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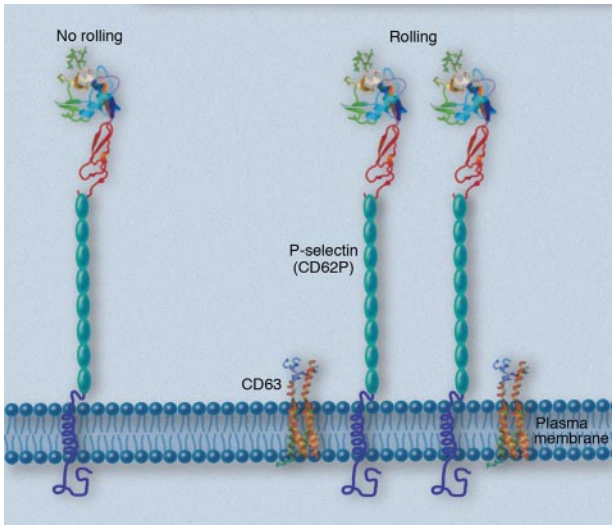
VASCULAR BIOLOGY

Comment on Doyle et al, page 4265

CD63 positions CD62P for rolling

Klaus Ley LA JOLLA INSTITUTE FOR ALLERGY AND IMMUNOLOGY

Doyle and colleagues identify the tetraspanin CD63 as an essential co-factor for endothelial P-selectin function.[1] In the absence of CD63 (by knockout in mice or knockdown in cultured endothelial cells), P-selectin (also known as CD62P) cannot mediate leukocyte tethering and rolling. This surprising finding suggests that P-selectin must be positioned in organized membrane microdomains to function.



The tetraspanin CD63 (orange, green, and blue molecule) is needed to organize P-selectin into small clusters to enable rolling. P-selectin (CD62P) lectin and EGF domains shown as ribbon diagrams based on crystal structure pdb 1G1R. Consensus repeats shown as turquoise ellipses (no structure available), transmembrane and cytoplasmic domain shown as putative α helix and unordered domain. Professional illustration by Marie Dauenheimer.

CD63 is a tetraspanin concentrated in endosomes and multivesicular bodies, where it seems to regulate trafficking of other transmembrane proteins.[2] In endothelial cells, CD63 is expressed in Weibel-Palade bodies, specialized secretory vesicles important in inflammation and hemostasis. The authors use immune electron microscopy and a proximity ligation assay to show that CD63 clusters with P-selectin (see figure). When CD63 expression is reduced by siRNA or eliminated by gene targeting, P-selectin expression on the endothelial surface is slightly reduced. More importantly, P-selectin clusters are smaller and, most remarkably, P-selectin becomes completely unable to support leukocyte rolling. The leukocyte recruitment defect in response to intraperitoneal injection of thioglycollate, a commonly used model of acute inflammation, is as severe in CD63-deficient mice as in P-selectin–deficient mice. Together with the cell biology data, this suggests that CD63 is absolutely essential for P-selectin function.

But why? Apparently, P-selectin must be organized in clusters to function, and this clustering is strictly CD63-dependent. It is well known that selectin interactions are polyvalent, presumably because the avidity of monovalent selectins is insufficient for ligand binding under flow. Indeed, P-selectin Glycoprotein Ligand-1 (PSGL-1), the main leukocyte ligand for P-selectin, is a constitutive dimer,[3] and the dimers are clustered on the tips of leukocyte microvilli.[4] The rolling is insensitive to reasonable changes in the number[5] and distribution[6] of microvilli on the cell surface.

Other tetraspanins are also known to organize leukocyte adhesion molecules into microdomains. For example, CD9 and CD151 have been reported to organize InterCellular Adhesion Molecule-1 and Vascular Cell Adhesion Molecule-1 in endothelial cells.[7] However, their requirement for leukocyte adhesion is not as stringent as that of CD63.

Beyond identifying the role of CD63 in endothelial cells, the report by Doyle et al also provides interesting data on P-selectin distribution on endothelial cells.[1] Although the site density (number of molecules per surface area) is known to be critical for leukocyte tethering, rolling, and signaling, such data have been scarce. More than 20 years ago, endothelial P-selectin expression was reported as 20 molecules per μm^2 under resting conditions and 50 molecules per μm^2 after histamine,[8] a treatment that promotes degranulation of Weibel-Palade bodies. In the present report, backscatter images were combined with secondary electron images to show that P-selectin and CD63 co-cluster on protruding surface structures of IL-4–treated endothelial cells. Each

of these clusters appears to contain 2 to 10 P-selectin molecules and a slightly smaller number of CD63 molecules. The data shown in the present paper are compatible with a site density of around 50 P-selectin molecules per μm^2. This amount of P-selectin expressed on these endothelial cells is sufficient to support rolling of THP-1 model leukocytes under flow. Importantly, this works only if CD63 is present and thus P-selectin is clustered.

The present report has important implications for the understanding of the inflammatory response. P-selectin expression on the endothelial surface in response to an inflammatory stimulus is so low that it barely works to support leukocyte adhesion. A modest decrease in surface expression and disruption of clustering induced by eliminating CD63 is sufficient to completely abolish P-selectin–mediated leukocyte recruitment. Like other biologic responses, leukocyte recruitment in inflammation has redundant mechanisms, and other leukocyte adhesion molecules can bypass the need for selectins. However, mice and people with defective selectin or selectin ligand function are susceptible to serious recurrent infections.

From a biomechanical standpoint, the report by Doyle et al provides information that enhances our understanding of how leukocyte adhesion under flow might work. In reconstituted in vitro systems, 20 molecules of P-selectin per μm^2 are commonly used as a minimum number that can support leukocyte rolling.[9] In such systems, P-selectin is used as a dimeric Fc fusion protein coated on a flat glass surface. Site density is measured by radiolabeled monoclonal antibody binding, which means that 20 sites per μm^2 are accessible to antibody and presumably to PSGL-1. The present work suggests that in endothelial cells in vivo, P-selectin may be clustered on little "hills," strategically positioned to bind PSGL-1 expressed on microvilli of a nearby leukocyte. CD63 is needed to organize endothelial P-selectin correctly and make it functional.

P-selectin is not only expressed in endothelial Weibel-Palade bodies, but also in α granules of platelets.[10] Whether CD63 is required for P-selectin clustering and function on the surface of activated platelets remains to be determined. Platelets express more P-selectin than endothelial cells, so perhaps the clustering and placement on the surface is not as critical as in endothelial cells. In addition, the cell biology of CD63 interaction with P-selectin remains to be worked out. Is the interaction direct or are other molecules involved? What is the nature of the cell membrane environment where P-selectin co-clusters with CD63?

The study by Doyle and colleagues represents a significant advance toward an understanding of the molecular requirements for leukocyte rolling. Efforts to model leukocyte rolling[5,11,12] have assumed randomly distributed P-selectin, which clearly does not capture the biologic situation on endothelial cells. P-selectin is clustered, and this clustering requires CD63.

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Klaus Ley

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