

Dances with leukocytes: how tetraspanin-enriched microdomains assemble to form endothelial adhesive platforms

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Rather than just providing an unstructured adhesive surface for leukocytes, cytokine-activated endothelial cells assemble preexisting tetraspanin-enriched microdomains to form endothelial adhesive platforms (EAPs) and endothelial docking structures. In this issue of the *Journal of Cell Biology*, Barreiro et al. (Barreiro, O., M. Zamai, M. Yáñez-Mó, E. Tejera, P. López-Romero, P.N. Monk, E. Gratton, V.R. Caiolfa, and F. Sánchez-Madrid. 2008. *J. Cell Biol.* 183:527–542) show how the immunoglobulin superfamily adhesion molecules intercellular adhesion molecule (ICAM)-1 and vascular cell adhesion molecule (VCAM)-1 form nanoclusters with the tetraspanins CD9 and CD151 in a physiologically relevant system. Furthermore, convincing biochemical data suggest that these structures are distinct from lipid rafts.

Most studies of leukocyte adhesion focus on leukocyte integrin activation by chemokine-triggered inside-out signaling (Ley et al., 2007) and consider an active role of endothelial cells only after adhesion has occurred and the leukocytes are ready to transmigrate (Imhof and Aurrand-Lions, 2004). The paper by Barreiro et al. (2008; see page 527 in this issue) dissects the molecular mechanisms of the active endothelial contribution to leukocyte adhesion. The authors focus on ICAM-1, the main ligand for the leukocyte $\alpha_L\beta_2$ integrin (LFA-1), and VCAM-1, the main ligand for the leukocyte $\alpha_4\beta_1$ integrin. In a previous study (Barreiro et al., 2005), the same group had shown that ICAM-1 and VCAM-1 both are recruited into endothelial docking structures even when incubated with leukocytes that express only one of the two integrins, $\alpha_L\beta_2$ or $\alpha_4\beta_1$. One plausible explanation was that the cytoskeletal tails of ICAM-1 and VCAM-1 might interact with the underlying cytoskeleton. In the current paper, Barreiro et al. (2008) show that VCAM-1 with a truncated cytoplasmic tail is still recruited into EAPs upon incubation of endothelial cells with $\alpha_L\beta_2$ -expressing K562 cells or primary T lymphoblasts in which

$\alpha_4\beta_1$ integrin had been inactivated, suggesting that cytoskeletal interaction is not required. A second possibility was that ICAM-1 and VCAM-1 formed heterodimers in endothelial cells. In the present study, fluorescence lifetime imaging microscopy with fluorescence resonance energy transfer (FLIM-FRET) studies showed no energy transfer between ICAM-1 tagged with GFP and VCAM-1 tagged with red fluorescent protein, suggesting that heterodimerization is less likely. Instead, FRAP experiments show that ICAM-1 and VCAM-1 are associated with the tetraspanins CD9 and CD151 in preformed tetraspanin-enriched microdomains (TEMs). Tetraspanins are a family of 33 hydrophobic, small transmembrane proteins, some of which organize these microdomains (Hemler, 2003). Immunoelectron microscopy on fixed endothelial cells suggests that the TEMs may be small, <100 nm in diameter. Light microscopy suggests a size of 300 nm, which is at the resolution limit of this technique. Remarkably, TEMs all contain the same amount of immunoreactivity, suggesting that they may represent controlled, preassembled units. Indeed, FLIM-FRET studies showed that the interaction strength between CD9 and ICAM-1, between CD151 and VCAM-1, and between the two tetraspanins was similar.

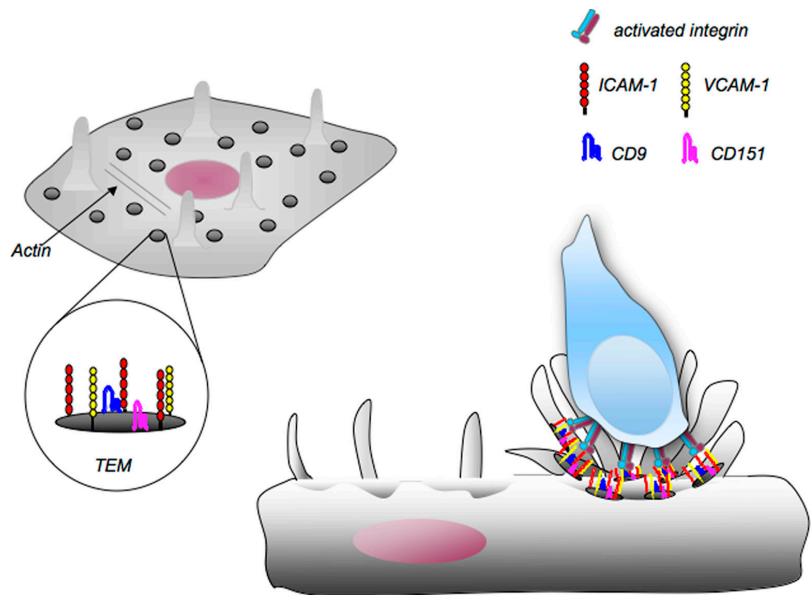
Although tetraspanins have been shown to associate with gangliosides (Claas et al., 2001) and remain incompletely soluble in detergent, the association between lipid rafts and tetraspanins remains unclear. Tetraspanin microdomains were found to be distinct from lipid rafts, because no association between tetraspanins and glycosylinositol phospholipid-linked proteins was found (Yang et al., 2004). The study by Barreiro et al. (2008) adds another important finding, that the TEMs show a specific size and cellular distribution distinct from lipid rafts.

When leukocytes adhere to endothelial cells and the docking structures are formed (Fig. 1), ICAM-1, VCAM-1, CD9, and CD151 all show reduced diffusivity and increased immobile fraction, suggesting that the TEM units stay intact and assemble to form the docking structures. Although the immobile fraction of ICAM-1 is higher and its diffusivity lower in ICAM-1- than in VCAM-1-mediated docking structures and vice versa, there

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Figure 1. **Preexisting TEMs assemble to form docking structure for adherent leukocytes.** TNF- α -stimulated endothelial cells show preassembled TEMs (left) that contain ICAM-1 (red), VCAM-1 (yellow), and the tetraspanins CD9 (blue) and CD151 (pink) as well as other molecules (not depicted). When an activated lymphoblast with activated integrins (light blue and purple heterodimers) adheres to the endothelial cell (right), the TEMs assemble into a characteristic docking structure that forms an elevated ring of microvilli.



is a measurable restriction of diffusion for all four molecules tested. This was shown by FRAP and confirmed by a second, more sensitive method called fluorescence correlation spectroscopy.

What does all this mean? First, Barreiro et al. (2008) provide an attractive model for how endothelial cells respond to leukocytes that initiate and then maintain adhesion. Rather than clustering of individual endothelial ICAM-1 and VCAM-1 molecules triggered by integrin rearrangement on the leukocyte, ICAM-1 and VCAM-1 are already preassembled in TEM structures, together with the tetraspanins CD9 and CD151. In that study, leukocyte–endothelial adhesion was tested in the absence of physiological shear stress, which may influence the results. Most leukocytes roll before they adhere, which may impact the formation of docking structures.

One wonders what else might be in the TEMs. Barreiro et al. (2008) report that platelet endothelial adhesion molecule (PECAM)–1, CD44, junctional adhesion molecule (JAM)–A, and ICAM-2, but not vascular endothelial (VE)–cadherin, are found in TEMs and EAPs. Of these molecules, PECAM-1 (Mamdouh et al., 2003), JAM-A (Woodfin et al., 2007), and ICAM-2 (Huang et al., 2006) have all been shown to be involved in transendothelial migration of leukocytes. In contrast, VE-cadherin was not found in EAPs. VE-cadherin localizes to interendothelial junctions, seems to hinder transmigration, and appears to get out of the way as transmigration proceeds (Shaw et al., 2001). It is therefore tempting to speculate that the EAP structures not only form docking structures, as shown by Barreiro et al. (2008), but also may begin to assemble the machinery necessary for transmigration. Alternative models of transmigration have been proposed, including a scenario where lymphoblasts extend “podosomes” to make dimples in the endothelial cell and probe for a route for transmigration (Carman et al., 2007). Another model suggests membrane recycling from a specialized compartment near the interendothelial junctions (Mamdouh et al., 2003). How these different models can be reconciled remains to be seen. There may be differences in different endothelial cells representing different vascular beds and also among different leuko-

cyte subsets. But the study by Barreiro et al. (2008) provides a solid foundation on which such future studies can be built.

Submitted: 24 September 2008

Accepted: 9 October 2008

References

- Barreiro, O., M. Yanez-Mo, M. Sala-Valdes, M.D. Gutierrez-Lopez, S. Ovalle, A. Higginbottom, P.N. Monk, C. Cabanas, and F. Sanchez-Madrid. 2005. Endothelial tetraspanin microdomains regulate leukocyte firm adhesion during extravasation. *Blood*. 105:2852–2861.
- Barreiro, O., M. Zamai, M. Yáñez-Mó, E. Tejera, P. López-Romero, P.N. Monk, E. Gratton, V.R. Caiola, and F. Sánchez-Madrid. 2008. Endothelial adhesion receptors are recruited to adherent leukocytes by inclusion in preformed tetraspanin nanoplateforms. *J. Cell Biol.* 183:527–542.
- Carman, C.V., P.T. Sage, T.E. Sciuto, M.A. de la Fuente, R.S. Geha, H.D. Ochs, H.F. Dvorak, A.M. Dvorak, and T.A. Springer. 2007. Transcellular diapedesis is initiated by invasive podosomes. *Immunity*. 26:784–797.
- Claas, C., C.S. Stipp, and M.E. Hemler. 2001. Evaluation of prototype transmembrane 4 superfamily protein complexes and their relation to lipid rafts. *J. Biol. Chem.* 276:7974–7984.
- Hemler, M.E. 2003. Tetraspanin proteins mediate cellular penetration, invasion, and fusion events and define a novel type of membrane microdomain. *Annu. Rev. Cell Dev. Biol.* 19:397–422.
- Huang, M.T., K.Y. Larbi, C. Scheiermann, A. Woodfin, N. Gerwin, D.O. Haskard, and S. Nourshargh. 2006. ICAM-2 mediates neutrophil transmigration in vivo: evidence for stimulus-specificity and a role in PECAM-1-independent transmigration. *Blood*. 107:4721–4727.
- Imhof, B.A., and M. Aurrand-Lions. 2004. Adhesion mechanisms regulating the migration of monocytes. *Nat. Rev. Immunol.* 4:432–444.
- Ley, K., C. Laudanna, M.I. Cybulsky, and S. Nourshargh. 2007. Getting to the site of inflammation: the leukocyte adhesion cascade updated. *Nat. Rev. Immunol.* 7:678–689.
- Mamdouh, Z., X. Chen, L.M. Pierini, F.R. Maxfield, and W.A. Muller. 2003. Targeted recycling of PECAM from endothelial surface-connected compartments during diapedesis. *Nature*. 421:748–753.
- Shaw, S.K., P.S. Bamba, B.N. Perkins, and F.W. Lusinskas. 2001. Real-time imaging of vascular endothelial-cadherin during leukocyte transmigration across endothelium. *J. Immunol.* 167:2323–2330.
- Woodfin, A., C.A. Reichel, A. Khandoga, M. Corada, M.B. Voisin, C. Scheiermann, D.O. Haskard, E. Dejana, F. Krombach, and S. Nourshargh. 2007. JAM-A mediates neutrophil transmigration in a stimulus-specific manner in vivo: evidence for sequential roles for JAM-A and PECAM-1 in neutrophil transmigration. *Blood*. 110:1848–1856.
- Yang, X., O.V. Kovalenko, W. Tang, C. Claas, C.S. Stipp, and M.E. Hemler. 2004. Palmitoylation supports assembly and function of integrin–tetraspanin complexes. *J. Cell Biol.* 167:1231–1240.