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Chemokine signaling to leukocytes at endothelial synapses under shear flow

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Circulating immune cells and hematopoietic progenitors exit blood vessels near specific target sites of antigen presentation, injury, infection, or inflammation. The vessel wall at these sites displays specific combinations of traffic signals that can recruit specific subsets of circulating leukocytes to traverse it. The initial attachment to the vessel wall, mediated by selectins, permits leukocytes to roll in the direction of flow, bringing them into proximity with endothelial-displayed chemoattractants (chemokines). These key regulatory elements bind specific G-protein coupled receptors (GPCRs) on attached leukocytes and trigger leukocyte integrins, within sub-seconds, to arrest on the blood vessel (Fig. 1). Following arrest, the leukocytes must locomote to, and correctly polarize their integrins and associated machineries at, intercellular endothelial junctions where they can squeeze through the blood vessel and migrate to the extravascular space (Fig. 2). This process involves complex rearrangements that remodel the junctions in a spatio-temporally regulated manner. Our recent studies reveal new exciting molecular details on how chemokine signals activate integrins on leukocytes under shear flow and how activated integrins mediate adhesion and transendothelial migration (TEM) across various endothelial beds.

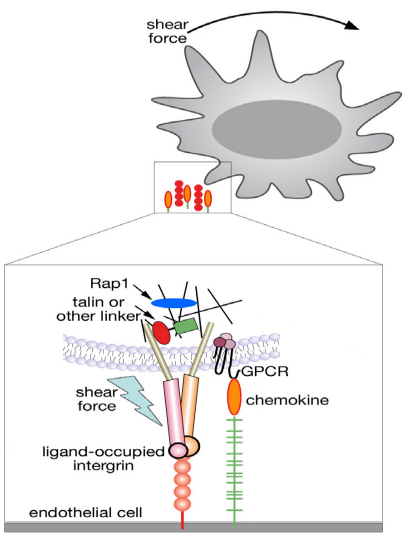

Fig. 1 *2D chemokine signaling to integrins, a new mode of localized integrin activation at leukocyte-endothelial synapses revealed by shear flow studies. A quartenary complex between integrin, ligand, chemokine and GPCR must form within milliseconds to arrest the rolling leukocyte on the vessel wall.*

Recent research findings and objectives

Using flow chamber assays simulating blood flow, we find that in addition to disrupting adhesive bonds, low shear forces directly stimulate leukocyte selectin recognition of surface-bound ligand by enhancing cellular transport and multi-contact formation. Associations of leukocyte-selectins and integrins with the actin cytoskeleton dramatically stabilize their adhesive interactions with respective ligands under shear. While chemokines destabilize selectin-mediated interactions by a novel *in situ* interference with selectin anchorage to the cytoskeleton, chemokines robustly activate integrins at confined endothelial contacts.

Although the molecular details of integrin activation at immune cell synapses have been elucidated, the mechanisms of integrin activation at chemokine-bearing endothelial synapses and its modulation by shear flow are just beginning to unfold. We find that activation of integrins by GPCR signals involves novel rearrangements dictated by ligand binding and proper integrin anchorage to the cortical actin cytoskeleton. These spatially confined events involve the cytoskeletal adaptors talin, paxillin and regulatory GTPases like Rap1 and RhoA. Integrin associated molecules such as tetraspanins also

modulate these rapid processes. Unexpectedly, surface-bound chemokines stimulate integrins through unique two-dimensional (2D) signals distinct from those transduced by their soluble counterparts (Fig. 1). Neither integrin affinity nor lateral mobility appears to be altered by these signals.

Characterizing integrin activation under shear, we have recently identified a new inherited adhesion deficiency syndrome, LAD-III, reflected in a severely impaired ability of leukocytes to arrest on vascular endothelium due to abnormal Rap1 signaling. New microbead assays reveal normal clustering and ligand binding properties of LAD-III integrins in shear-free conditions, pointing to a role for Rap1 in integrin activation by ligand and shear force signals. Integrin activation by chemokines at leukocyte-endothelial synapses appears to involve novel alterations in mechanical properties of ligand-occupied integrins actively induced by shear flow.

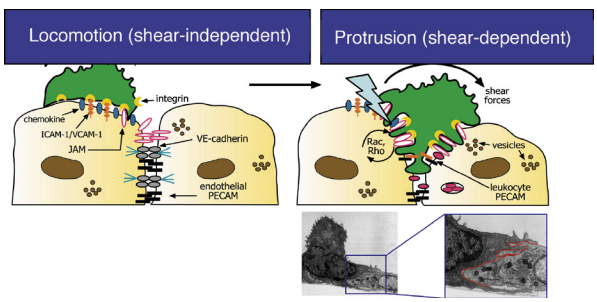

Fig. 2 *Current view on the possible role of shear in integrin outside-in signaling promoting transendothelial migration. The leading edge of leukocytes positioned at endothelial junctions rearranges under shear forces to trigger protrusions and gap formation between underlying endothelial cells. EM micrograph of PBL migrating across SDF-1α presenting TNFα-stimulated endothelium is depicted.*

Current studies are aimed at identifying how integrin stretching by shear triggers actin remodeling GTPases and actomyosin contractility in leukocytes adhered to vessel walls. These latter machineries may underlie the key role played by shear, GPCR signals and integrins in lymphocyte TEM. Leukocyte TEM across endothelial barriers depends on the ability of the leukocytes to extend protrusions into various endothelial compartments (Fig. 2). Notably, although lymphocyte TEM occurs exclusively at endothelial junctions, we find that neutrophils migrate right through inflamed endothelial cells. These unique transcellular routes involve specialized endosomal machineries which may predominate in TEM of specialized immune subsets and possibly metastatic tumors across vessels comprised of impermeable junctions such as the blood brain barrier.

Selected Publications

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