Chemokine signaling to lymphocyte integrins at endothelial and extravascular contacts

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Circulating immune cells and hematopoietic progenitors must exit blood vessels near specific lymphoid organs and sites of inflammation. The vessel wall at these sites displays specific combinations of traffic adhesion molecules (selectins and integrin ligands) as well as chemotactic cytokines (chemokines) which operate in sequence to recruit only specific circulating subsets with proper receptors to these signals. Leukocyte integrins have evolved to undergo rapid conformational switches under the specialized mechanical context of blood flow. Using special flow chambers which simulate this flow we dissect how these molecules and their cytoplasmic associations with the cell cytoskeleton dynamically modulate integrin adhesiveness and thereby mediate leukocyte adhesion to and motility over and through blood vessels. Videomicroscopy of immune cells interacting with vessel-derived endothelial cells and subcellular staining of both adhesion receptors and their specific cytoplasmic regulators together with ultrastructural electron microscopic analysis allow us to propose new pathways by which chemokines and integrin ligands signal to lymphocytes to across the blood vessel walls and identify new therapeutic targets to attenuate immune cell function in autoimmunity, allergy, injury, atherosclerosis and organ rejection.

Recent research findings and objectives

A. Chemokine activation of leukocyte integrins involves the Rap-1 activator, CalDAG-GEFI

The ability of rolling leukocytes to arrest on target endothelial sites depends on the rapid activation of their integrins. Studying LFA-1 activation as a paradigm for leukocyte integrins, we find that rapid LFA-1 affinity modulation by endothelial-displayed chemokines involves local G-protein coupled receptor (GPCR) signals which trigger inside-out and outside-in (ligand induced) rearrangements of the integrin headpiece within millisecond contacts (Fig. 1). These events are mediated by the GTPase Rap-1 and require proper integrin anchorage to the cortical actin cytoskeleton via talin. An inherited integrin activation deficiency identified by us, called LAD-III (Leukocyte Adhesion Deficiency III), is linked to improper activation of Rap-1 due to reduced expression of the Rap-1 GEF, CalDAG-GEFI. This GEF is bidirectionally activated via PLC mediated hydrolysis of PIP2 and one of its two secondary messenger products, DAG. De novo generation of PIP2 involves chemokine activation of a second GTPase, RhoA. These and other results collectively suggest the existence of preformed multicomponent signalosomes specialized in either VLA-4 or LFA-1 activation by chemokines and integrin ligands.

B. External forces are critical for rapid integrin activation by both GPCR and TCR signals

Lymphocyte motility in lymph nodes (LN) is critically regulated by chemokines. Surface-bound lymph node chemokines that decorate LN stroma, but not their soluble counterparts, promote robust and sustained lymphocyte motility. LN chemokines induce compartmentalized clustering of the integrins LFA-1 and VLA-4, but keep integrins nonadhesive in shear-free environments to minimize antigen independent lymphocyte sticking to ICAM-1 expressed by lymphocytes, dendritic cells and stroma. Application of shear stress readily triggers integrin-mediated adhesion, suggesting that external forces are robust switches of integrin adhesiveness. Internal myosin contractile forces may, on the other hand, weakly activate integrin subsets on lymphocytes migrating on extracellular matrix ligands and chemokines.

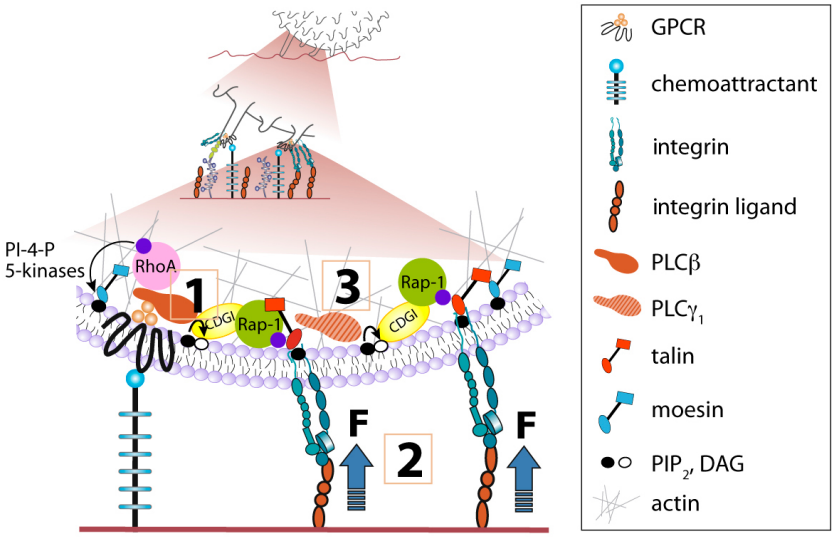

Fig. 1 *The LFA-1-GPCR signalosome in T cells, a preformed assembly of signaling molecules which integrate rapid inside-out chemokine triggered signals (1) with outside-in signals from ligand occupied integrins (2,3), through activation of GTPases and the key integrin regulator, talin. The different postulated steps are marked in numbers. PIP2 lipids serve as substrates for PLCβ and PLCγ1 upstream of the Rap-1 GEF, CalDAG-GEFI. PIP2 also keeps talin in an active conformation. Shear forces (F) may catalyze LFA-1 facilitated PLCγ activation. A different signaling cascade applies to VLA-4 activation, postulated to involve a structurally distinct talin containing signalosome.*

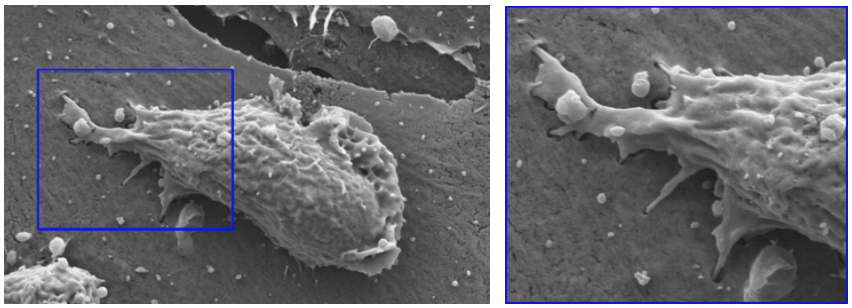

Fig. 2 *Scanning electron microscopy of a T cell crawling on inflamed endothelial cell in vitro. Apical chemokines and integrin ligands trigger numerous submicron focal contacts underneath the crawling lymphocyte, a subset of which generate invasive filopodia. Upon reaching endothelial junctions, these filopodia are stretched out underneath the thin endothelial cell and allow the lymphocyte to integrate guidance cues from the basal endothelial membrane, resulting in successful transendothelial migration (TEM).*

Recent findings also suggest a positive role for external forces in rapid LFA-1 activation by TCR signals, critical for lymphocyte arrest on antigen presenting dendritic cells. Unlike chemokine signals, TCR signals fail to trigger high affinity extended LFA-1 conformations. We predict that only small subsets of LFA-1 anchored to the cytoskeleton can undergo unique activation by external forces. Other mobile LFA-1 molecules are subject to secondary TCR signals that activate actomyosin machineries and generate internal forces on these integrin molecules as they organize in large clusters.

C. Millipede-like crawling of lymphocytes on activated endothelium is mediated by chemokine triggered invasive filopodia: role in transendothelial migration (TEM)

Endothelial chemokines are also instrumental in integrin mediated lymphocyte crawling from sites of arrest to TEM sites. To address how chemokine signals promote rapid crawling while keeping LFA-1 resistance to detachment by disruptive shear forces, we stained LFA-1 subsets on T cells during active crawling and TEM. Endothelial chemokines were found to induce high affinity LFA-1 engaged with endothelial ICAM-1 at numerous submicron dots underneath the entire crawling lymphocyte. These short lived dots contain focal adhesion proteins such as vinculin, talin and phosphorylated ERMs and serve as nucleating cores of filopodial attachments. Notably, shear forces applied on crawling lymphocytes render their filopodia invasive into the endothelial cell body and thereby facilitate rapid extension of subluminal lamellipodia, enriched with new high affinity LFA-1 contacts with subluminal ICAM-1. We propose that high affinity LFA-1 dots are the quantal units of millipede-like lymphocytes crawling on and below endothelial barriers. Current studies are aimed at delineating the endothelial machineries that proactively regulate these LFA-1-ICAM-1 driven invaginations during transendothelial migration. The complex interplay between the T cell GTPases Rap-1, Rac1 CDC42 and their upstream GEFs in this motility is also investigated. Specific attention is focused on regulatory roles of shear stress in the dynamic activation of these GTPases as well as in their downstream effectors in both resting and effector T cells.

Selected publications (last 3 yrs)

Shamri, R., Grabovsky, V., Gauguet, J.M., Feigelson, S., Manevich, E., Kolanus, W., Robinson, M.K., Staunton, D.E., von-Andrian, U.H. and Alon, R. (2005) Lymphocyte arrest on endothelial ICAM-1 requires a subsecond induction of an extended LFA-1 conformation by an endothelial-bound chemokine signal. Nature Immunology 6, 497-506.

Alon, R., Feigelson, S., Manevich, E., Rose, D.M., Schmitz, J., Overby. D.R., Winter, E., Grabovsky, V., Shinder, V., Matthews, B.D., Sokolovsky-Eisenberg, M., Ingber, D.E., Benoit, M., and Ginsberg, M.H. (2005) $\alpha_4\beta_1$-dependent adhesion strengthening under mechanical strain is regulated by paxillin association with the α_4 cytoplasmic domain J. Cell. Biol. 171, 1073-1084.

Shulman, Z. Pasvolsky, R., Woolf, E., Grabovsky, V. Feigelson, S.W., Erez, N., Fukui, Y., and Alon, R. (2006) DOCK2 regulates chemokine-triggered lateral lymphocyte motility but not transendothelial migration. Blood 108, 2150-2158.

Pasvolsky, R., Feigelson, S.W., Kilic, S.S., Simon, A.J., Tal-Lapidot, G., Grabovsky, V., Crittenden, J.R., Amariglio, N., Safran, M., Graybiel, A.M., Rechavi, G., Ben-Dor, S., Etzioni, A., and Alon, R. (2007) A LAD-III syndrome is associated with defective expression of the Rap-1 activator CalDAG-GEFI in lymphocytes, neutrophils, and platelets. J. Exp. Med. 204, 1571-1582.

Dwir, O., Grabovsky, V., Pasvolsky, R., Manevich, E., Shamri, R., Gutwein, P., Feigelson, S.W., Altevogt, P., and Alon, R. (2007) Membranal cholesterol is not required for L-selectin adhesiveness in primary lymphocytes but controls a chemokine-induced destabilization of L-selectin rolling adhesions. J. Immunol. 179, 1030-1038.

Woolf, E., Grigorova, I., Sagiv, A., Grabovsky, V., Feigelson, S.W., Shulman, Z., Hartmann, T., Sixt, M., Cyster, J.C., and Alon, R. (2007) Immobilized lymph node chemokines promote sustained T lymphocyte motility but fail to trigger stable integrin adhesiveness in the absence of shear forces. Nature Immunol. 8, 1076-1085.

Manevich, E., Grabovsky V., Feigelson, S.W., and Alon, R. (2007) Talin1 and paxillin facilitate distinct steps in rapid VLA-4-mediated adhesion strengthening to VCAM-1. J. Biol. Chem. 282, 25338-25348.

Alon, R. and Dustin, M.L. (2007) Force as a facilitator of integrin conformational changes during leukocyte arrest on blood vessels and antigen-presenting cells. Immunity 26, 17-27.

Pasvolsky, R., Grabovsky, V.,Giagulli, C., Shulman, Z., Shamri, R., Feigelson, S.W., Laudanna, C.,and Alon, R. (2008) RhoA is involved in LFA-1 extension triggered by CXCL12 but not in a novel outside-in LFA-1 activation facilitated by CXCL9. J. Immunol., 180, 2815-2823.

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