibited by Sulfated Glycans.** *J Biol Chem* 1991, 266:5371–5374.

48. • ARUFFO A, KOLANUS W, WALZ G, FREDMAN P, SEED B: **CD62/P-Selectin Recognition of Myeloid and Tumor Cell Sulfatides.** *Cell* 1991, 67:35–44.
Evidence that the P-selectin can recognize and bind to a small sulfated glycolipid that does not contain sialic acid. The binding appears to be calcium-independent, however, and may represent a distinct recognition pathway.

49. •• FUKUDA M: **Leukosialin, a Major *O*-Glycan-containing Sialylglycoprotein Defining Leukocyte Differentiation and Malignancy.** *Glycobiology* 1991, 1:347–356.
An up-to-date review, including discussion of the expression of lactosaminoglycans on myeloid cells.

50. • ASADA M, FURUKAWA K, KANTOR C, GAHMBERG CG, KOBATA A: **Structural Study of the Sugar Chains of Human Leukocyte Cell Adhesion Molecules CD11/CD18.** *Biochemistry* 1991, 30:1561–1571.
The sialyl-Lewis^x motif is present on a neutrophil adhesion protein of the integrin family. The possibility of a tripartite recognition system involving a cross-over between the selectin and integrin families must be considered.

51. VARKI A: **Diversity in the Sialyic Acids.** *Glycobiology* 1992, 2:25–40.

52. GENG JG, BEVILACQUA MP, MOORE KL, MCINTYRE TM, PRESCOTT SM, KIM JM, BLISS GA, ZIMMERMAN GA, MCEVER RP: **Rapid Neutrophil Adhesion to Activated Endothelium Mediated by GMP-140.** *Nature* 1990, 343:757–760.

53. GAMBLE JR, SKINNER MP, BERNDT MC, VADAS MA: **Prevention of Activated Neutrophil Adhesion to Endothelium by Soluble Adhesion Protein GMP140.** *Science* 1990, 249:414–417.

54. • WATSON SR, FENNIE C, LASKY LA: **Neutrophil Influx into an Inflammatory Site Inhibited by a Soluble Homing Receptor-IgG Chimera.** *Nature* 1991, 349:164–167.
Demonstration in the intact animal of the potential role of the L-selectin in determining early neutrophil exit into inflammatory sites. The results predict that the L-selectin ligand must be expressed by acutely inflamed endothelia.

55. • SMITH CW, KISHIMOTO TK, ABBASS O, HUGHES B, ROTHLEIN R, MCINTIRE LV, BUTCHER E, ANDERSON DC: **Chemotactic Factors Regulate Lectin Adhesion Molecule 1 (LECAM-1)-dependent Neutrophil Adhesion to Cytokine-stimulated Endothelial Cells *In Vitro*.** *J Clin Invest* 1991, 87:609–618.
One of three related papers by the same group (see also [69]) that conclude that the L-selectin (LECAM-1) must be involved in the integrin-independent binding of neutrophils to activated endothelium.

56. •• PICKER LJ, KISHIMOTO TK, SMITH CW, WARNOCK RA, BUTCHER EC: **ELAM-1 is an Adhesion Molecule for Skin-homing T Cells.** *Nature* 1991, 349:796–799.
Demonstration that the E-selectin (ELAM-1) is involved in the extravasation not only of phagocytic cells in acute inflammation, but also of a subpopulation of T cells during chronic inflammation.

57. • LUSCINSKAS FW, CYBULSKY MI, KIELY J-M, PECKINS CS, DAVIS VM, GIMBRONE MA JR: **Cytokine-activated Human Endothelial Monolayers Support Enhanced Neutrophil Transmigration Via a Mechanism Involving both Endothelial-leukocyte Adhesion Molecule-1 and Intercellular Adhesion Molecule-1.** *J Immunol* 1991, 146:1617–1625.
Careful dissection of the role of multiple adhesion systems in neutrophil–endothelium interactions.

58. KYAN-AUNG U, HASKARD DO, POSTON RN, THORNHILL MH, LEE TH: **Endothelial Leukocyte Adhesion Molecule-1 and Intercellular Adhesion Molecule-1 Mediate the Adhesion of Eosinophils to Endothelial Cells *In Vitro* and are Expressed by Endothelium in Allergic Cutaneous Inflammation *In Vivo*.** *J Immunol* 1991, 146:521–528.

59. •• SHIMIZU Y, SHAW S, GRABER N, GOPAL TV, HORGAN KJ, VAN SEVENTER GA, NEWMAN W: **Activation-independent Binding of Human Memory T Cells to Adhesion Molecule ELAM-1.** *Nature* 1991, 349:799–802.
Evidence that E-selectin (ELAM-1) is important in the steady-state recognition of memory T cells by chronically inflamed endothelium.

60. • KOCH AE, BURROWS JC, HAINES GK, CARLOS TM, HARLAN JM, LEIBOVICH SJ: **Immunolocalization of Endothelial and Leukocyte Adhesion Molecules in Human Rheumatoid and Osteoarthritic Synovial Tissues.** *Lab Invest* 1991, 64:313–320.
Evidence that E-selectin (ELAM-1) is expressed constitutively by endothelium in chronic arthritic diseases. Raises the hope that interference with the recognition of leucocytes could be of therapeutic value against these common and debilitating diseases.

61. • SPERTINI O, KANSAS GS, MUNRO JM, GRIFFIN JD, TEDDER TF: **Regulation of Leukocyte Migration by Activation of the Leukocyte Adhesion Molecule-1 (LAM-1) Selectin.** *Nature* 1991, 349:691–694.
This study shows that mysterious 'activation' events such as previously described for the integrins also occur with the L-selectin (LAM-1). This manifests itself as an improved affinity of pre-existing receptors on the cell surface. The rapidity of the effect is remarkable and supports the notion that it is biologically relevant. However, because PPME (a phosphorylated yeast mannan) was used instead of a sialylated ligand, the possibility that mannose-6-phosphate receptors were also being detected cannot be ruled out.

62. • PATEL KD, ZIMMERMAN GA, PRESCOTT SM, MCEVER RP, MCINTYRE TM: **Oxygen Radicals Induce Human Endothelial Cells to Express GMP-140 and Bind Neutrophils.** *J Cell Biol* 1991, 112:749–759.
Depending upon the type of stimulus, the kinetics of expression of the P-selectin on the cell surface can be very different.

63. • WONG CS, GAMBLE JR, SKINNER MP, LUCAS CM, BERNDT MC, VADAS MA: **Adhesion Protein GMP140 Inhibits Superoxide Anion Release by Human Neutrophils.** *Proc Natl Acad Sci U S A* 1991, 88:2397–2401.
Evidence that binding of a selectin may deliver a signal to the cells recognized.

64. KUIJPERS TW, HAKKERT BC, HOOGERWERF M, LEEUWENBERG JFM, ROOS D: **Role of Endothelial Leukocyte Adhesion Molecule-1 and Platelet-activating Factor in Neutrophil Adherence to IL-1-prestimulated Endothelial Cells: Endothelial Leukocyte Adhesion Molecule-1-mediated CD18 Activation.** *J Immunol* 1991, 147:1369–1374.

65. •• LAWRENCE MB, SPRINGER TA: **Leukocytes Roll on a Selectin at Physiologic Flow Rates: Distinction from and Prerequisite for Adhesion Through Integrins.** *Cell* 1991, 65:859–873.
An elegant *in vitro* study showing the role of shear force in determining the relative importance of the selectins and integrins on model membranes. The results fit well with studies carried out in intact systems.

66. • LEY K, GAEHTGENS P, FENNIE C, SINGER MS, LASKY LA, ROSEN SD: **Lectin-like Cell Adhesion Molecule 1 Mediates Leukocyte Rolling in Mesenteric Venules *In Vivo*.** *Blood* 1991, 77:2553–2555.
Direct demonstration of a role for the L-selectin in the 'rolling' of neutrophils prior to their arrest and extravasation from the blood stream. This implies that the L-selectin ligand is expressed rapidly on recently activated endothelial surfaces.

67. •• VON ANDRIAN UH, CHAMBERS JD, MCEVOY LM, BARGATZE RF, ARFORS K-E, BUTCHER EC: **Two-step Model of Leukocyte-Endothelial Cell Interaction in Inflammation: Distinct Roles for LECAM-1 and the Leukocyte b_2 Integrins *In Vivo*.** *Proc Natl Acad Sci U S A* 1991, 88:7538–7542.

A refinement of a model proposed earlier by this group in which the selectins and integrins act sequentially and cooperatively. The model is well supported by most other recent studies.

68. ANDERSON DC, ABBASSI O, KISHIMOTO TK, KOENIG JM, MCINTIRE LV, SMITH CW: **Diminished Lectin-, Epidermal Growth Factor-, Complement Binding Domain-cell Adhesion Molecule-1 on Neonatal Neutrophils Underlies Their Impaired CD18-independent Adhesion to Endothelial Cells *In Vitro*.** *J Immunol* 1991, 146:3372–3379.

69. KISHIMOTO TK, WARNOCK RA, JUTILA MA, BUTCHER EC, LANE C, ANDERSON DC, SMITH CW: **Antibodies Against Human Neutrophil LECAM-1 (LAM-1/Leu-8/DREG-56 Antigen) and Endothelial Cell ELAM-1 Inhibit a Common CD18-independent Adhesion Pathway *In Vitro*.** *Blood* 1991, 78:805–811.

70. LO SK, LEE S, RAMOS RA, LOBB R, ROSA M, CHI-ROSSO G, WRIGHT SD: **Endothelial-leukocyte Adhesion Molecule 1 Stimulates the Adhesive Activity of Leukocyte Integrin CR3 (CD11b/CD18, Mac-1, a_mb_2) on Human Neutrophils.** *J Exp Med* 1991, 173:1493–1500.

71. BOCHNER BS, LUSCINSKAS FW, GIMBRONE MA JR, NEWMAN W, STERBINSKY SA, DERSE-ANTHONY CP, KLUNK D, SCHLEIMER RP: **Adhesion of Human Basophils, Eosinophils, and Neutrophils to Interleukin 1-activated Human Vascular Endothelial Cells: Contributions of Endothelial Cell Adhesion Molecules.** *J Exp Med* 1991, 173:1553–1556.

72. CARLOS T, KOVACH N, SCHWARTZ B, ROSA M, NEWMAN B, WAYNER E, BENJAMIN C, OSBORN L, LOBB R, HARLAN J: **Human Monocytes Bind to Two Cytokine-induced Adhesive Ligands on Cultured Human Endothelial Cells: Endothelial-Leukocyte Adhesion Molecule-1 and Vascular Cell Adhesion Molecule-1.** *Blood* 1991, 77:2266–2271.

73. • SHIMIZU Y, NEWMAN W, GOPAL TV, HORGAN KJ, GRABER N, BEALL LD, VAN SEVENTER GA, SHAW S: **Four Molecular Pathways of T Cell Adhesion to Endothelial Cells: Roles of LFA-1, VCAM-1, and ELAM-1 and Changes in Pathway Hierarchy Under Different Activation Conditions.** *J Cell Biol* 1991, 113:1203–1212.
Dissection of the relative roles of three different systems in the adhesion of T-cells to endothelium.

74. WELLER PF, RAND TH, GOELZ SE, CHI-ROSSO G, LOBB RR: **Human Eosinophil Adherence to Vascular Endothelium Mediated by Binding to Vascular Cell Adhesion Molecule 1 and Endothelial Leukocyte Adhesion Molecule 1.** *Proc Natl Acad Sci U S A* 1991, 88:7430–7433.

75. • LEUNG DYM, POBER JS, COTRAN RS: **Expression of Endothelial-Leukocyte Adhesion Molecule-1 in Elicited Late Phase Allergic Reactions.** *J Clin Invest* 1991, 87:1805–1809.
Demonstration in human subjects that the local migration of inflammatory cells into allergic lesions may be mediated by cytokine-induced expression of E-selectin (ELAM-1).

76. •• HUBER AR, KUNKEL SL, TODD RF III, WEISS SJ: **Regulation of Transendothelial Neutrophil Migration by Endogenous Interleukin-8.** *Science* 1991, 254:99–102.
Demonstration of a key role for this cytokine in the 'cross-talk' between the endothelium and the neutrophil following the initial recognition events.

77. • SPERTINI O, LUSCINSKAS FW, KANSAS GS, MUNRO JM, GRIFFIN JD, GIMBRONE MA JR, TEDDER TF: **Leukocyte Adhesion Molecule-1 (LAM-1, L-Selectin) Interacts with an Inducible Endothelial Cell Ligand to Support Leukocyte Adhesion.** *J Immunol* 1991, 147:2565–2573.
Further evidence that an L-selectin ligand is detectable on activated endothelium. Assay conditions were critical in differentiating selectin-mediated binding from integrin-mediated binding.

78. ABBASSI O, LANE CL, KRATER S, KISHIMOTO TK, ANDERSON DC, MCINTIRE LV, SMITH CW: **Canine Neutrophil Margination Mediated by Lectin Adhesion Molecule-1 *In Vitro*.** *J Immunol* 1991, 147:2107–2115.

79. •• LORANT DE, PATEL KD, MCINTYRE TM, MCEVER RP, PRESCOTT SM, ZIMMERMAN GA: **Coexpression of GMP-140 and PAF by Endothelium Stimulated by Histamine or Thrombin: a Juxtacrine System for Adhesion and Activation of Neutrophils.** *J Cell Biol* 1991, 115:223–234.
A well documented demonstration of juxtacrine 'cross-talk' between activated endothelium and adherent neutrophils, involving platelet-activating factor.

80. •• MULLIGAN MS, VARANI J, DAME MK, LANE CL, SMITH CW, ANDERSON DC, WARD PA: **Role of Endothelial-leukocyte Adhesion Molecule 1 (ELAM-1) in Neutrophil-mediated Lung Injury in Rats.** *J Clin Invest* 1991, 88:1396–1406.
A well controlled study showing the importance of the E-selectin in facilitating neutrophil-mediated lung injury in an intact animal system. This strengthens the belief that pharmaceutical interference with the selectin system could have significant therapeutic value against naturally occurring diseases.

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A Varki, Cancer Center 0063, University of California, San Diego, 9500 Gilman Drive, La Jolla, California 92093-0063, USA.