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The molecular basis of leukocyte adhesion to and migration through vascular endothelium

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Objectives of research

Immune cells emigrate from the bloodstream to effector sites in response to specific adhesive and activation signals displayed on the surface of blood vessels near these sites. Specific combinations of multiple traffic signals recruit different types of leukocytes in a multi-step manner (Fig. 1). The attachment of circulating leukocytes to the vessel wall is labile, permitting leukocytes to roll in the direction of flow (step 1) and bringing them into proximity with activating chemoattractants, or chemokines. These chemokines bind G-protein coupled receptors (GPCRs) and trigger, within subseconds, (step 2) the activation of integrins, which can then firmly bind their endothelial ligands (step 3) and come to temporary arrest. Once arrested, the leukocyte undergoes extensive cytoskeleton remodeling, which allows it to cross the endothelial lining of the blood vessel into the target tissue (step 4). Our recent studies revealed exciting new molecular insight into these individual steps.

Major findings

Using flow chamber assays which simulate physiological blood flow, we have found that lymphocyte transendothelial migration does not implicate a response to chemoattractant gradients across the endothelial barrier, as previously suggested. Rather, adherent leukocytes integrate local G-protein activating signals with mechanosignals exerted on these leukocytes by the shear flow. In collaboration with Dr. Vera Shinder of the electron microscopy unit, we observed very close apposition between the membranes of the transmigrating leukocyte and its endothelial barrier, suggesting highly coordinated cytoskeletal remodeling of these cells under shear flow (Fig. 2). We termed this new mode of transendothelial migration chemo-rheo-taxis (rheo-flow) and found it to apply to many types of leukocytes. Our recent results suggest that the specific GPCR subsets involved in this mechanosensitive process, must bind immobilized rather than soluble chemoattractants to trigger migration.

The initial leukocyte recruitment to the endothelial site is controlled by the selectin adhesion family. Binding of the leukocyte selectin, L-selectin, to carbohydrate ligands expressed on endothelial scaffold proteins are the fastest cell-cell recognition events known in nature and share high tolerance to applied shear forces i.e. moderate destabilization by disruptive force. We have recently identified a major role for the cytoplasmic tail of L-selectin in maintaining this high

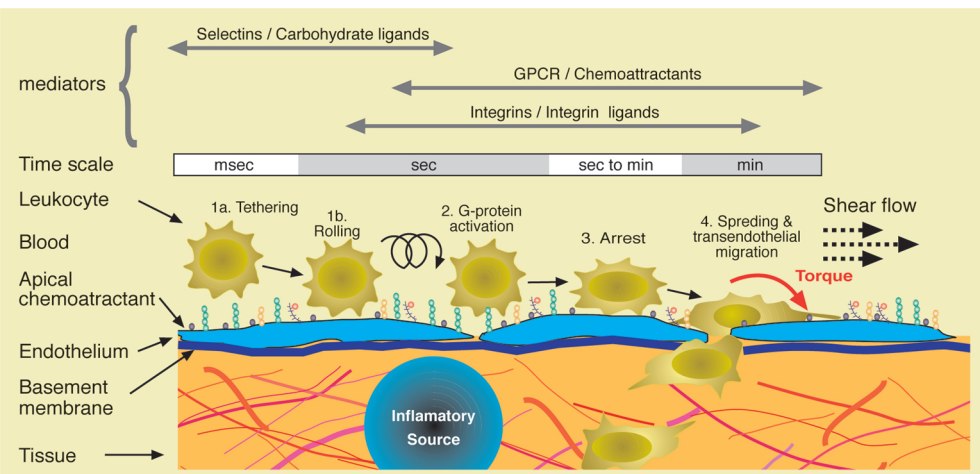

Fig. 1 *The four step model for leukocyte recruitment. Selectins, chemoattractants (chemokines) and integrins coordinate in capturing a circulating leukocyte to the vessel wall at specific sites. Further leukocyte exposure to apical endothelial chemokines and shear flow triggers their potential to undergo transendothelial migration without disrupting the integrity of the endothelial barrier.*

tolerance. Cytoskeletal anchorage of selectins allows a rapid (submillisecond) stabilization of L-selectin tethers. Our results suggest that this stabilization of leukocyte capture and rolling adhesions depends on restriction of lateral movement of selectins and integrin subsets at adhesive contact zones. Our recent results also extend the classical role suggested for endothelial chemokines as triggers of integrin avidity. We find that many chemokines suppress selectin-mediated leukocyte rolling by interfering in situ with a subsecond generation of high avidity selectin/ligand contacts. In contrast to the proadhesive and promigratory activities of chemokines, this chemokine-suppression does not involve G_i-protein signaling. Since chemokines exert opposite activities on selectin and integrin adhesions, a proper balance between these activities seems to dictate whether selectin rolling is bridged to integrin-mediated arrest on vascular endothelium.

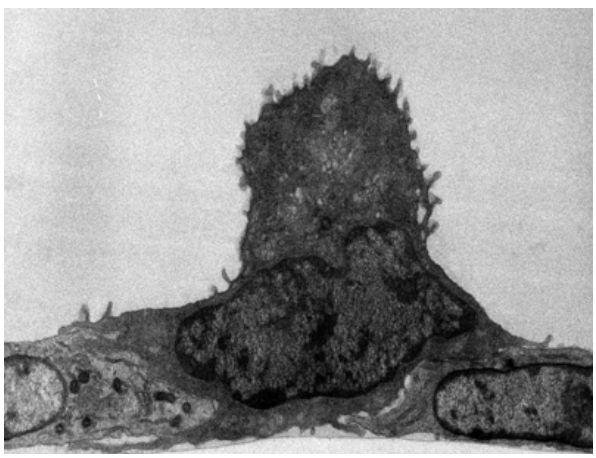

Fig. 2 *Ultrastructural analysis of a T lymphocyte transmigrating an inflamed endothelial barrier under physiological shear flow. A cross section of a lymphocyte in the process of diapedesis between adjacent cytokine-activated endothelial cells is depicted.*

To optimally respond to chemokines and generate high avidity contacts on vascular endothelium, integrins must exist at high affinity conformations which prolong the leukocyte contact and exposure to endothelial chemokine signals. In addition, integrins must undergo rapid clustering upon binding their endothelial ligands. We find that cytoskeletal constraints of either VLA-4 or LFA-1 integrins must be released to allow optimal enhancement of integrin avidity and adhesion strengthening. Our studies reveal a key role in this process for PKC-induced phosphorylation of specific cytoskeletal regulators. We have recently found that specific tetraspans, integrin associated membranal proteins, localize PKC and GPCR activities to membranal compartments containing specific integrins. We also find that the distribution of integrins to cholesterol-rich rafts determine their crosstalk with specific GPCR machineries, enriched in these compartments. The involvement of specific Rho family GTPases in this crosstalk, and their role in rapid adhesion strengthening and migration of leukocytes under shear flow, are the focus of our current studies.

Selected Publications

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