Immune Network

# Inhibition of Leukocyte Adhesion by Developmental Endothelial Locus-1 (Del-1)

*Eun Young Choi\**

Experimental Immunology Branch, National Cancer Institute, National Institutes of Health, Bethesda, Maryland, USA

The leukocyte adhesion to endothelium is pivotal in leukocyte recruitment which takes place during inflammatory, auto-immune and infectious conditions. The interaction between leukocytes and endothelium requires an array of adhesion molecules expressed on leukocytes and endothelial cells, thereby promoting leukocyte recruitment into sites of inflammation and tissue injury. Intervention with the adhesion molecules provides a platform for development of anti-inflammatory therapeutics. This review will focus on developmental endothelial locus-1 (Del-1), an endogenous inhibitor of leukocyte adhesion.
[Immune Network 2009;9(5):153-157]

## INTRODUCTION

### Leukocyte-endothelial Interactions

Leukocytes circulating in the blood need to migrate into tissues where a pathogen- or nonpathogen-derived antigen or inflammation is present. In particular, leukocyte recruitment is pivotal during infection caused by bacterial, viral, fungal or parasitic pathogens, during inflammatory disorders, and in the course of autoimmune diseases. All leukocytes participating in innate or adaptive immunity have the ability to migrate to the sites of inflammation or tissue injury by crossing endothelial barriers between blood and tissues (1,2). The process of leukocyte recruitment comprises a tightly regulated cascade of adhesive interactions between leukocytes and endothelial cells; (i) leukocyte capture by activated endothelium, (ii) leukocyte rolling on the endothelium, (iii) slow rolling of leukocytes on the endothelium, (iv) leukocyte activation by chemokines on the endothelium, (v) firm adhesion of leukocytes onto the endothelium, (vi) post-adhesion leukocyte crawling or locomotion, and (vii) transendothelial migration or diapedesis. Distinct families of adhesion molecules control which cells are to be correctly recruited to the right place at the right time (Fig. 1) (3,4).

## ADHESION MOLECULES THAT REGULATE LEUKOCYTE-ENDOTHELIAL INTERACTIONS

At sites of inflammation, the endothelium is locally activated by cytokines to express adhesion molecules called selectins on its surface where the leukocytes are captured from the blood and start rolling along the endothelial surface, which is the initial step of the leukocyte-endothelial interactions. Capturing and rolling are primarily mediated by the interaction between endothelial selectins and their glycosylated leukocyte selectin ligands. Under certain circumstances, however, capturing and rolling are mediated by the interaction between leukocyte integrins $\alpha_4\beta_1$ (VLA-4) and $\alpha_4\beta_7$ and their endothelial ligands VCAM-1 and MadCAM-1, both of which are members of immunoglobulin superfamily (5-9).

The leukocyte rolling on the vessel wall allows the interaction of G-protein coupled receptors (GPCRs) on the leukocyte surface with the specific chemokines which are clustered and immobilized via binding to glycosaminoglycans in the endothelial glycocalyx, thereby priming the GPCRs (10-12). Activation of the GPCRs triggers an inside-out signaling cascade which induces integrin clustering at contact site and rapid conformational change in integrins to an active state. This allows efficient binding of leukocyte $\beta1$ and $\beta2$ integrins, i.e., LFA-1, Mac-1 or VLA-4, to immunoglobulin superfamily receptors, ICAM-1, -2 or VCAM-1, on the endothelium, resulting in leukocyte arrest and firm adhesion to the vessel wall

## Leukocyte recruitment:
## Leukocyte-endothelial interactions
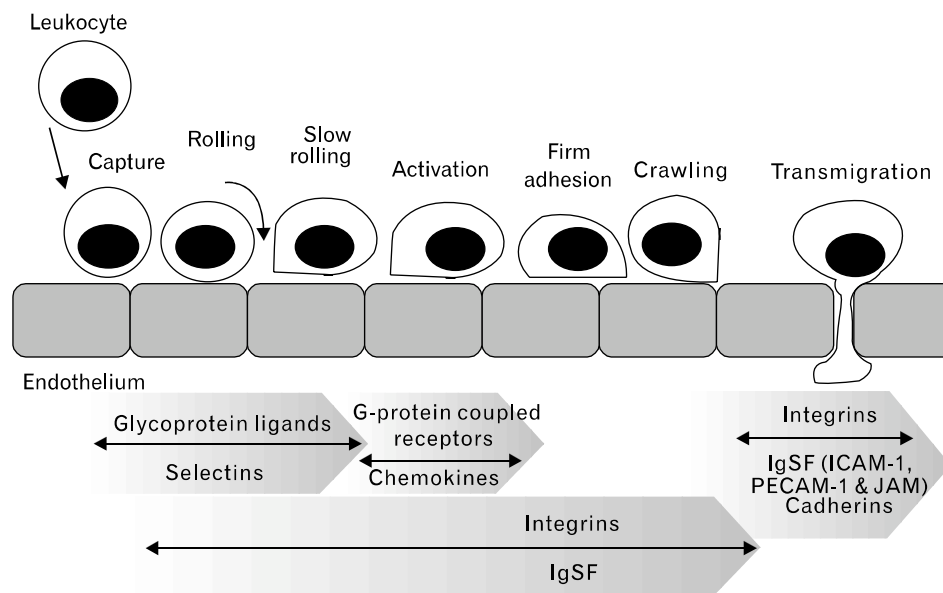


**Figure 1.** A schematic diagram of the process of leukocyte recruitment.

(13,14). Selectin-mediated initial leukocyte rolling and sub-sequent integrin activation may also cooperate to mediate an additional step termed slow rolling prior to the step of firm adhesion (8,15).

Once firmly adhered, leukocytes crawl or locomote along the endothelial surface to find a junction between two endo-thelial cells (paracellular route) or to find a way to pass through one endothelial cell (transcellular route). Formation of transmigratory cups by leukocyte integrin LFA-1 and its en-dothelial ligand ICAM-1 is essential in both paracellular and transcellular routes (16-19). Ligation of ICAM-1 by leukocyte integrins, followed by intracellular signaling events, opens up endothelial junctions (20-23). Transmigration of leukocytes through the endothelial layer is achieved by a chain of adhe-sive events which are orchestrated by a number of adhesion molecules. Several homophilic and heterophilic interactions between the leukocyte/endothelial apical and junctional ad-hesion molecules take place sequentially (24-26). Molecules directly acting in the transmigration include leukocyte in-tegrins, endothelial immunoglobulin superfamily members (ICAM-1, -2, JAM-A, -B, -C and PECAM-1) and a non-im-munoglobulin molecule CD99 (27-31).

The aforementioned classical leukocyte-endothelial inter-actions in the leukocyte recruitment do not occur in all tissues. Non-classical leukocyte endothelial interactions are regulated by the tissue-specific microvasculature environment with unusual combinations of distinct endothelial adhesion molecules and chemokines, and tissue-specific signaling path-ways (32-35). For example, leukocyte migration to the liver primarily takes place in the hepatic sinusoids, not in the post-capillary venules as is the case with many other tissues. As the hepatic sinusoids have non-classical endothelial adhesion molecules and a set of unique predominant chemokines on the vascular endothelium, unusual leukocyte adhesion mole-cules are employed (33,35-37).

Although immune cell migration is critical for protective im-mune responses against pathogens, the accumulation of leu-kocytes in the tissues could trigger significant release of cyto-toxic mediators from leukocytes, leading to tissue damage and finally a wide spectrum of inflammatory conditions. Thus, inhibition of excessive or misdirected leukocyte recruit-ment provides a means for anti-inflammatory therapeutics.

## DEVELOPMENTAL ENDOTHELIAL LOCUS-1 (DEL-1): STRUCTURE AND EXPRESSION

Del-1 (also termed Edil3) is a 52 kDa glycoprotein secreted by endothelial cells and associates with the endothelial cell

**Figure 2.** A domain structure of Del-1.

surface and extracellular matrix, but seems not to circulate in the blood (38-40) (Choi unpublished). Human Del-1 shares more than 97% amino acid identity with the mouse counterpart. Del-1 consists of a signal sequence at its N-terminus, three epidermal growth factor (EGF)-like repeats and two discoidin I-like domains at its C-terminus (Fig. 2) (40). It has been shown that the discoidin domains are responsible for binding to sulfated glycan and phosphatidyl serine-rich membrane (39,40). On the other hand, the second EGF like repeat contains an Arg-Gly-Asp (RGD) motif that enables Del-1 to bind integrin $\alpha_v\beta_3$, mediating adhesion of endothelial cells to Del-1 (40).

Expression of Del-1 was initially observed in embryonic cells including endothelium and thymus, and subsequent studies showed its expression in adult endothelial cells, some subsets of macrophages and hypertrophic chondrocytes, It was recently reported, however, that Del-1 is expressed in adult mice in a tissue-specific manner: strong expression in the lung, brain and eye, a very little in the kidney, and none in the intestine, liver, heart, spleen, whole blood and bone marrow cells (38).

## INHIBITION OF LEUKOCYTE-ENDOTHELIAL INTERACTION BY DEL-1

A role for Del-1 may be inferred from its expression in adult vascular endothelium. Recently, it was demonstrated that Del-1 functions as an endogenous inhibitor of a major leukocyte adhesion receptor LFA-1 to prevent leukocyte adhesion to the endothelium (38). Unlike many adhesion molecules including selectins and Ig superfamily members promoting leukocyte adhesion on the endothelium, Del-1 inhibits the process of leukocyte binding to the endothelium, thereby suppressing entrance of leukocytes to inflamed tissues.

Del-1's loss of function phenotype is characterized by an increase in leukocyte adhesion on the vascular endothelium and increased inflammatory cell recruitment. Del-1 deficient mice showed more leukocytes adhering on the post-capillary vasculature under both non-inflamed and inflamed conditions, than wild type mice, as demonstrated by intravital mi-

croscopy (38). Increased adhesion of leukocytes to the vascular endothelium was a prelude to an increase in leukocyte migration into the inflamed tissue. Intranasal administration of LPS to the Del-1 deficient mice to induce lung inflammation yielded more neutrophil accumulation in bronchoalveolar lavage fluid, as opposed to control mice (38). This phenotype was reversed by the absence of LFA-1 on leukocytes. Upon LPS-elicited lung inflammation, LFA-1/Del-1 double deficient mice display very low leukocyte recruitment, indicating that the phenotype of Del-1 deficiency is dependent on the presence of LFA-1 (38).

Given that Del-1 is a ligand for LFA-1, it is intriguing that Del-1 inhibits the function of LFA-1 (38). Other LFA-1 ligands including ICAM-1 are known to enhance leukocyte adhesion. However, Del-1 is an exception. Under physiologic flow conditions, addition of Del-1 to the system where leukocytes adhere onto immobilized ICAM-1 via interaction with activated LFA-1 represses the adhesion of leukocytes (38). This implies that Del-1 may compete with ICAM-1 for the binding to LFA-1. Studies on the mechanism by which Del-1 inhibits LFA-1 functions are underway.

Del-1-mediated inhibition of the interaction between LFA-1 and ICAM-1 further implies that Del-1 could be used as a therapeutic tool for inflammatory diseases. In such a case, a fair amount of exogenous Del-1 is worth to employ, given that ICAM-1 is up-regulated in the tissues during the course of inflammation. Following LPS-elicited lung inflammation, Del-1 deficient mice supplemented with soluble Del-1 behaved in a comparable fashion to the wild type mice in terms of inflammatory cell recruitment into the lung tissues, recapitulating Del-1's potential as anti-inflammatory therapeutics (38).

Modulation of Del-1 expression could also represent a candidate therapeutic strategy for autoimmune and chronic inflammatory disease. Del-1 mRNA message was down-regulated in the inflamed tissues and the endothelial cells upon induction of inflammation by TNF-$\alpha$ stimulation (38). It is reasonable therefore that as a resident anti-inflammatory molecule, such a decrease in Del-1 expression may render the body's normal inflammatory response more competent. It is tempting to speculate that constitutively expressed Del-1 in certain tissues may function as a gate keeper within the tissues against unspecified potential weak inflammatory agents. As a result, normal healthy subjects should not respond to the agents, thereby preventing the initiation of immune response. However, those who have defects in the ex-

pression and/or function of Del-1 may show hypersensitivity. Taken together, Del-1 holds promise for treating diseases related to excessive or deficient recruitment of leukocytes. If excessive recruitment is required (i.e., infectious disease), a strategy to inhibit Del-1 should be applied. Conversely, if excessive leukocyte is the culprit, a strategy to boost Del-1 should be applied.

## ACKNOWLEDGEMENTS

## CONFLICTS OF INTEREST

The authors have no financial conflict of interest.

## REFERENCES

1. von Andrian UH, Mackay CR: T-cell function and migration. Two sides of the same coin. N Engl J Med 343;1020-1034, 2000
2. Luster AD, Alon R, von Andrian UH: Immune cell migration in inflammation: present and future therapeutic targets. Nat Immunol 6;1182-1190, 2005
3. Ley K, Laudanna C, Cybulsky MI, Nourshargh S: Getting to the site of inflammation: the leukocyte adhesion cascade updated. Nat Rev Immunol 7;678-689, 2007
4. Zarbock A, Ley K: New insights into leukocyte recruitment by intravital microscopy. Curr Top Microbiol Immunol 334; 129-152, 2009
5. McEver RP, Cummings RD: Perspectives series; cell adhesion in vascular biology. Role of PSGL-1 binding to selectins in leukocyte recruitment. J Clin Invest 100;485-491, 1997
6. Alon R, Feizi T, Yuen CT, Fuhlbrigge RC, Springer TA: Glycolipid ligands for selectins support leukocyte tethering and rolling under physiologic flow conditions. J Immunol 154;5356-5366, 1995
7. Berlin C, Bargatze RF, Campbell JJ, von Andrian UH, Szabo MC, Hasslen SR, Nelson RD, Berg EL, Erlandsen SL, Butcher EC: Alpha 4 integrins mediate lymphocyte attachment and rolling under physiologic flow. Cell 80;413-422, 1995
8. Sigal A, Bleijs DA, Grabovsky V, van Vliet SJ, Dwir O, Figdor CG, van Kooyk Y, Alon R: The LFA-1 integrin supports rolling adhesions on ICAM-1 under physiological shear flow in a permissive cellular environment. J Immunol 165;442-452, 2000
9. Marshall BT, Long M, Piper JW, Yago T, McEver RP, Zhu C: Direct observation of catch bonds involving cell-adhesion molecules. Nature 423;190-193, 2003
10. Adams DH, Lloyd AR: Chemokines: leucocyte recruitment and activation cytokines. Lancet 349;490-495, 1997
11. Campbell JJ, Qin S, Bacon KB, Mackay CR, Butcher EC: Biology of chemokine and classical chemoattractant receptors: differential requirements for adhesion-triggering versus chemotactic responses in lymphoid cells. J Cell Biol 134;255-266, 1996
12. Johnson Z, Proudfoot AE, Handel TM: Interaction of chemokines and glycosaminoglycans: a new twist in the regulation of chemokine function with opportunities for therapeutic intervention. Cytokine Growth Factor Rev 16;625-636, 2005
13. Campbell JJ, Hedrick J, Zlotnik A, Siani MA, Thompson DA, Butcher EC: Chemokines and the arrest of lymphocytes rolling under flow conditions. Science 279;381-384, 1998
14. Tanaka Y, Adams DH, Shaw S: Regulation of leukocyte recruitment by proadhesive cytokines immobilized on endothelial proteoglycan. Curr Top Microbiol Immunol 184;99-106, 1993
15. Dunne JL, Ballantyne CM, Beaudet AL, Ley K: Control of leukocyte rolling velocity in TNF-alpha-induced inflammation by LFA-1 and Mac-1. Blood 99;336-341, 2002
16. Phillipson M, Heit B, Colarusso P, Liu L, Ballantyne CM, Kubes P: Intraluminal crawling of neutrophils to emigration sites; a molecularly distinct process from adhesion in the recruitment cascade. J Exp Med 203;2569-2575, 2006
17. Schenkel AR, Mamdouh Z, Muller WA: Locomotion of monocytes on endothelium is a critical step during extravasation. Nat Immunol 5;393-400, 2004
18. Barreiro O, Yanez-Mo M, Serrador JM, Montoya MC, Vicente-Manzanares M, Tejedor R, Furthmayr H, Sanchez-Madrid F: Dynamic interaction of VCAM-1 and ICAM-1 with moesin and ezrin in a novel endothelial docking structure for adherent leukocytes. J Cell Biol 157;1233-1245, 2002
19. Carman CV, Springer TA: A transmigratory cup in leukocyte diapedesis both through individual vascular endothelial cells and between them. J Cell Biol 167;377-388, 2004
20. Huang AJ, Manning JE, Bandak TM, Ratau MC, Hanser KR, Silverstein SC: Endothelial cell cytosolic free calcium regulates neutrophil migration across monolayers of endothelial cells. J Cell Biol 120;1371-1380, 1993
21. Millan J, Ridley AJ: Rho GTPases and leucocyte-induced endothelial remodelling. Biochem J 385;329-337, 2005
22. Wee H, Oh HM, Jo JH, Jun CD: ICAM-1/LFA-1 interaction contributes to the induction of endothelial cell-cell separation; implication for enhanced leukocyte diapedesis. Exp Mol Med 41;341-348, 2009
23. Wittchen ES: Endothelial signaling in paracellular and transcellular leukocyte transmigration. Front Biosci 14;2522-2545, 2009
24. Lou O, Alcaide P, Luscinskas FW, Muller WA: CD99 is a key mediator of the transendothelial migration of neutrophils. J Immunol 178;1136-1143, 2007
25. Bixel MG, Petri B, Khandoga AG, Khandoga A, Wolburg-Buchholz K, Wolburg H, März S, Krombach F, Vestweber D: A CD99-related antigen on endothelial cells mediates neutrophil but not lymphocyte extravasation in vivo. Blood 109;5327-5336, 2007

26. Wakelin MW, Sanz MJ, Dewar A, Albelda SM, Larkin SW, Boughton-Smith N, Williams TJ, Nourshargh S: An anti-platelet-endothelial cell adhesion molecule-1 antibody inhibits leukocyte extravasation from mesenteric microvessels in vivo by blocking the passage through the basement membrane. J Exp Med 184;229-239, 1996

27. Schenkel AR, Mamdouh Z, Chen X, Liebman RM, Muller WA: CD99 plays a major role in the migration of monocytes through endothelial junctions. Nat Immunol 3;143-150, 2002

28. Woodfin A, Voisin MB, Imhof BA, Dejana E, Engelhardt B, Nourshargh S: Endothelial cell activation leads to neutrophil transmigration as supported by the sequential roles of ICAM-2, JAM-A, and PECAM-1. Blood 113;6246-6257, 2009

29. Garrido-Urbani S, Bradfield PF, Lee BP, Imhof BA: Vascular and epithelial junctions: a barrier for leucocyte migration. Biochem Soc Trans 36;203-211, 2008

30. Pfeiffer F, Kumar V, Butz S, Vestweber D, Imhof BA, Stein JV, Engelhardt B: Distinct molecular composition of blood and lymphatic vascular endothelial cell junctions establishes specific functional barriers within the peripheral lymph node. Eur J Immunol 38;2142-2155, 2008

31. Wegmann F, Petri B, Khandoga AG, Moser C, Khandoga A, Volkery S, Li H, Nasdala I, Brandau O, Fässler R, Butz S, Krombach F, Vestweber D: ESAM supports neutrophil extravasation, activation of Rho, and VEGF-induced vascular permeability. J Exp Med 203;1671-1677, 2006

32. Butcher EC, Picker LJ: Lymphocyte homing and homeostasis. Science 272;60-66, 1996

33. Lalor PF, Adams DH: The liver: a model of organ-specific lymphocyte recruitment. Expert Rev Mol Med 4;1-16, 2002

34. Robert C, Kupper TS: Inflammatory skin diseases, T cells, and immune surveillance. N Engl J Med 341;1817-1828, 1999

35. Shetty S, Lalor PF, Adams DH: Lymphocyte recruitment to the liver: molecular insights into the pathogenesis of liver injury and hepatitis. Toxicology 254;136-146, 2008

36. Lee WY, Kubes P: Leukocyte adhesion in the liver: distinct adhesion paradigm from other organs. J Hepatol 48;504-512, 2008

37. Wong J, Johnston B, Lee SS, Bullard DC, Smith CW, Beaudet AL, Kubes P: A minimal role for selectins in the recruitment of leukocytes into the inflamed liver microvasculature. J Clin Invest 99;2782-2790, 1997

38. Choi EY, Chavakis E, Czabanka MA, Langer HF, Fraemohs L, Economopoulou M, Kundu RK, Orlandi A, Zheng YY, Prieto DA, Ballantyne CM, Constant SL, Aird WC, Papayannopoulou T, Gahmberg CG, Udey MC, Vajkoczy P, Quertermous T, Dimmeler S, Weber C, Chavakis T: Del-1, an endogenous leukocyte-endothelial adhesion inhibitor, limits inflammatory cell recruitment. Science 322;1101-1104, 2008

39. Hidai C, Kawana M, Kitano H, Kokubun S: Discoidin domain of Del1 protein contributes to its deposition in the extracellular matrix. Cell Tissue Res 330;83-95, 2007

40. Hidai C, Zupancic T, Penta K, Mikhail A, Kawana M, Quertermous EE, Aoka Y, Fukagawa M, Matsui Y, Platika D, Auerbach R, Hogan BL, Snodgrass R, Quertermous T: Cloning and characterization of developmental endothelial locus-1: an embryonic endothelial cell protein that binds the alphavbeta3 integrin receptor. Genes Dev 12;21-33, 1998