Regulation of leukocyte L-selectin expression
by Aiyappa Muthanna Palecanda

A thesis submitted am partial fulfillment off the requirements for the degree of Doctor off Philosophy in Veterinary Science
Montana State University
© Copyright by Aiyappa Muthanna Palecanda (1993)

Abstract:
Neutrophil emigration into sites of inflammation and the nonrandom recirculation of lymphocytes are essential immunological phenomena controlled, in part, by specific receptor interactions between the leukocyte and vascular endothelium. L-selectin is an adhesion molecule expressed on all leukocytes and is required for the initial interaction of these cells with the endothelium prior to extravasation. Activation of leukocytes results in rapid shedding of L-selectin from the cell surface, which has been proposed to be a mechanism of release from the endothelium prior to emigration into sites of inflammation. Here a novel pathway leading to the shedding of L-selectin is described. I show that chemical crosslinking agents induce a rapid activation-independent shedding of leukocyte L-selectin. Specific crosslinking of L-selectin with monoclonal antibodies or treatment of leukocytes with the polysaccharide fucoidin also induce downregulation of L-selectin, which cannot be explained by activation alone. Importantly, L-selectin ligand interactions in vivo induce L-selectin downregulation. An irreversible inhibitor of serine proteases completely blocks both crosslinking- and activation-induced downregulation of L-selectin. Therefore, a similar serine protease is likely involved in both pathways leading to L-selectin shedding. Based on these observations, a new model for the regulation of leukocyte/endothelial interactions is presented.

REGULATION OF LEUKOCYTE L-SELECTIN EXPRESSION

by

Aiyappa Muthanna Palecanda

A thesis submitted in partial fulfillment
of the requirements for the degree
of
Doctor of Philosophy
in
Veterinary Science

MONTANA STATE UNIVERSITY
Bozeman, Montana
December 1993

REGULATION OF LEUKOCYTE L-SELECTIN EXPRESSION

Aiyappa Muthanna Palecanda

Advisor: Mark A. Jutila, Ph.D.

Montana State University

1993

Abstract

Neutrophil emigration into sites of inflammation and the nonrandom recirculation of lymphocytes are essential immunological phenomena controlled, in part, by specific receptor interactions between the leukocyte and vascular endothelium. L-selectin is an adhesion molecule expressed on all leukocytes and is required for the initial interaction of these cells with the endothelium prior to extravasation. Activation of leukocytes results in rapid shedding of L-selectin from the cell surface, which has been proposed to be a mechanism of release from the endothelium prior to emigration into sites of inflammation. Here a novel pathway leading to the shedding of L-selectin is described. I show that chemical crosslinking agents induce a rapid activation-independent shedding of leukocyte L-selectin. Specific crosslinking of L-selectin with monoclonal antibodies or treatment of leukocytes with the polysaccharide fucoidin also induce downregulation of L-selectin, which cannot be explained by activation alone. Importantly, L-selectin ligand interactions *in vivo* induce L-selectin downregulation. An irreversible inhibitor of serine proteases

completely blocks both crosslinking- and activation-induced downregulation of L-selectin. Therefore, a similar serine protease is likely involved in both pathways leading to L-selectin shedding. Based on these observations, a new model for the regulation of leukocyte/endothelial interactions is presented.

D378
P174

APPROVAL

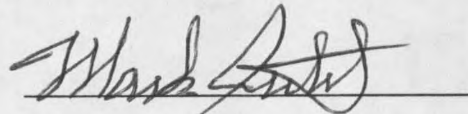
of a thesis submitted by

Aiyappa Muthanna Palecanda

This thesis has been read by each member of the thesis committee and has been found to be satisfactory regarding content, English usage, format, citations, bibliographic style, and consistency, and is ready for submission to the College of Graduate Studies.

11/26/93

Date

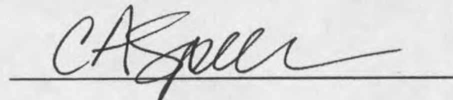


Chairperson, Graduate Committee

Approved for the Major Department

11/20/93

Date

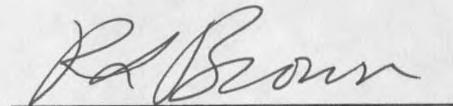


Head, Major Department

Approved for the College of Graduate Studies

11/23/93

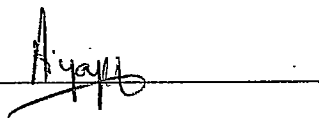
Date



Graduate Dean

STATEMENT OF PERMISSION TO USE

In presenting this thesis in partial fulfillment of the requirements for a doctoral degree at Montana State University, I agree that the Library shall make it available to borrowers under rules of the Library. I further agree that copying of this thesis is allowable only for scholarly purposes, consistent with "fair use" as prescribed in the U.S. Copyright Law. Requests for extensive copying or reproduction of this thesis should be referred to University Microfilms International, 300 North Zeeb Road, Ann Arbor, Michigan 48106, to whom I have granted "the exclusive right to reproduce and distribute my dissertation for sale in and from microform or electronic format, along with the right to reproduce and distribute my abstract in any format in whole or in part".

Signature 

Date 11/16/93

I dedicate this thesis to my parents, the late Mrs. Gangamma Muthanna and Mr. Palecanda Poovaiah Muthanna, and to my brother Palecanda Shyam.

ACKNOWLEDGMENTS

I would like to thank the members of my doctoral committee, Dr. Bruce L. Granger, Dr. Al Jesaitis, Dr. Mark A. Jutila, Dr. Norman D. Reed, and Dr. C. A. Speer for their helpful suggestions and recommendations. I particularly want to thank my advisor and committee chairman Dr. Mark A. Jutila, who provided constructive criticism, guidance, and encouragement on a continual basis. I also wish to express my sincere gratitude to Dr. Norman D. Reed, for his faith in me during the earliest part of my doctoral program and for his continued help and guidance throughout my doctoral program.

Special thanks to Davin Jutila, Kathy Jutila, Gayle Watts, Sandy Kurk, and Ginger Perry, for their friendship and excellent technical assistance. I would also like to thank two of my colleagues, Bruce Walcheck and Rob Bargatze, for a pleasant and congenial work atmosphere. My thanks to Dana Hoover for editing and correction of manuscripts and the dissertation.

A special appreciation to the departmental secretaries, Joan, Linda and Bert for their help in getting through all the necessary paperwork. I would like to thank Gayle Callis and Andy Blixt for their assistance in tissue sectioning and photography.

I wish to acknowledge the special support and encouragement I received from my brothers, Shyam and Arun, and my sisters, Gita, Jyothi, Bollacca, Mallika and Swaroop. A very special thanks to my wife, Lakshmi, for her understanding and encouragement.

TABLE OF CONTENTS

PAGE

LIST OF TABLES.. x

LIST OF FIGURES.. xi

ABSTRACT.. xiii

1. INTRODUCTION.. 1

Identification of L-selectin.. 2
Structure of L-selectin.. 5
Function of L-selectin.. 7
Regulation of L-selectin 11
My Hypothesis.. 13
References.. 14

2. ACTIVATION INDEPENDENT SHEDDING OF LEUKOCYTE L-SELECTIN IS INDUCIBLE BY CROSSLINKING AGENTS.. 24

Introduction.. 24
Materials and Methods.. 26
Antibodies.. 26
Preparation of leukocyte suspensions and flow cytometric analysis......... 26

Crosslinking of leukocyte L-selectin.. 27
Antibody crosslinking of L-selectin.. 28
Western blot SDS-PAGE analysis.. 28
ELISA analysis.. 29
Results.. 29
Activation-independent down-regulation of leukocyte L-selectin can be induced by chemical crosslinking agents.. 29
Treatment of human leukocytes with BS^3 causes shedding of L-selectin.. 32
Crosslinking of L-selectin with specific monoclonal antibodies causes loss of surface expression of L-selectin.. 34
Activation-independent shedding of L-selectin occurs *in vivo*.. 37
Discussion.. 39.
References.. 42

3. **LIGAND INTERACTIONS *IN VIVO* AND ANTIBODY CROSSLINKING OR FUCOIDIN TREATMENT *IN VITRO* LEADS TO DOWNREGULATION OF L-SELECTIN EXPRESSION**.. 46

Introduction.. 46
Materials and Methods.. 49
Antibodies and polysaccharides.. 49
Preparation of cell suspension.. 49
Flow cytometric analysis.. 49
Chemical crosslinking.. 50
Antibody crosslinking of L-selectin.. 50
Immunofluorescence microscopy.. 51
Crosslinking of L-selectin with fucoidin and other polysaccharides.. 51
In vivo trafficking of L-selectin transfected L1/2 cells.. 52

Results 52
Crosslinking of primary anti-L-selectin mAbs is required to induce downregulation of neutrophil L-selectin 52
Treatment of neutrophils or lymphocytes with the sulfated polysaccharide fucoidin can also induce downregulation of L-selectin 58
Crosslinker-induced shedding is a property of L- but not E-selectin 61
In vivo trafficking of L1/2 cells results in a loss in the surface expression of L-selectin 62
Discussion 67
References 71

4 ROLE OF A DIISOPROPYL FLUOROPHOSPHATE (DFP) INHIBITABLE, ACTIVATION-DEPENDENT, MEMBRANE-ASSOCIATED, PUTATIVE PROTEASE IN THE SHEDDING OF LEUKOCYTE L-SELECTIN 78

Introduction 78
Materials and Methods 80
Antibodies 80
Preparation of leukocyte suspensions and flow cytometric analysis 80
Leukocyte activation 81
Chemical crosslinking 81
Protease inhibitor treatment 81
Labeling of activation induced [^{3}H] Diisopropyl-Fluorophosphate (^{3}H-DFP) binding proteins 82
Sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE) and autoradiography 83
Results 83
Activation-induced L-selectin shedding is completely inhibited by DFP 83
Crosslinking-induced L-selectin shedding is also completely inhibited by DFP 85
Upregulation of surface markers for cellular activation are not affected by DFP 87

DFP inhibits a protease which is exposed/accessible only upon cellular activation or chemical crosslinking.. 88
Cellular activation exposes previously unexpressed or hidden DFP binding membrane-associated proteins..91
Discussion..93
References..96

5. CONCLUSIONS.. 100
Model.. 104

LIST OF TABLES

TABLE PAGE

1. Chemical crosslinking of mouse and human leukocyte surface proteins causes loss of L-selectin expression.............................. 31
2. Crosslinked lysine residues must be greater than 6 Angstroms apart to induce L-selectin down-regulation.................................. 34
3. Cross-linking with specific mAb at 37°C causes loss of surface expression of leukocyte homing receptor L-selectin........................36
4. Effect of primary antibodies crosslinked with whole molecule second stage antibody on the surface expression of L-selectin and Mac-1.. 55
5. Exposure of neutrophils and lymphocytes to soluble fucoidin induces L-selectin down-regulation at 37°C................................ 59
6. Polysaccharides that do not affect surface expression of L-selectin........ 60
7. Treatment of the L1/2 L-selectin transfectants with anti-L-selectin antibodies plus second stage or fucoidin induces down-regulation at 37°C.. 64

LIST OF FIGURES

FIGURE PAGE

1. Chemical cross-linking of mouse and human leukocytes causes L-selectin down-regulation........ 33
2. Chemical crosslinking of human leukocytes caused L-selectin shedding from the cell surface........ 35
3. The human leukocyte homing receptor L-selectin is downregulated upon specific cross-linking at 37°C........ 37
4. Shed L-selectin can be detected in the plasma of healthy adults........ 38
5. Primary anti-L-selectin antibodies alone do not induce L-selectin down-regulation........ 54
6. Primary anti-L-selectin antibodies plus second stage induce downregulation of L-selectin at 37°C........ 56
7. Primary anti-L-selectin antibodies, followed by whole molecule, but not Fab', second stage, induces L-selectin down-regulation at 37°C........ 57
8. The human leukocyte homing receptor L-selectin aggregates to form patches upon specific antibody crosslinking........ 58
9. Immobilized fucoidin induces downregulation of L-selectin expression.. 61
10. Chemical crosslinking causes L-selectin, but not E-selectin, downregulation........ 63
11. L-selectin L1/2 transfectants, injected i.v. into mice and allowed to recirculate, rapidly downregulate L-selectin expression........ 65
12. Anti-L-selectin antibodies block downregulation of L-selectin on L-selectin transfectants allowed to recirculate in vivo........ 66
13. DFP, but not other protease inhibitors, block L-selectin shedding in response to PMA or FMLP treatment of human leukocytes........ 84
14. DFP, but not other serine protease inhibitors, block L-selectin shedding in response to BS^3 crosslinking........ 86
15. DFP has no effect on the activation-induced upregulation of certain surface markers for cellular activation........ 88

16. Presence of DFP is required during crosslinking or activation to block L-selectin shedding on human neutrophils........................ 89
17. Presence of DFP is required during activation or crosslinking to block L-selectin shedding on mouse bone-marrow neutrophils......... 90
18. Activation exposes membrane associated DFP binding proteins............92
19. Activation exposes membrane-associated 90 kD DFP binding proteins on both lymphocytes and neutrophils................................93

ABSTRACT

Neutrophil emigration into sites of inflammation and the nonrandom recirculation of lymphocytes are essential immunological phenomena controlled, in part, by specific receptor interactions between the leukocyte and vascular endothelium. L-selectin is an adhesion molecule expressed on all leukocytes and is required for the initial interaction of these cells with the endothelium prior to extravasation. Activation of leukocytes results in rapid shedding of L-selectin from the cell surface, which has been proposed to be a mechanism of release from the endothelium prior to emigration into sites of inflammation. Here a novel pathway leading to the shedding of L-selectin is described. I show that chemical crosslinking agents induce a rapid activation-independent shedding of leukocyte L-selectin. Specific crosslinking of L-selectin with monoclonal antibodies or treatment of leukocytes with the polysaccharide fucoidin also induce downregulation of L-selectin, which cannot be explained by activation alone. Importantly, L-selectin ligand interactions *in vivo* induce L-selectin downregulation. An irreversible inhibitor of serine proteases completely blocks both crosslinking- and activation-induced downregulation of L-selectin. Therefore, a similar serine protease is likely involved in both pathways leading to L-selectin shedding. Based on these observations, a new model for the regulation of leukocyte/endothelial interactions is presented.

CHAPTER 1

INTRODUCTION

Lymphocyte extravasation into secondary lymphoid tissues and neutrophil recruitment into sites of inflammation are essential immunological phenomena exemplifying immune surveillance and protective immune responses to injury. The first step in leukocyte extravasation is adherence to the vascular endothelium. This critical step is mediated by L-selectin on the leukocyte and its ligands, either constitutively expressed on the peripheral-lymph-node high endothelial venules (PLN-HEV) or on venules in response to inflammatory stimuli. The expression of L-selectin and its ligand needs to be stringently regulated to initiate the leukocyte/endothelial interaction only at sites requiring leukocyte extravasation, such as sites of inflammation and secondary lymphoid organs. L-selectin is constitutively expressed on all leukocytes and is rapidly shed from the leukocyte surface upon activation with inflammatory chemotactic agents, such C5a, which attract cells within the vascular bed to the underlying inflamed tissue. Activation-induced L-selectin shedding has been suggested as a requirement for the release of leukocytes from the endothelium in order for these cells to continue with migration. My hypothesis is that if L-selectin shedding is required for leukocyte extravasation, then an activation-independent mechanism of L-selectin shedding may be occurring at sites of leukocyte extravasation in the absence of inflammation, such as the normal migration of lymphocytes into secondary lymphoid

organs in the periphery. My dissertation has focused on the effects of receptor crosslinking and *in vivo* trafficking on L-selectin expression, and the study of mechanisms involved in the activation and activation-independent downregulation of leukocyte L-selectin. Each chapter in my dissertation deals with experiments performed to decipher the various aspects of L-selectin regulation, beginning with an introduction pertaining to the data presented in that chapter and closing with a discussion and a list of cited references. The final chapter will assimilate all key findings. This introduction gives an overview of L-selectin and ends with the presentation of my hypothesis.

Identification of L-selectin

The immune system has to keep constant surveillance of the various microenvironments in the body to detect antigen insult. Lymphocytes which are capable of detecting and responding to diverse antigens were shown by *in vivo* experiments to recirculate from blood to secondary lymphoid organs and back to blood via the thoracic duct (1,2). The extravasation of lymphocytes from blood is nonrandom and occurs at specialized high endothelial venules (HEV) in secondary lymphoid organs (reviewed in 3-9). There are subsets of lymphocytes that only bind HEV in certain tissues, and this specificity is maintained across species barriers (10,11).

There are at least four different lymphocyte HEV specificities: one for peripheral lymphoid tissues, a second for Peyer's patches, a third for lung-associated lymphoid tissues, and a fourth at sites of chronic inflammation, such as the inflamed synovium or skin (10,12-15). The attachment of lymphocytes to tissue-specific endothelium is mediated by receptor/counter-receptor pairs (10). The *in vivo* interaction of lymphocyte subsets with

different HEV is reflected in an elegant *in vitro* model for lymphocyte/endothelial-cell interaction developed by Stamper and Woodruff in 1976 (16). This unique specificity of lymphocyte/HEV binding gave rise to the term "homing" and was predicted to be mediated by receptor/counter-receptor pairs expressed by both cell types. The lymphocyte molecules responsible for this specificity were termed "homing receptors." Thus, the receptor involved in lymphocyte homing to peripheral lymph nodes was called the peripheral-lymph-node homing receptor (17).

Based on the observation that certain mouse lymphoid cell lines bind with absolute specificity to peripheral-lymph-node HEV while others bind to mucosal HEV, attempts were made to generate monoclonal antibodies against these receptors on lymphocytes (10). Immunization of rats with a mouse B-cell lymphoma that binds to peripheral lymph node HEV but not to Peyer's patch (mucosal) HEV was used to generate the monoclonal antibody MEL-14 (18). MEL-14 reacted with a cell-surface determinant present on all lymphocytes that bound peripheral HEV. Also, MEL-14 blocked adherence of lymphomas and normal cells to peripheral-lymph-node HEV *in vitro* and also blocked lymphocyte migration into the peripheral lymph nodes *in vivo* (17-19).

MEL-14 binds to a 90 kD glycoprotein from both peripheral HEV binding lymphomas and normal lymphocytes (18). This 90 kD glycoprotein is called the peripheral-lymph-node homing receptor (PLNHR) or $gp90^{MEL\text{-}14}$. Expression of the MEL-14 antigen is not restricted to lymphocytes, since the mAb MEL-14 stains granulocytes and monocytes and immunoprecipitates a 100 kD glycoprotein from the neutrophil surface (20). The $gp^{90MEL\text{-}14}$ on neutrophils was also shown to mediate adhesion of neutrophils to inflamed endothelium (20-22). Independently, the human homologue of the mouse $gp90^{MEL\text{-}14}$ antigen was identified as the pan leukocyte markers Leu-8, TQ-1, and DREG, found on most circulating human lymphocytes, neutrophils, and monocytes (23-28).

Based on the partial amino-acid sequence of $gp90^{MEL-14}$, an oligonucleotide probe was derived and used to isolate the cDNA clone encoding the core polypeptide of $gp90^{MEL-14}$ (29,30). The human homologue of $gp90^{MEL-14}$ was independently isolated by different laboratories through the hybridization selection screening of human lymphocyte cDNA library with mouse $gp90^{MEL-14}$ cDNA (31,32), immunoscreening of transfected cDNA clones (27), and by differential hybridization (33). The molecular cloning of mouse $gp90^{MEL-14}$ revealed a glycoprotein with tandem interaction domains containing, from the NH_2-terminal, a separate carbohydrate binding (lectin) domain, an epidermal growth-factor-like (EGF) domain, and duplicated repeats homologous to complementary regulatory proteins (CRP) (27,29-33).

The characteristic protein mosaic architecture of $gp90^{MEL-14}$ is also found in two other independently studied cell-surface glycoproteins, identifying a novel family of receptors recently named selectins (34). There are currently three members in the selectin family: 1) L-selectin, which is the peripheral-lymph-node homing receptor or $gp90^{MEL-14}$, has been called Leu-8, LAM-1, TQ-1, LECAM-1, LEC-CAM-1, and DREG (in this dissertation it will be referred to as L-selectin), 2) E-selectin, which is expressed on cytokine-activated endothelial cells and is thought to mediate their binding to leukocytes at sites of inflammation (35,36), and 3) P-selectin (also known as CD62, GMP140, or PADGEM), which is stored in Weibel-Palade bodies of endothelial cells and alpha-granules of platelets, is rapidly mobilized to the cell surface after activation and promotes binding of these cells to monocytes and neutrophils (37,38). All three members play critically important roles in mediating cell/cell interaction in the vasculature (29,30,35,39).

Structure of L-selectin

The deduced transmembrane protein of L-selectin is 334 amino acids long with an unusually long hydrophobic leader sequence of 38 amino acids, a hydrophobic transmembrane region followed by a cluster of positively-charged residues, and a hydrophilic cytoplasmic tail of 18 amino acids (29,30). Hydropathy plot of the protein predict regions of hydrophilicity concentrated in the NH_2-terminal 150 amino acids and a membrane proximal 20 amino acids. The intervening extracytoplasmic portion consists of a relatively uncharged neutral stretch, which includes the EGF and CRP domains (29,30). The mature protein begins with a tryptophan, which is unusual as an amino terminal residue (29,30). It has ten potential asparagine-linked glycosylation sites and no sites for O-linked glycosylation in the deduced sequence (29,30). This is consistent with the antibody affinity purification and biochemical analysis of L-selectin from the murine T-lymphoma cell line EL-4 which indicated that the core protein with a maximum size of 46.5 kD is modified by N-linked carbohydrates accounting for 45% of L-selectin mass (30). The mature protein contains 22 cysteine residues accounting for 6.6% of the total amino acids (29,30).

The NH_2-terminal lectin domain of mouse L-selectin is homologous to domains found in a diverse series of calcium-dependent animal lectins (40,41). The lectin domain has a large number (sixteen) of lysine residues (29,30). The EGF-like domain of L-selectin consists of a single copy homologue of EGF-like sequences found in various proteins such as growth factors, developmental gene products, extracellular matrix proteins, cell-surface receptors, blood-clotting factors, and plasminogen activators (29,30). The EGF-like domain contains all six consensus cysteines and the glycine residues characteristic of this structure. The complement binding motif consists of two 62-amino-acid repeats (29,30).

This motif is found in a number of complementary regulatory proteins that bind C3 and C4 and other proteins, such as the IL-2 receptor. The presence of the three distinct motifs in L-selectin suggests that the gene for L-selectin may have evolved through exon shuffling (29,30).

The human homologue of mouse L-selectin shows an identical domain organization: an NH_2-terminal lectin domain, followed by an EGF-like domain, two complement binding repeats, a transmembrane domain, and a cytoplasmic tail (31-33). The overall sequence identity between the mouse and the human L-selectin is 86% in the lectin domain, 82% in the EGF-like domain, and 74% and 60% in the first and second CRP domains respectively. Also, almost identical amino-acid sequences are found in the transmembrane (95% identity) and surrounding regions of both human and mouse L-selectin cDNA sequences (31-33). The human L-selectin molecule immunoprecipitated from lymphocytes has a molecular mass of 74 kD, whereas the molecule on neutrophils is 90 kD (42). The neutrophil and lymphocyte mRNA transcripts have the same size by northern blot analysis; therefore, the difference in molecular mass is thought to be due to post-translational modification (42).

Recently in our laboratory, Bruce Walcheck et al. (1992) cloned the lectin domain of the bovine homologue of the human L-selectin (84). The nucleotide sequence of the bovine L-selectin lectin domain revealed a nucleotide identity of 84.2% and 80.4% with the human and mouse homologues, respectively. The predicted amino-acid sequence of the bovine L-selectin lectin domain has an 81.6% and 76.3% identity with the human and mouse lectin domains, respectively. The only difference in the bovine lectin domain was the presence of a third N-linked glycosylation site. The remaining two N-linked glycosylation sites were conserved between cow, mouse, and human (84).

Function of L-selectin

As mentioned above, two important adhesive functions are mediated by L-selectin: 1) lymphocyte adhesion to peripheral lymphoid tissues, and 2) neutrophil/endothelial interactions. Anti-L-selectin antibodies inhibit lymphocyte migration into peripheral lymph nodes *in vivo* and the adherence of lymphocytes to PLN-HEV in *in vitro* adherence assays. Soluble, affinity-purified, lymphocyte L-selectin or a recombinant protein can bind PLN-HEV and block lymphocyte localization (4,18,43). Anti-L-selectin antibodies also inhibit neutrophil localization to sites of acute inflammation, including the dermis and peritoneum of mice, and block neutrophil adhesion to cytokine-stimulated endothelial cells *in vitro* (44-48). Recently, a soluble L-selectin-IgG chimeric molecule was also shown to almost completely block the neutrophil influx into thioglycollate-inflamed mouse peritoneum *in vivo* (49). Early studies showed that certain monosaccharides, like D-mannose-6-phosphate and D-fructose-1-phosphate, inhibit lymphocyte binding to PLN-HEV in rat, mouse, and humans (50-52). Also, the mannose-6-phosphate-rich phosphomannan monoester (PPME) and the fucose-rich polysaccharide fucoidin inhibited lymphocyte/PLN-HEV binding (53,54). Using PPME-derivatized microbeads, it was shown that a calcium-dependent, lectin-like receptor on the lymphocyte surface was required for lymphocyte binding to PLN-HEV (53,54). The monoclonal antibody MEL-14 blocked PPME binding to lymphocytes, and it was predicted that the lectin-like molecule was the same as L-selectin (53).

A direct ELISA-based assay has confirmed the binding of the purified or recombinant L-selectin to the mannose-6-phosphate-rich polysaccharide PPME in a calcium-dependent, mannose-6-phosphate- and fructose-6-phosphate-inhibitable manner (55). The ELISA assay also showed that fucoidin, a potent inhibitor of lymphocyte binding to HEV,

competes for PPME binding of L-selectin (55). As discussed above, molecular cloning of L-selectin cDNA confirmed the presence of a C-type lectin domain in the NH2-terminal of L-selectin (55); thus, L-selectin is a mammalian lectin.

The lectin domain of L-selectin contains a large number of lysine residues: sixteen in the mouse and twelve in the human, indicating a high concentration of positive charge (31-33). This is in agreement with the requirement for sialic acid on the endothelial ligand and the fact that all the known sugars that bind L-selectin are anionic (56,57). The human and mouse L-selectin share an overall similarity in deduced primary sequence (77% at the protein level and 79% at the nucleotide level) and exhibit very similar carbohydrate binding activity (58).

L-selectin has also been demonstrated to mediate leukocyte rolling along the endothelium (59,60). This process has been proposed as a means of slowing the leukocyte before it comes to a complete stop at sites of inflammation. Neutrophil rolling *in vivo* can be completely abrogated by the infusion of anti-L-selectin antibodies, a L-selectin-IgG chimera, and various sulfated carbohydrates, such as dextran sulfate, fucoidin, and sulfatides (a sulfated glycolipid)--all inhibitors of L-selectin (59, 61-64). Thus, L-selectin appears to mediate a high avidity interaction that occurs under significant shear.

L-selectin-mediated rolling of L-selectin cDNA transfected mouse pre-B cell line 300.19 in exteriorized rat mesenteric venules has been shown by Kansas et al. (1993) to be dependent on the presence of the cytoplasmic tail of L-selectin (84). Transfectants lacking eleven amino acids at the carboxy terminal of L-selectin failed to bind PLN-HEV *in vitro* and also failed to roll on endothelium *in vivo*. The rolling was also abrogated if cells were pretreated with cytochalasin B, which disrupts actin microfilaments (84). However, the transfectants lacking the eleven COOH-terminal amino acids did bind PPME (84). These data suggest that the cytoplasmic domain of L-selectin may be involved in an interaction

with the cytoskeleton to mediate leukocyte rolling that is independent of ligand recognition.

The proposed ligands for L-selectin are the antigens identified by the antibody MECA-79, and glycoproteins immunoisolated by a L-selectin-IgG chimeric molecule, one of which has been recently cloned and named GlyCAM-1 (65-67). Of the two, the best characterized ligands for L-selectin are the glycoproteins identified by the monoclonal antibody MECA-79, which recognizes a peripheral lymphnode-specific HEV antigen found in both mouse and man (65). MECA-79 also inhibits lymphocyte binding to PLN-HEV, but not substantially to HEV in Peyer's patches. The antigens identified by MECA-79 are called the peripheral-lymph-node addressin or PNad (65).

Immunoisolated PNad coated onto glass slides selectively binds lymphocytes and lymphoid cell lines that can bind PLN-HEV (65). Lymphocyte binding to purified PNad is calcium-dependent and abrogated by neuraminidase treatment (65). Binding of mouse and human lymphocytes to purified PNad is inhibitable with anti-L-selectin antibodies or MECA-79. Also, a mouse pre-B cell line transfected with human L-selectin cDNA bound purified PNad, whereas the untransfected parent line could not. All these data confirm that L-selectin and PNad are receptor/ligand pairs. Using MECA-79 as an immunoadsorbent, a number of glycoproteins of distinct molecular weight (50, 90, 105, 115, 170, and 200 kD) were isolated. It remains to be seen whether one or all of these glycoproteins are the ligand for L-selectin (65).

Other putative ligands for L-selectin include Sgp50 and Sgp90 (66), which are sulfated, fucosylated, and sialylated glycoproteins isolated by affinity columns using L-selectin-IgG chimeric molecule from PLN-HEV. These components (Sgp50 and Sgp90) are not detected in other lymphoid organs, such as spleen, thymus, or Peyer's patches. The binding of the L-selectin-IgG chimera to Sgp50 and Sgp90 is calcium dependent and inhibited by MEL-14, PPME, and treatment of the Sgps with sialidase (66).These Sgps are also precipitated by the anti-peripheral-lymph-node-addressin (PNad) antibody MECA-79. N-glycanase

treatment of Sgp50 and Sgp90 does not diminish their molecular weight, indicating the absence of N-linked carbohydrate chains (66).

N-terminal amino-acid microsequencing of purified Sgp50 was used to clone a cDNA encoding the protein proposed to be the ligand for L-selectin (67). The cDNA encodes a novel, serine/threonine-rich, mucin-like glycoprotein now called GlyCAM-1/(glycosylation-dependent cell-adhesion molecule) (67). GlyCAM-1 is HEV-associated, and contains predominantly O-linked carbohydrate chains with a requirement for sulfation to bind L-selectin (68).

Recent reports have established that E-, P-, and L -selectin all recognize the fucosylated and sialylated tetrasaccharide called sialyl Lewis X and related carbohydrates (69-74). Therefore, all selectins are suspected to bind ligands that are distinctive modifications of the core carbohydrate, such as sialyl Lewis X.

Recently, using a recombinant L-selectin as an immunohistochemical probe, potential ligands for L-selectin have been demonstrated on myelinated regions of the central but not the peripheral nervous system (75). Based on antibody blocking studies, L-selectin has been shown to mediate binding of lymphocytes to myelinated regions of mouse central nervous system. These studies raise the possibility that a L-selectin-dependent mechanism may be a factor in the pathogenesis of certain central-nervous-system demyelinating diseases (75). It is interesting to note that treatment of rats with fucoidin or mannose-6-phosphate--two carbohydrate-based inhibitors of L-selectin, has been reported to prevent or delay the induction of experimental autoimmune encephalomyelitis in rats (76,77).

Regulation of L-selectin

L-selectin is rapidly shed from the cell surface upon activation of mouse neutrophils with various inflammatory mediators, and the shed form is 5-10 kD smaller in size than the membrane-associated form. (78). Mouse lymphocytes activated in vitro by exposure to phorbol esters shed a soluble L-selectin that is 12 kD smaller than the intact receptor (79). Similarly, L-selectin is also released upon stimulation of human lymphocytes (23,28). The loss of L-selectin expression has been proposed to be important for the release of the leukocyte from the vascular endothelium in order to enter the underlying tissues (78). This model is consistent with leukocyte entry into sites of inflammation since neutrophils that are found in inflamed lesions are L-selectin negative (78). However, the concentration of inflammatory mediators used in the *in vitro* assays to cause L-selectin downregulation is high and are unlikely to be attained *in vivo*. Also, activation of leukocytes *in vitro* leads to a total loss of cell-surface expression of L-selectin; whereas, lymphocytes which enter peripheral lymph nodes *via* L-selectin-dependent adhesion pathways do not show appreciable loss of L-selectin expression (85). Therefore, an alternate mechanism for the downregulation of L-selectin expression may exist.

Upon activation, neutrophils upregulate other adhesion receptors of the integrin family, particularly CD11b/CD18 (expression of which is obligatory for neutrophil localization at sites of inflammation) (78). This intricately controlled and inverse regulation of L-selectin and CD11b/CD18 is proposed necessary for the neutrophils to detach from the endothelium and begin the process of transendothelial migration into inflammatory sites (78). The downregulation of L-selectin has been hypothesized to be mediated by a surface protease that becomes functional upon activation of the neutrophil. Recent reports have shown that low-dose chymotrypsin treatment of leukocytes can cause shedding of L-selectin *in vitro*

(45). The presence of lysine and tyrosine residues near the plasma membrane on the extracytoplasmic portion of the L-selectin molecule lend support to the model that activation of the neutrophil may indeed "turn on" a serine protease-like enzyme to cleave and release L-selectin.

It has been suggested that a PI-linked form of L-selectin may be expressed, and, therefore, a phospholipase may be involved in the regulation of L-selectin expression (80). This hypothesis was based on isolation of two cDNA clones of different lengths from T-cell libraries which encode either a longer transmembrane protein or a shorter PI-linked form of L-selectin. PI-PLC treatment of COS cells transfected with the shorter clone diminished reactivity to anti-L-selectin antibodies by FACs analysis, whereas PI-PLC treatment of COS cells transfected with the longer clone had no effect on L-selectin expression (80). However, the presence of PI-linked L-selectin on the leukocyte surface and its functional significance is controversial. Recently, Ord et al. found no splice site in the genomic DNA that would yield a mRNA encoding a PI-linked form of L-selectin (81). Also, leukocytes, isolated from patients with paroxysmal nocturnal hemoglobinuria (PNH) who have defective expression of PI-linked proteins, had normal levels of L-selectin expression (81). Therefore, the significance of a PI-linked form of L-selectin and its regulation remains to be determined.

Recently, Tedder et al. showed that activation of lymphocytes with anti-CD3 monoclonal antibodies causes a transient increase in the affinity of L-selectin to bind PPME (82). Similarly, neutrophil activation with cytokines, such as TNF, also transiently increases PPME binding by L-selectin. In both of these cases, the cell-surface expression of L-selectin did not increase, suggesting a conformational change in L-selectin that enhances receptor affinity. These authors propose that leukocyte lineage-specific activation *in vivo* may be the regulatory mechanism that induces enhanced ligand binding by L-selectin prior to being shed from the cell surface (82).

My Hypothesis

The prevailing hypothesis is that shedding of L-selectin is required for the release of the bound leukocyte from the endothelium and extravasation into the underlying tissues. If gross activation of the leukocyte is required for total shedding of L-selectin and this in turn is required for extravasation, then this hypothesis fails to address the following issues: (1) lymphocytes require L-selectin to bind HEV, but do not become L-selectin negative upon entering uninflamed lymph nodes (79); (2) neutrophils bind uninflamed HEV via L-selectin in *ex vivo* binding assays, but do not enter uninflamed lymphoid tissues *in vivo*, suggesting that neutrophils must release from the HEV and re-enter circulation; (3) our recent finding that L-selectin can be detected in the plasma of individuals who do not show any overt signs of acute inflammation indicates that L-selectin may be shed in the absence of inflammatory mediators *in vivo*. Therefore, a mechanism of L-selectin downregulation that does not rely on overt activation of the cell may exist.

I propose that crosslinking of L-selectin to its endothelial ligand causes or enhances downregulation. This hypothesis is consistent with shedding being involved in the circumstance listed above. For example, the entry of lymphocytes into normal lymphoid tissue would result in crosslinking of L-selectin to its ligand on the PLN-HEV. This in turn would cause shedding of L-selectin and releasing of the lymphocyte from the endothelium in order to proceed with the process of extravasation. The presence of L-selectin-positive lymphocytes in lymphoid tissue could be explained by shedding occurring only at the sites of leukocyte/endothelial-cell contact and not over the entire cell surface as seen in overtly activated cells. Similarly, the L-selectin-mediated neutrophil-rolling phenomenon observed *in vivo* would result in crosslinking of L-selectin to its ligand on the endothelium, which

causes shedding at sites of neutrophil/endothelium contact enabling the neutrophil to continue rolling. If inflammatory mediators are present, then upregulation of CD11b/CD18 occurs which would mediate firm attachment and emigration. The lack of neutrophil extravasation into uninflamed lymphoid tissue, even though neutrophil L-selectin binds HEV, could also be explained by our hypothesis. The binding of neutrophils to HEV would result in crosslinking of L-selectin and subsequent releasing of neutrophils from the HEV. The presence of L-selectin in the plasma of individuals showing no signs of overt inflammation may be due to the shedding of L-selectin induced by constant crosslinking occurring under physiological conditions.

References

1. Gowans, J.L., and E.J. Knight. 1964. The route of recirculation of lymphocytes in the rat. *Proc. R. Soc. Lond. B. Biol. Sci.* 159:257-282.

2. Marchesi, V.T., and J.L. Gowans. 1984. The migration of lymphocytes through the endothelium of venules in lymph nodes. *Proc. R. Soc. Lond. B. Biol. Sci.* 159:475-484.

3. Berg, E.L., L.A. Goldstein, M.A. Jutila, M. Nakache, L.J. Picker, P.R. Streeter, W. Wu, D. Zhou, and E.C. Butcher. 1989. Homing receptors and vascular addressins: Cell adhesion molecules that direct lymphocyte traffic. *Immunol. Rev.* 108:5-18.

4. Butcher, E.C., 1990. Cellular and molecular mechanisms that direct leukocyte traffic. *Amer. J. Pathol.* 136:3-11.

5. Rosen, S.D. 1989. Lymphocyte homing: progress and prospects. *Curr. Opin. Cell. Biol.* 1:913-919.

6. Stoolman, L.M. 1989. Adhesion molecules controlling lymphocyte migration. *Cell.* 56:907-910.

7. Woodruff, J.J., L.M. Clarke, and Y.H. Chin. 1987. Specific cell-adhesion mechanisms determining migration pathways of recirculating lymphocytes. *Annu. Rev. Immunol.* 5:201-222.

8. Yednock, T.A., and S.D. Rosen. 1989. Lymphocyte homing. *Adv. Immunol.* 44:313-378.

9. Picker, L.J. and E.C. Butcher. 1992. Physiological and molecular mechanisms of lymphocyte homing. *Annu. Rev. Immunol.* 10:561-591.

10. Butcher, E.C., R.G. Scollay, and I.L. Weissman. 1980. Organ specificity of lymphocyte migration: mediation by highly selective lymphocyte interaction with organ specific determinants on high endothelial venules. *Eur. J. Immunol.* 10:556-561.

11. Cahill, R.N., D.C. Poskitt, H. Frost, and Z. Trnka. 1977. Two distinct pools of recirculating T-lymphocytes: migratory characteristics of nodal and intestinal T lymphocytes. *J. Exp. Med.* 145:420-428.

12. Chin, Y.H., R. Rasmussen, A.G. Cakiroglu, and J.J. Woodruff. 1984. Lymphocyte recognition of lymph node high endothelium. VI. Evidence of distinct structures mediating binding to high endothelial cells of lymph nodes and Peyer's patches. *J. Immunol.* 136:2556-2561.

13. Geoffroy, J.S., T.A. Yednock, J.L. Curtis, and S.D. Rosen. 1988. Further evidence for a distinct lung associated lymphocyte homing specificity. *FASEB. J.* 2: A667.

14. Jalkanen, S., A.C. Steere, R.I. Fox, and E.C. Butcher. 1986. A distinct endothelial cell recognition system that controls lymphocyte traffic into inflamed synovium. *Science.* 233:556-558.

15. Picker, L.J., S.A. Michie, L.S. Rott, and E.C. Butcher. 1990. A unique phenotype of skin-associated lymphocytes in humans: Preferential expression of the HECA-452 epitope by benign and malignant T-cells at cutaneous sites. A*m. J. Path.* 136:1053-1068.

16. Stamper, H.B., and J.J. Woodruff. 1976. Lymphocyte homing into lymph nodes:

in vitro demonstration of the selective affinities of recirculating lymphocytes for high-endothelial venules. *J. Exp. Med.* 144:828-833.

17. Butcher, E.C. 1986. The regulation of lymphocyte traffic. *Curr. Top. Microbiol. Immunol.* 128:85-122.

18. Gallatin, W. M., I.L Weissman, and E.C. Butcher. 1983. A cell surface molecule involved in organ specific homing of lymphocytes. *Nature (London).* 304:30-34.

19. Mountz, J., W. Gause, F. Finkelman, and A. Steinberg. 1988. Prevention of lymphadenopathy in MRL-lpr/lpr mice by blocking peripheral lymph node homing with MEL-14 in vivo. *J. Immunol.* 140:2943-2949.

20. Lewinsohn, D.M., R.F. Bargatze, and E.C. Butcher. 1987. Leukocyte endothelial cell recognition: Evidence of a common molecular mechanism shared by neutrophils, lymphocytes and other leukocytes. *J. Immunol.* 138: 4313-4321.

21. Jutila, M.A., L. Rott, E.L. Berg, and E.C. Butcher. 1989. Function and regulation of the Mel-14 antigen in vivo: Comparison with LFA-1 and Mac-1. *J. Immunol.* 143: 3318-3324.

22. Jutila, M.A., D.M. Lewinsohn, E.L. Berg, and E.C. Butcher. 1988. Homing receptors in lymphocyte, neutrophil, and monocyte interactions with endothelial cells. Leukocyte adhesion molecules: Structure, function, and regulation. T.A Springer, ed., New York, Springer-Verlag. 227-235.

23. Kishimoto, T.K., M.A. Jutila, and E.C. Butcher. 1990. Identification of the human peripheral homing receptor: A rapidly down-regulated adhesion molecule. *Proc. Natl. Acad. Sci. USA.* 87: 2244-2248.

24. Lanier, L.L., E.G. Engleman, P. Gatenby, G.F. Babcock, N.L. Warner, and L.A. Herzenberg. 1983. Correlation of functional properties of human lymphoid cell subsets and surface marker phenotypes using multiparameter analysis and flow cytometry. *Immunol Rev.* 74:143-160.

25. Kansas, G.S., G.S. Wood, D.M. Fishwild, and E.G. Engleman. 1985. Functional characterization of human T-lymphocyte subsets distinguished by monoclonal anti-Leu-8. *J. Immunol.* 134:2995-3002.

26. Poletti, A., R. Manconi, and P. De Paoli. 1988. Double labeling immunohistologic and flow cytometric analysis of human B cells with particular reference to Leu-8 expression. *Hum. Pathol.*. 19:1001-1007.

27. Camerini, D., S.P. James, I Stamenkovic, and B. Seed. 1989. Leu-8/TQ1 is the human equivalent of Mel-14 lymph node homing receptor. *Nature (Lond.)*. 342:78-82.

28. Tedder, T.F., C.A. Penta, H.B. Levine, and A.S. Freedman. 1990. Expression of the human leukocyte adhesion molecule, LAM1. Identity with the TQ1 and Leu-8 differentiation antigens. *J. Immunol.* 144:532-540.

29. Lasky, L.A., Singer, M.S., Yednock, T.A., Dowbenko, D., Fennie, C., Rodriguez, H., Nguyen, T., Stachel, S., and Rosen, S.D. 1989. Cloning of a lymphocyte homing receptor reveals a lectin domain. *Cell* 56: 1045-1055.

30. Siegelman, M.H., van de Rijn, M., and Weissman, I.L. 1989. Mouse lymph node homing receptor cDNA encodes a glycoprotein revealing tandem interaction domains. *Science (Wash. D.C.)*. 243: 1165-1172.

31. Bowen, B.R., T. Nguyen, and L.A. Lasky. 1989. Characterization of a human homologue of the murine peripheral lymph node homing receptor. *J. Cell. Biol.* 109:421-427.

32. Siegelman, M.H., and I.L. Weissman. 1989. Human homologue of mouse lymph node homing receptor: evolutionary conservation at tandem cell interaction domains. *Proc. Natl. Acad. Sci. USA* 86:5562-5566.

33. Tedder, T.F., C.M. Isaacs, T.J. Ernst, G.D. Demetri, D.A. Adler, and C.M. Disteche. 1989. Isolation and chromosomal localization of cDNAs encoding a novel human lymphocyte cell surface molecule, LAM1. J. Exp. Med. 170:123-133.

34. Springer, T. A. 1990. Adhesion receptors of the immune system. Nature 346, 425-434.

35. Bevilacqua, M. P., S. Stengelin, M.A. Gimbrone, Jr., and B. Seed. 1989. Endothelial leukocyte adhesion molecule 1: an inducible receptor for neutrophils related to complementary regulatory proteins and lectins. *Science (Wash. DC)* 243

:1160-1165.

36. Bevilacqua, M. P., J.S. Pober, D.L. Mendrick, R.S. Cotran, and M.A. Gimbrone, Jr. 1987. Identification of an inducible endothelial leukocyte adhesion molecule, ELAM-1. *Proc. Natl. Acad. Sci. USA*. 84:9238-9242.

37. Larsen, E., A. Celi, B.C. Furie, J.K. Erban, R. Bonfanti, D.D. Wagner, and B. Furie. 1989. PADGEM protein : a receptor that that mediates the interaction of activated platelets with neutrophils and monocytes. Cell 63, 467-474 .

38. Geng, J.G., M.P. Bevilacqua, K.L. Moore, T.M. McIntyre, S.M. Prescott, J.M. Kim, G.A. Bliss, G.A. Zimmerman, and R.P. McIver. 1990. Rapid adhesion of neutrophils to activated endothelium mediated by GMP-140. *Nature (London)* 343:757-760.

39. Johnston, G.I., R.G. Cook, and R.P. McIver. 1989. Cloning of GMP-140, a granule membrane protein of platelets and the endothelium: sequence similarity to proteins involved in cell adhesion and inflammation. *Cell*. 56:1033-1044.

40. Drickamer, K., and V. McCreary. 1987. Exon structure of a mannose-binding protein gene reflects its evolutionary relationship to the asialoglycoprotein receptor and nonfibrillar collagens. *J. Biol. Chem.* 262:2582-5289.

41. Drickamer, K. 1988. Two distinct classes of carbohydrate-recognition domains in animal lectins. *J. Biol. Chem.* 263:9557-9560.

42. Ord. D.C., T.J. Ernst, L.J. Zhou, A. Rambaldi, O. Spertini, J. Griffin, and T.F. Tedder. 1990. Structure of the gene encoding the human leukocyte adhesion molecule-1 (TQ1, Leu-8) of lymphocytes and neutrophils. *J. Biol. Chem.* 265:7760-7767.

43. Imai, Y., M.S. Singer, C. Fennie, L.A. Lasky, and S.D. Rosen. 1991. Identification of a carbohydrate based endothelial ligand for a lymphocyte homing receptor. *J. Cell Biol.* 113:1213-1221.

44. Jutila. M. A., L. Rott, E.L. Berg, and E.C. Butcher. 1989. Function and regulation of the Mel-14 antigen in vivo: Comparison with LFA-1 and Mac-1. *J. Immunol.* 143: 3318-3324.

45. Jutila. M.A., T.K. Kishimoto, and M. Finken. 1990. Low dose Chymotrypsin treatment inhibits neutrophil migration into sites of inflammation in vivo: Effects on Mac-1 and Mel-14 adhesion protein expression and function. *Cell. Immunol.* 132:201-214.

46. Smith, C. W., T.K. Kishimoto, O. Abbass, B. Hughes, R. Rothlein, L.V. McIntire, E.C. Butcher, and D.C. Anderson. 1991. Chemotactic factors regulate lectin adhesion molecule-1 (LECAM-1)-dependent neutrophil adhesion to cytokine-stimulated endothelial cells in vitro. *J. Clin. Invest.* 7: 609-618.

47. Hallman, R., M.A. Jutila, C.W. Smith, D.C. Anderson, T.K. Kishimoto, and E.C. Butcher. 1991. The peripheral lymph node homing receptor, LECAM-1, is involved in CD18-independent adhesion of human neutrophils to endothelium. *Biochem. Biophys. Res. Commun.* 174: 236-243.

48. Spertini, O., F.W. Luscinskas, G.S. Kansas, M.J. Munro, J.D. Griffin, M.A. Gimbrone, Jr., and T.F. Tedder. 1991. Leukocyte adhesion molecule-1 (LAM-1, L-Selectin) interacts with an inducible endothelial ligand to support leukocyte adhesion. *J. Immunol.* 147:2565-2573.

49. Watson, S. R., Y. Imai, C. Fennie, J.S. Geoffroy, and S.D. Rosen, 1990. A homing receptor-IgG chimera as a probe for adhesive ligands of lymph node high endothelial venules. *J. Cell Biol.* 110:2221-2229.

50. Stoolman, L. M., T.S. Tenforde, and S.D. Rosen. 1984. Phosphomannosyl receptors may participate in adhesion interaction between lymphocytes and high endothelial venules. *J. Cell. Biol.* 99:1535-1540.

51. Stoolman, L.M., and S.D. Rosen. 1983. Possible role of cell-surface carbohydrate-binding molecules in lymphocyte recirculation. *J. Cell. Biol.* 96:722-729.

52. Stoolman, L.M., T.A. Yednock, and S.D. Rosen. 1987. Homing receptors on human and rodent lymphocytes--evidence for a conserved carbohydrate-binding specificity. *Blood.* 70:1842-1850.

53. Yednock, T. A., L.M. Stoolman, and S.D. Rosen. 1987. Phosphomannosyl derivatized beads detect a receptor involved with lymphocyte homing. *J. cell Biol.* 104:713-723.

54. Yednock, T.A., E.C. Butcher, L.M. Stoolman, and S.D. Rosen. 1987. Receptors involved in lymphocyte homing: relationship between a carbohydrate-binding receptor and the MEL-14 antigen. *J. Cell. Biol.* 104:725-731.

55. Imai, Y., D.D. True, M.S. Singer, and S.D. Rosen. 1990. Direct demonstration of lectin activity of gp90^{mel}, a lymphocyte homing receptor.*J. Cell Biol.* 111, 1225-1232.

56. Rosen, S.D., M.S. Singer, T.A. Yednock, and L.M. Stoolman. 1985. Involvement of sialic acid on endothelial cells in organ-specific lymphocyte recirculation. *Science* 228:1005-1007.

57. Rosen, S.D., S.-I. Chi, D.D. True, M.S. Singer, and T.A. Yednock. 1989. Intravenously injected sialidase inactivates attachment sites for lymphocytes on high endothelial venules. *J. Immunol.* 142:1895-1902.

58. Rosen, S.D. 1990. The LEC-CAMs: An emerging family of cell-cell receptors based upon carbohydrate recognition. *Am. J. Respir. Cell Mol. Biol.* 3, 397-402.

59. Ley, K., P. Gaehtgens, C. Fennie, M.S. Singer, L.A. Lasky, and S.D. Rosen. 1991. Lectin like adhesion molecule-1 mediates leukocyte rolling in mesenteric venules *in vivo*. *Blood* 77: 2553.

60. von Andrian, U. H., J.D. Chambers, L. McEvoy, R.F. Bargatze, K.E. Arfors, and E.C. Butcher. 1991. Two step model of leukocyte endothelial interaction: Distinct roles for LECAM-1 and the beta-2 integrins in vivo. *Proc. Natl. Acad. Sci. USA* 88:7538-7542.

61. Perry, M.A., and D.N. Granger. 1991. Role of CD11/CD18 in shear rate-dependent leukocyte-endothelial cell interactions in cat mesenteric venules. *J. Clin. Invest.* 87, 1798-1804.

62. Ley, K.,M. Cerrito, and K.E. Arfors. 1991. Sulfated polysaccharides inhibit leukocyte rolling in rabbit mesentery venules. *Am. J. Physiol.* H1667-H1673.

63. Tangelder, G.J., and K.E. Arfors. 1991. Inhibition of leukocyte rolling in venules by protamine and polysaccharides. *Blood.* 77, 1565-1571.

64. Von Andrian, U., P. Hansell, J.D. Chambers, E.M. Berger, I.T. Filho, E.C. Butcher, and K.E. Arfors. 1992. L-selectin function is required for ß2-integrin-mediated neutrophil adhesion at physiological shear rates in vivo. *Am. J. Physiol.* 263:H1034-H1039

65. Berg, E.L., M.K. Robinson, R.A. Warnock, and E.C. Butcher. 1991. The human peripheral lymph node vascular addressin is a ligand for LECAM-1, the peripheral lymph node homing receptor. *J. Cell Biol.* 114:234-249.

66. Imai, Y., M.S. Singer, C. Fennie, L.A. Lasky, and S.D. Rosen. 1991. Identification of a carbohydrate based endothelial ligand for a lymphocyte homing receptor. *J. Cell Biol.* 113, 1213-1221.

67. Lasky, L.A., M.S. Singer, D. Dowbenko, Y. Imai, W.J. Henzel, C. Grimley, C. Fennie, N. Gillett, S.R. Watson, and S.D. Rosen. 1992. An endothelial ligand for L-selectin is a novel mucin-like molecule. *Cell* 69:927-934.

68. Imai, Y., L.A. Lasky, and S.D. Rosen. 1993. Sulfation requirement for GlyCAM-1, an endothelial ligand for L-selectin. *Nature (London)* 361:555-557.

69. Lowe, J.B., L.M. Stoolman, R.P. Nair, R.D. Larsen, T.L. Berhend, and R.M. Marks. 1990. ELAM-1 dependent cell adhesion to vascular endothelium determined by a transfected human fucosyltransferase cDNA. *Cell* 63:475-484.

70. Walz, G., A. Aruffo, W. Kolanus, M. Bevilacqua, and B. Seed. 1990. Recognition by ELAM-1 of the sialyl-Lex determinant on myeloid and tumor cells. *Science (Wash. D.C.)* 250:1132-1135.

71. Tiemeyer, M., S.J. Swiedler, M. Ishihara, M. Moreland, H. Schweingruber, P. Hirtzer, and B.K. Brandley. 1991. Carbohydrate ligands for endothelial-leukocyte adhesion molecule-1. *Proc. Natl. Acad. Sci. USA* 88:1138-1142.

72. Polley, M.J., M.L. Phillips, E. Wayner, E. Nudleman, A.K. Singhal, S. Hakomori, and J.C. Paulson. 1991. CD62 and endothelial cell-adhesion molecule 1 (ELAM-1) recognize the same carbohydrate ligand, sialyl-Lewis x. *Proc. Natl. Acad. Sci. USA* 88:6224-6228.

73. Berg. E.L., J. Magnani, A.R. Warnock, M.K. Robinson, and E.C. Butcher. 1992. Comparison of L-selectin and E-selectin ligand specificities: The L-selectin

can bind the E-selectin ligands sialyl Lex and sialyl Lea. *Biochem. Biophys. Res. Commun.* 184:1048-1055.

74. Foxall, C., S.R. Watson, D. Dowbenko, C. Fennie, L.A. Lasky, M. Kiso, A. Hasigawa, D. Asa, and B.K. Brandley. 1992. The three members of the selectin receptor family recognize a common carbohydrate epitope, the sialyl Lewis x oligosaccharide. *J. Cell Biol.* 117:895-902.

75. Huang, K., J.S. Geoffroy, M.S. Singer, and S.D. Rosen. 1991. A lymphocyte homing receptor (L-selectin) mediates the in vitro attachment of lymphocytes to myelinated tracts of the central nervous system. *J. Clin. Invest.* 88:1778-1783.

76. Willenborg, D.O., and C.R. Parish. 1988. Inhibition of allergic encephalomyelitis in rats by treatment with sulfated polysaccharides. *J. Immunol.* 140:3401-3405.

77. Willenborg, D.O., C.R. Parish, and W.B. Cowden. 1989. Phosphosugars are potent inhibitors of central nervous system inflammation. *FASEB J.* 3:1968-1971.

78. Kishimoto, T.K., M.A. Jutila, E.L. Berg, and E.C. Butcher. 1989. Neutrophil Mac-1 and Mel-14 adhesion proteins inversely regulated by chemotactic factors. *Science (Wash. D.C.)* 245:1238-1241.

79. Jung, T.M., and M.O. Dailey. 1990. Rapid modulation of homing receptors (gp90MEL-14) induced by activators of protein kinase C. Receptor shedding due to accelerated proteolytic cleavage at the cell surface. *J. Immunol.* 144:3130-3136.

80. Camerini, D., S.P. James, I. Stamenkovic, and B. Seed. 1989. Leu-8/TQ1 is the human equivalent of the Mel-14 lymph node homing receptor. *Nature* 342:78.

81. Ord, D.C., T.J. Ernst, L.J. Zhou, A. Rambaldi, O. Spertini, J. Griffin, and T.F. Tedder. 1990. Structure of the gene encoding the human leukocyte adhesion molecule-1 (TQ1, Leu-8) of lymphocytes and neutrophils. *J. Immunol.* 265:7760.

82. Spertini, O., G.S. Kansas, J.M. Munro, J.D. Griffin, and T.F. Tedder. 1991. Regulation of leukocyte migration by activation of the leukocyte adhesion molecule-1 (LAM-1) selectin. *Nature (London)* 349:691-694.

83. Kansas, G.S., K. Ley, J.M. Munro, and T.F. Tedder. 1993. Regulation of leukocyte rolling and adhesion to endothelial venules through the cytoplasmic domain of L-selectin. *J. Exp. Med.* 177:833-838.

84. Walcheck, B., M. White, S. Kurk, T.K. Kishimoto, and M.A. Jutila. 1992. Characterization of the bovine lymph node homing receptor: a lectin cell adhesion molecule (LECAM). Eur. J. Immunol. 22:469-476.

85. Pilarski, L.M., E.L. Turley, A.R.E. Shaw, W.M. Gallatin, M.P. Laderoute, R. Gillitzer, I.G.R. Beckman, and H. Zola. 1991. FMC46, a cell protrusion associated leukocyte adhesion molecule-1 epitope on human lymphocytes and thymocytes. *J. Immunol.* 47: 136-143.