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

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The leukocyte adhesion to endothelium is pivotal in leukocyte recruitment which takes place during inflammatory, autoimmune and infectious conditions. The interaction between leukocytes and endothelial cells 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.

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 $\beta_1$ and $\beta_2$ 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.
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Leukocyte recruitment: Leukocyte-endothelial interactions

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

(13,14). Selectin-mediated initial leukocyte rolling and subsequent 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 endothelial 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 endothelial 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 adhesive events which are orchestrated by a number of adhesion molecules. Several homophilic and heterophilic interactions between the leukocyte/endothelial apical and junctional adhesion molecules take place sequentially (24-26). Molecules directly acting in the transmigration include leukocyte integrins, endothelial immunoglobulin superfamily members (ICAM-1, -2, JAM-A, -B, -C and PECAM-1) and a non-immunoglobulin molecule CD99 (27-31).

The aforementioned classical leukocyte-endothelial interactions 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 pathways (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 molecules are employed (33,35-37).

Although immune cell migration is critical for protective immune responses against pathogens, the accumulation of leukocytes in the tissues could trigger significant release of cytotoxic mediators from leukocytes, leading to tissue damage and finally a wide spectrum of inflammatory conditions. Thus, inhibition of excessive or misdirected leukocyte recruitment 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
in the intestine, liver, heart, spleen, whole blood and bone marrow of adult mice in a tissue-specific manner: strong expression in these tissues was recently reported, however, that Del-1 is expressed in subsets of macrophages and hypertrophic chondrocytes. Multiple studies showed its expression in adult endothelial cells, some subsets of macrophages and hypertrophic chondrocytes, 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 further implies that Del-1 might 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-α 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.

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CONFLICTS OF INTEREST

The authors have no financial conflict of interest.

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