

LEUKOCYTE ADHESION TO THE VASCULATURE

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I. Leukocyte-Endothelial Cell Adhesion Molecules

The recruitment of leukocytes from the blood stream to extravascular tissue is a critical event in host defense against microbial invasion and in the repair of tissue damage. Studies by intravital microscopy have established a sequence of events involved in phagocyte emigration at sites of inflammation. In response to extravascular stimuli such as bacterial-derived chemoattractants or endogenous lipid and peptide mediators, signals are generated that activate both the leukocyte and the endothelial cell. As a consequence of activation, one or both cell types become adhesive, leading to transient adhesion of the leukocyte to endothelium. The result of these initial adhesive interactions and shear forces due to blood flow is leukocyte "rolling" along the vessel wall. With further stimulation, some of the rolling leukocytes adhere firmly, or "stick," and then diapedese between endothelial cells to emigrate through tissue in response to chemoattractants.

These adhesive interactions - rolling, sticking, and diapedesis - are mediated by cell surface molecules expressed on the leukocytes and endothelial cells (reviewed in detail in references 1 and 2). Adhesion molecules involved in leukocyte-endothelial adherence can generally be placed in three categories: integrins, members of the immunoglobulin superfamily (IgSF), and selectins. Leukocyte integrin receptors interact primarily with IgSF ligands on the endothelial cell; selectin receptors expressed on both the leukocyte and the endothelial cell recognize specific carbohydrate counter-structures. Known integrin/IgSF interactions include the beta1 integrin VLA-4 with vascular cell adhesion molecule-1 (VCAM-1); the beta2 integrins LFA-1 with intercellular adhesion molecule-1 (ICAM-1) or ICAM-2 and Mac-1 with ICAM-1; the beta7 integrin alpha4beta7 with the mucosal address in MAdCAM-1 (or to a lesser extent with VCAM-1); and the integrin alphaVbeta3 with platelet-endothelial cell adhesion molecule-1 (PECAM-1). Endothelial E-selectin, endothelial P-selectin and leukocyte L-selectin all recognize the sialylated, fucosylated tetrasaccharide, sialyl Lewis X (SLeX), as well as other sialylated, fucosylated carbohydrate structures. Some ligands for L-selectin are also sulfated. The proteins that bear the carbohydrate ligands for the selectins include leukocyte PSGL-1 (P-selectin glycoprotein-1), which binds to P- and E-selectin; ESL-1 (E-selectin ligand-1), which binds to E-selectin; and CD34 on high endothelial venules (HEV) of peripheral nodes and MAdCAM-1 on mucosal HEV that bear carbohydrate moieties recognized by leukocyte L-selectin.

Other pathways of leukocyte adhesion to endothelium have also been described including binding of Mac-1 to iC3b deposited on endothelium, "bridging" by fibrinogen bound to endothelial ICAM-1 and leukocyte Mac-1, and binding of leukocyte VLA-4 to the CS-1 fragment of fibronectin expressed on the luminal surface of endothelium. In particular, a homophilic interaction of leukocyte PECAM-1 with endothelial PECAM-1 that is localized at inter-endothelial cell junctions appears to be critical for phagocyte diapedesis between endothelial cells. Table 1 lists the leukocyte-endothelial adhesion

pathways that have been confirmed *in vivo* using adhesion-blocking reagents in animal models.

II. Adhesion Cascade

Studies *in vitro* and *in vivo* have led to a model of leukocyte-endothelial interactions defined as an adhesion cascade (Figure 1). Briefly, initial contact of leukocyte with the inflamed vessel wall results in selectin-mediated, low-affinity adhesion that initially 'tethers' the leukocyte and then is manifested as rolling

Table 1. Leukocyte-Endothelial Adhesion Pathways Demonstrated *In Vivo*

Integrins	Counter-Structure(s)
LFA-1 ($\alpha_L\beta_2$; CD11a/CD18)	ICAM-1
Mac-1 ($\alpha_M\beta_2$; CD11b/CD18)	ICAM-1
$\alpha_4\beta_7$	MAdCAM-1
VLA-4 (CD49d/CD29)	VCAM-1
Ig Superfamily	
ICAM-1 (CD54)	LFA-1, Mac-1
PECAM-1 (CD31)	PECAM-1
MAdCAM-1	$\alpha_4\beta_7$, L-Selectin
VCAM-1 (CD106)	VLA-4
Selectins	
E-Selectin (CD62E)	Sialylated, fucosylated glycoconjugates (e.g., SLeX, CD15s)
P-Selectin (CD62P)	Sialylated, fucosylated glycoconjugates (e.g., SLeX)
L-Selectin (CD62L)	Sialylated glycoconjugates expressed on CD34 and MAdCAM-1

along the surface of the endothelium under conditions of flow. With this initial tethering and rolling, the leukocyte integrins can then be activated by soluble or surface-bound agonists to bind to their IgSF endothelial ligands. Integrin activation produces firm 'sticking' that is resistant to shear forces, allowing subsequent leukocyte migration across the endothelium and then diapedesis between endothelium via integrin/IgSF or PECAM-1/PECAM-1 interactions. Subsequent migration through subendothelial tissue involves integrin binding to matrix components.

There are several important caveats to consider with regards to this current model. First, rolling may not be a prerequisite for emigration under conditions of reduced shear forces, i.e., when there is stasis. A second concern is that the model was developed for leukocyte emigration in the systemic microcirculation where emigration occurs predominantly in post-capillary venules. Its validity in the pulmonary microcirculation

where emigration occurs in capillaries or in the arterial circulation where shear forces may be considerably greater remains untested. Third, under some conditions leukocytes are able to roll on VCAM-1 via VLA-4, an integrin/IgSF interaction. Finally, although this model likely applies to leukocytes other than neutrophils and lymphocytes, e.g., monocytes, this remains yet to be shown *in vivo*.

III. Leukocyte Adhesion Deficiency Syndromes

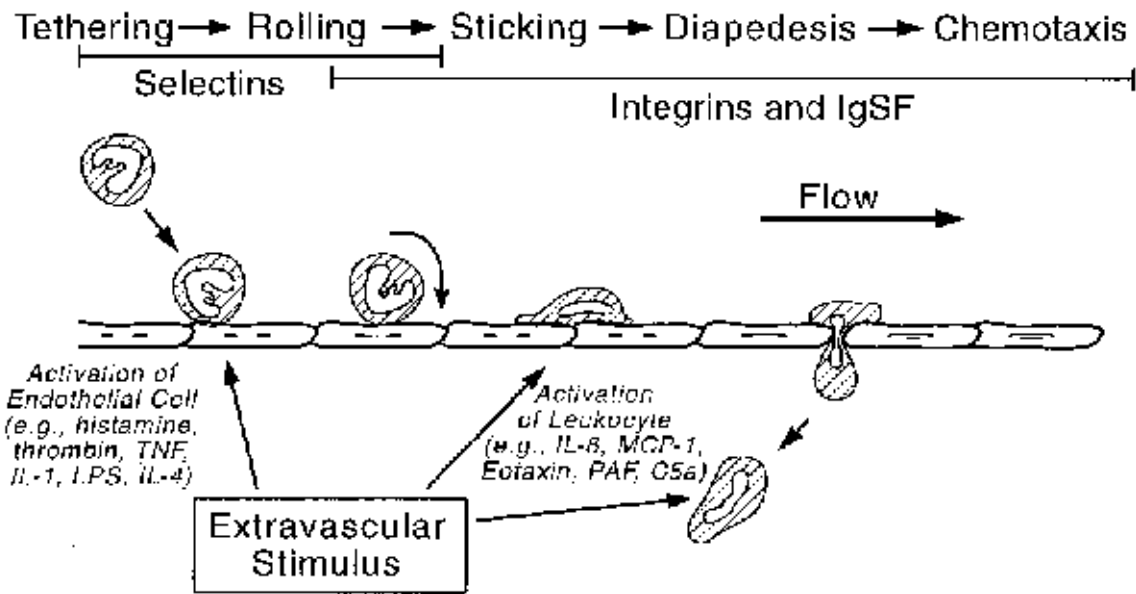
LAD I

As with other questions in biology, important insights into the molecular basis of leukocyte-endothelial interactions have come from the investigation of genetic deficiency syndromes (Table 2). Congenital deficiency of CD11/CD18 due to heterogeneous mutations in the common beta2 subunit - the Leukocyte Adhesion Deficiency syndrome Type I (LAD I) - is associated with a profound defect in neutrophil and monocyte emigration to sites of inflammation in the systemic circulation (reviewed in 3,4). Studies *in vitro* and *in vivo* indicate that the interaction of the beta2 integrin receptor complex, CD11/CD18, with ICAM-1 or other endothelial ligands is responsible for a major component of neutrophil sticking to endothelium. The inability of LAD I neutrophils to stick to and diapedese between endothelium accounts for the clinical features of the syndrome - recurrent bacterial and fungal infections without pus formation, neutrophilic leukocytosis, chronic periodontitis, and delayed wound healing.

Observations in LAD I patients demonstrated the presence of mononuclear leukocytes and eosinophils in inflamed tissues and thereby suggested the presence of an alternate adhesion pathway to CD11/CD18 - ICAM-1 for these cell types. Studies *in vitro* and *in vivo* indicate that the beta1 integrin receptor VLA-4 and its cytokine-induced endothelial ligand VCAM-1 constitute this second pathway of adherence. Notably, amongst circulating leukocytes, only neutrophils do not express significant VLA-4. The unique absence of VLA-4 on circulating neutrophils most likely accounts for the inability of these cells to emigrate in LAD I syndrome, i.e., the CD11/CD18-deficient neutrophils cannot utilize the alternate VLA-4/VCAM-1 pathway.

LAD II

In contrast to sticking and diapedesis, the phenomenon of leukocyte



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Figure 1. Adhesion Cascade.

Table 2: Clinical Manifestations of LAD I and LAD II

rolling is not dependent upon integrin receptors but involves primarily the interaction of selectin receptors with their carbohydrate counter-structures.

	LAD I	LAD II
Delayed separation of umbilical cord		+ -
Recurrent infections	++	+
Neutrophilia	+	+
Impaired phagocyte emigration	+	+
Impaired antibody response	+	-
Impaired cell-mediated immunity		+/- -
Developmental abnormalities	-	+

This conclusion is supported by recent observations in two patients with LAD Type II syndrome.⁽⁵⁾ The two LAD II patients have a congenital defect of fucose metabolism that results in a complete deficiency of SLeX, the fucosylated carbohydrate structure recognized by E- and P-selectins (and likely also L-selectin). These patients also exhibit a defect in neutrophil emigration to tissue.⁽⁶⁾ Observations by intravital microscopy using fluorescein-labeled LAD I or LAD II human neutrophils in inflamed rabbit mesenteric vessels support the current model of adhesion cascade with defects in either rolling or sticking reducing emigration. LAD I cells were able to roll but did not stick or emigrate; LAD II cells were unable to roll or emigrate under conditions of flow, but stuck and emigrated when flow was reduced.⁽⁷⁾ The ability of LAD II neutrophils to adhere when flow is reduced and the apparently normal immune function of LAD II cells may account for the milder clinical phenotype of LAD II vs. LAD I.

Through the technique of gene targeting, a variety of adhesion molecule-deficient mice have been generated for the study of leukocyte-endothelial interactions (Table 3). In general, the phenotype of the deficient adult animals has been consistent with results obtained previously with blocking monoclonal antibodies (MAbs).

IV. Regulation of Adhesion

Quantitative and qualitative alterations of adhesion molecules play an important role in these adhesive interactions.^(1,2) Adhesion proteins are induced on endothelium by diverse inflammatory stimuli. In cultured human endothelial cells, P-selectin is rapidly mobilized from subcellular stores in Weibel-Palade bodies to the luminal surface in response to thrombin or histamine. Stimulation of endothelial cells by a variety of stimuli also induces surface expression of platelet-activating factor (PAF), a lipid mediator that can activate CD11/CD18. E-selectin, VCAM-1, and ICAM-1 are induced over a period of hours following stimulation of endothelium by interleukin-1 (IL-1), tumor necrosis factor-alpha

Table 3: Adhesion Molecule–Deficient Mice

Deficiency	Findings
β_2 (CD18)	<ul style="list-style-type: none"> • partial deficiency with mild leukocytosis, reduced emigration, delay in transplant rejection⁽⁸⁾ • develop psoriasiform skin disorder on PL/J background⁽⁹⁾
VLA-4	<ul style="list-style-type: none"> • embryonic lethal due to failure of allantois to fuse (early) or epicardial defect (late)⁽¹⁰⁾
ICAM-1	<ul style="list-style-type: none"> • moderate granulocytosis; impaired neutrophil emigration; decreased contact hypersensitivity⁽¹¹⁾ • protected against ischemic renal injury⁽¹²⁾, cerebral ischemic-reperfusion injury⁽¹³⁾, and septic shock⁽¹⁴⁾
VCAM-1	<ul style="list-style-type: none"> • majority die as embryo from failure of allantois to fuse to chorion; rare mice survive as adults with elevated circulating mononuclear leukocytes⁽¹⁵⁾
E-Selectin	<ul style="list-style-type: none"> • no developmental abnormalities; no abnormalities in circulating leukocyte counts or emigration⁽¹⁶⁾ • addition of anti-P-Selectin Mab produces marked defect in neutrophil emigration⁽¹⁶⁾
P-Selectin	<ul style="list-style-type: none"> • elevated circulating neutrophil counts; reduced neutrophil and platelet rolling; reduced early phagocyte emigration⁽¹⁷⁻²⁰⁾ • reduced leukocyte recruitment in contact hypersensitivity⁽²¹⁾ and mild defect in hemostasis⁽²²⁾

- L-Selectin
 - normal circulating neutrophil counts; reduced lymphocyte homing; reduced neutrophil rolling; impaired early neutrophil emigration(23,18)
 - reduced leukocyte emigration to DTH and contact hypersensitivity sites; resistance to endotoxic shock(24)
- P-Selectin + ICAM-1
 - absent trauma-induced leukocyte rolling(25)
 - absent neutrophil emigration into inflamed peritoneum but normal emigration into inflamed alveoli(26)
- E-Selectin + P-Selectin
 - extreme leukocytosis; susceptibility to opportunistic bacterial infections; altered hematopoiesis with elevated IL-3 and GM-CSF levels(27)

(TNF-alpha), and lipopolysaccharide (LPS). (In mice P-selectin is also induced over several hours by LPS and cytokines.) Interferon-gamma induces ICAM-1 and potentiates the induction of E-selectin, while interleukin-4 (IL-4) induces VCAM-1 but not ICAM-1 or E-selectin.

Leukocyte integrin receptors must be in a low binding state in order for cells to circulate, but able to rapidly increase their binding to the vasculature at sites of inflammation and immune reaction. Adhesivity of leukocytes is determined by both quantitative and qualitative changes. Activation of neutrophils by chemoattractants or by some cytokines causes shedding of L-selectin and translocation of CD11b/CD18 from secondary or tertiary granules to the plasma membrane. However, for leukocyte integrins, qualitative changes in the receptor are more important than quantitative changes. The process by which leukocyte activation triggers a change in integrin receptor avidity is termed "inside-out" signaling. The precise molecular basis of avidity modulation remains an area of intense research in integrin receptor biology.⁽²⁸⁾ The increase in avidity of leukocyte integrins is determined by changes in receptor conformation that directly alter affinity and/or by post-receptor events involving interactions of integrin cytoplasmic domains with various cytoskeletal components to form adhesion complexes.

V. Selective Recruitment

Selective recruitment of leukocyte subpopulations to sites of inflammation or immune reaction is a process involving the combination of cytokines or other mediators that induce endothelial selectin or IgSF molecules and chemokines and other chemoattractants that activate the leukocyte integrins.^(1,2,29) For example, in asthma, expression of P- or E-selectin in concert with the preferential induction of VCAM-1 by IL-4 released by mast cells or Th2 lymphocytes and activation of eosinophil integrins by the chemokine eotaxin released from lung cells would promote eosinophil influx. Similarly, in atherosclerosis induction of P-selectin, VCAM-1 or ICAM-1 in conjunction with production of the chemokine monocyte-chemoattractant protein (MCP-1) by arterial wall cells would favor monocyte accumulation.

VI. Consequences of Leukocyte Adherence to Endothelium

Although critical for host defense and repair, under some circumstances leukocyte adherence to endothelium may provoke vascular and tissue damage. Leukocytes tightly adherent to the vessel wall may release toxic products that injure endothelium, producing permeability edema or thrombosis. Emigrated leukocytes may similarly damage adjacent tissue. Inhibition of leukocyte adherence - "anti-adhesion" therapy - has the potential to reduce vascular damage by preventing the formation of a protected microenvironment between adherent leukocytes and endothelium that is inaccessible to endogenous inhibitors or by inhibiting leukocyte traffic to tissue. Potential therapeutics include antagonists of adhesion receptor-ligand interactions (e.g., humanized MAbs, oligosaccharides, peptides, chimeric receptors) or agents that inhibit the induction, mobilization, or activation of leukocyte or endothelial cell adhesion molecules. The efficacy of anti-adhesion therapy has been demonstrated in a variety of animal models with now more than 100 publications on this topic. Several of these anti-adhesion therapies are currently in clinical trials including: SLeX oligosaccharides in myocardial infarction and chronic pulmonary thrombotic obstruction, CD18 MAb and anti-VLA-4 MAb in multiple sclerosis, anti-E-selectin Mab in septic shock, and anti-ICAM-1 MAb in renal allograft recipients, burn patients, stroke patients, and patients with refractory rheumatoid arthritis.

References

1. Springer TA. Traffic signals for lymphocyte recirculation and leukocyte emigration: the multistep paradigm. *Cell* 76: 301, 1994.
2. Carlos TM, Harlan JM. Leukocyte-endothelial adhesion molecules. *Blood* 84: 2068, 1994.
3. Anderson CD, Schmalstieg FC, Finegold MJ, et al. The severe and moderate phenotypes of heritable Mac-1, LFA-1 deficiency: their quantitative definition and relation to leukocyte dysfunction and clinical features. *J Infect Dis* 152: 668, 1985.
4. Harlan JH. Leukocyte adhesion deficiency syndrome: insights into the molecular basis of leukocyte emigration. *Clin Immunol Immunopathol* 67: S16, 1993.
5. Etzioni A, Frydman M, Pollack S, Avidor I, Phillips ML, Paulson JC, Gershoni-Baruch R. Brief report: recurrent severe infections caused by a novel leukocyte adhesion deficiency. *N Engl J Med* 327: 1789, 1992.
6. Price TH, Ochs HD, Gershoni-Baruch R, Harlan JM, Etzioni A. In vivo neutrophil and lymphocyte function studies in a patient with leukocyte adhesion deficiency type II. *Blood* 84: 1635, 1994.
7. von Andrian UH, Berger EM, Ramezani L, Chambers JD, Ochs HD, Harlan JM, Paulson JC, Etzioni A, Arfors KE. In vivo behavior of neutrophils from two patients with distinct inherited leukocyte adhesion deficiency syndromes. *J Clin Invest* 91: 2893, 1993.
8. Wilson RW, Ballantyne CM, Smith CW, Montgomery C, Bradley A, OsBrien WE, Beaudet AL. Gene targeting yields a CD18-mutant mouse for study of inflammation. *J Immunol* 151: 1571, 1993.

9. Bullard DC, Scharffetter-Kochanek K, McArthur MJ, Chosay JG, McBride ME, Montgomery CA, Beaudet AL. A polygenic mouse model of psoriasiform skin disease in CD18-deficient mice. *Proc Natl Acad Sci USA* 93: 2116, 1996.
10. Yang JT, Rayburn H, Hynes RO. Cell adhesion events mediated by $\alpha 4$ integrins are essential in placental and cardiac development. *Develop* 121: 549, 1995.
11. Sligh Jr JE, Ballantyne CM, Rich SS, Hawkins HK, Smith CW, Bradley A, Beaudet AL. Inflammatory and immune responses are impaired in mice deficient in intercellular adhesion molecule 1. *Proc Natl Acad Sci USA* 90: 8529, 1993.
12. Kelly KJ, Williams Jr WW, Colvin RB, Meehan SM, Springer TA, Gutierrez-Ramos JC, Bonventre JV. Inter-cellular adhesion molecule-1-deficient mice are protected against ischemic renal injury. *J Clin Invest* 97: 1056, 1996.
13. Connolly Jr ES, Winfree CJ, Springer TA, Naka Y, Liao H, Yan SD, Stern DM, Solomon RA, Gutierrez-Ramos JC, Pinsky DJ. Cerebral protection in homozygous null ICAM-1 mice after middle cerebral artery occlusion: role of neutrophil adhesion in the pathogenesis of stroke. *J Clin Invest* 97: 209, 1996.
14. Xu H, Gonzalo JA, St Pierre Y, Williams IR, Kupper TS, Contran RS, Springer TA, Gutierrez-Ramos JC. Leukocytosis and resistance to septic shock in intercellular adhesion molecule 1-deficient mice. *J Exp Med* 180: 95, 1994.
15. Gurtner GC, Davis V, Li H, McCoy MJ, Sharpe A, Cybulsky MI. Targeted disruption of the murine VCAM-1 gene: essential role of VCAM-1 in chorioallantoic fusion and placentation. *Genes Dev* 9: 1, 1995.
16. Labow MA, Norton CR, Rumberger JM, Lombard-Gillooly KM, Shuster DJ, Hubbard J, Bertko R, Knaack PA, Terry RW, Harbison ML, Kontgen F, Stewart CL, McIntyre KW, Will PC, Burns DK, Wolitzky BA. Characterization of E-selectin-deficient mice: demonstration of overlapping function of the endothelial selectins. *Immunity* 1: 709, 1994.
17. Mayadas TN, Johnson RC, Rayburn H, Hynes RO, Wagner DD. Leukocyte rolling and extravasation are severely compromised in P selectin-deficient mice. *Cell* 74: 541, 1993.
18. Ley K, Bullard DC, Arbonson ML, Bosse R, Vestweber D, Tedder TF, Beaudet AL. Sequential contribution of L- and P-selectin to leukocyte rolling in vivo. *J Exp Med* 181: 669, 1995.
19. Johnson RC, Mayadas TN, Frenette PS, Mebius RE, Subramaniam M, Lacasce A, Hynes RO, Wagner DD. Blood cell dynamics in P-selectin-deficient mice. *Blood* 86: 1106, 1995.
20. Frenette PS, Johnson RC, Hynes RO, Wagner DD. Platelets roll on stimulated endothelium in vivo: an interaction mediated by endothelial P-selectin. *Proc Natl Acad Sci USA* 92: 7450, 1995.
21. Subramaniam M, Saffaripour S, Watson SR, Mayadas TN, Hynes RO, Wagner DD. Reduced recruitment of inflammatory cells in a contact hypersensitivity response in P-selectin-deficient mice. *J Exp Med* 181: 2277, 1995.
22. Subramaniam M, Frenette PS, Saffaripour S, Johnson RC, Hynes RO, Wagner DD. Defects in hemostasis in P-selectin-deficient mice. *Blood* 87: 1238, 1996.

23. ArbonJs ML, Ord DC, Ley K, Ratch H, Maynard-Curry C, Otten G, Capon DJ, Tedder TF. Lymphocyte homing and leukocyte rolling and migration are impaired in L-selectin-deficient mice. *Immunity* 1: 247, 1994.
24. Tedder TF, Steeber DA, Pizcueta P. L-selectin-deficient mice have impaired leukocyte recruitment into inflammatory sites. *J Exp Med* 181: 2259, 1995.
25. Kunkel EJ, Jung U, Bullard DC, Norman KE, Wolitzky BA, Vestweber D, Beaudet AL, Ley K. Absence of trauma-induced leukocyte rolling in mice deficient in both P-selectin and intercellular adhesion molecule 1. *J Exp Med* 183: 57, 1996.
26. Bullard DC, Qin L, Lorenzo I, Quinlin WM, Doyle NA, Bosse R, Vestweber D, Doerschuk CM, Beaudet AL. P-selectin/ICAM-1 double mutant mice: acute emigration of neutrophils into the peritoneum is completely absent but is normal into pulmonary alveoli. *J Clin Invest* 95: 1782, 1995.
27. Frenette PS, Mayadas TN, Rayburn H, Hynes RO, Wagner DD. Susceptibility to infection and altered hematopoiesis in mice deficient in both P- and E-selectins. *Cell* 84: 563, 1996.
28. Stewart M, Thiel M, Hogg N. Leukocyte integrins. *Curr Op Cell Biol* 7: 690, 1995.
29. Butcher EC. Leukocyte-endothelial cell recognition: three (or more) steps to specificity and diversity. *Cell* 67: 1033, 1991.