Chemokine signaling to leukocyte integrins at endothelial and extravascular contacts

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Circulating immune cells and hematopoietic progenitors must exit blood vessels near specific target sites of injury, inflammation or tissue repair. The vessel wall at these sites displays specific combinations of traffic signals, i.e., adhesion molecules (selectins, integrins) and chemotactic cytokines (chemokines) which operate in sequence to recruit only specific circulating subsets with proper receptors to these signals. As these processes take place under shear stress, these traffic molecules have evolved to operate under specialized kinetic and mechanical contexts. Using special flow chambers which simulate blood flow in the circulation we attempt to dissect how these molecules and their cytoplasmic associations with the cell cytoskeleton mediate cell adhesion and exit through blood vessels. Videomicroscopy of immune cells interacting with vessel cells and real time subcellular staining of both adhesion receptors and their specific cytoplasmic regulators allow us to follow spatially and temporally how these molecules mediate leukocyte exit across the blood vessel walls. This information is key for the development and implementation of therapeutic tools to fight autoimmunity, allergy, heart injury, atherosclerosis, organ rejection and metastasis.

Recent research findings and objectives

A. Chemokine activation of leukocyte integrins at endothelial contacts under shear stress

The ability of rolling leukocytes to arrest on target endothelial sites depends on rapid activation of their integrins. Studying LFA-1 activation as a paradigm for leukocyte integrins, we find that rapid LFA-1 activation by endothelial-displayed chemokines involves local GPCR signals which trigger conformational rearrangements of the integrin within millisecond contacts (Fig. 1). These spatially confined events involve the cytoskeletal regulatory GTPases RhoA and Rap-1 and require proper integrin anchorage to the cortical actin cytoskeleton via cytoskeletal linkers. An inherited integrin activation deficiency identified by us, termed LAD-III, is linked to improper activation of Rap-1 by various signals, a result of impaired expression of the main hematopoietic cell Rap-1 GEF, CalDAG-GEF-I. A possible crosstalk between Rap-1, RhoA, talin and their associated effectors is currently under investigation in both normal and malignant lymphocytes.

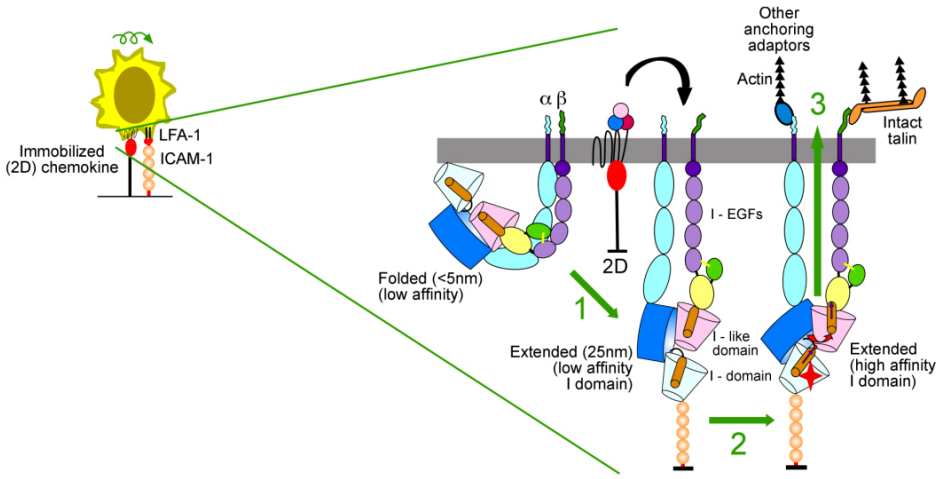

Fig. 1 *A proposed model for rapid integrin activation by endothelial-immobilized chemokines. Bidirectional activation switches the integrin (LFA-1 in this demonstration) from an inactive bent state to an extended state within 0.1 sec (step 1). This critical event primes the integrin to bind its endothelial ligand and undergo a further conformational shift (step 2, depicted by the red star). This activation of the integrin head piece causes further separation of the integrin subunit tails (step 3). Associations of the integrin subunit tails with talin and additional adaptors provide critical mechanical stabilization of the nascent adhesive bond.*

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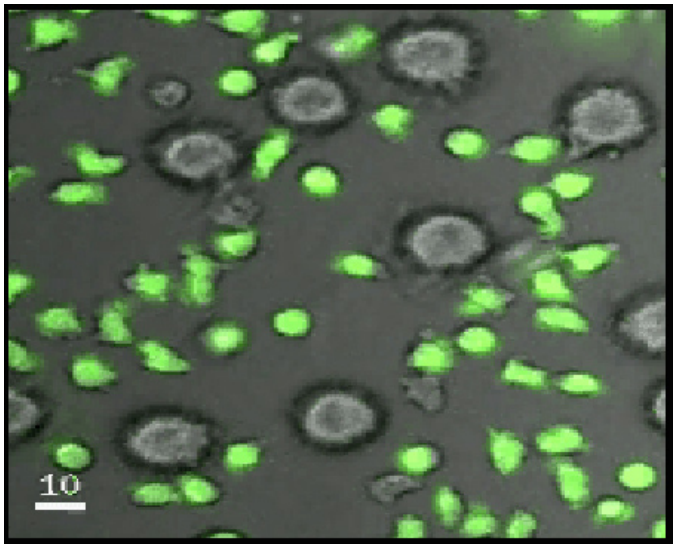

Fig. 2 *In vitro imaging of T cells (labeled with the inert dye BCECF) locomoting over an extracellular matrix presenting immobilized CCL21, the key chemokine found in lymph node T zones. T cells are rapidly locomoting around monocyte-differentiated dendritic cells (grey), derived from a matching donor, and make stable contacts in the presence of an antigen.*

B. Paxillin binding and phosphorylation sites on VLA-4 cytoplasmic tails regulate mechanical properties of the integrin bonds critical for lymphocyte adhesion to inflamed vessels under shear stress

Using flow chamber assays, we find that associations of the major lymphocyte integrin VLA-4 with the adaptors paxillin and talin dramatically stabilize its adhesive interactions with respective ligands without altering integrin affinity or avidity to ligands under shear-free conditions. This is the first example that integrins on circulating cells operating under shear flow must employ mechanical stabilization of their individual bonds rather than mere clustering. Experiments with T cells in which talin or paxillin transcription has been silenced further suggest that these two adaptors regulate distinct and complementary roles in VLA-4 adhesiveness. Strikingly, neither of these effectors regulate VLA-4 avidity to surface bound VCAM-1, highlighting their role as specialized mechanical adaptors rather than general conformational regulators of VLA-4. Current work aims at identifying how specific serine phosphorylation sites further contribute to VLA-4 affinity, activation through rearrangement by ligand, anchorage, and mechanical stabilization of adhesive bonds in intact and chemokine-activated T cells.

C. The role of Rac, Rap and Rab GTPases in lymphocyte adhesion, transendothelial migration (TEM) and interstitial locomotion

Rac GTPases are key regulators of leukocyte motility. In lymphocytes, chemokine-mediated Rac activation depends on the CDM adaptor DOCK2. Using a novel ex vivo model for real time analysis of murine leukocyte subsets, we recently identified a specialized role for this adaptor in chemokine-triggered lymphocyte motility on endothelial and extracellular matrix barriers but not in chemokine-triggered integrin-mediated adhesiveness. DOCK2 is also dispensable for transmigration of lymphocytes through a chemokine-presenting endothelial barrier. Our studies point to a key role of a small subset of high affinity LFA-1 in lymphocyte TEM. High affinity LFA-1 subsets are dynamically triggered by endothelial chemokines. We have identified minute levels of high affinity LFA-1 within a perinuclear region of T cells polarized on apical endothelial chemokines. Our working hypothesis is that these subsets are mobilized to the leading edge of transmigrating T cells by Rab GTPases, and thereby activate LFA-1 via a DOCK2 independent but Rap-1 dependent events. Specific regulatory roles of shear stress in these as well as in downstream integrin stretching events are also investigated.

D. Promotion of lymphocyte motility and scanning of dendritic cells by matrix associated chemokines

Lymphocytes entering the T cell zone in peripheral lymph nodes are exposed to high levels of CCR7 and CXCR4 binding chemokines. Our recent work suggests that these extravascular chemokines preferentially operate to trigger T cell motility and encounter of antigen presenting dendritic cells (Dcs) in matrix-presented (two-dimensional) rather than soluble states. We have recently developed an in vitro live imaging set-up to follow how human T cells locomoting on matrix presented chemokines encounter DCs via distinct compartments and subsets of T cell integrins (Figure 2). This analysis reveals a complex role of chemokines in T cell polarization and activity states of LFA-1, and suggests that promigratory environmental chemokines rather than DC derived chemokines mobilize lymphocytes to successfully encounter antigen presenting DCs.

Selected publications (last 3 yrs)

Alon, R., Aker, M., Feigelson, S., Sokolovsky-Eisenberg, M., Staunton, D.E., Cinamon, G., Grabovsky, V., Shamri, R., and Etzioni, A. (2003) A novel genetic leukocyte adhesion deficiency in subsecond triggering of integrin avidity by endothelial chemokines results in impaired leukocyte arrest on vascular endothelium under shear flow. Blood 101, 4437-4445.

Dwir, O., Solomon, A., Mangan, S., Kansas, G.S., Schwarz, U.S. and Alon, R. (2003) Avidity enhancement of L-selectin bonds by flow: shear-promoted rotation of leukocytes turn labile bonds into functional tethers. J. Cell. Biol. 163, 649-659.

Feigelson, S., Grabovsky, V., Shamri, R., Levy, S. and Alon, R. (2003) The CD81 tetraspanin facilitates instantaneous VLA-4 rearrangements promoting leukocyte adhesion to VCAM-1 under shear flow. J. Biol. Chem. 278, 51203-51212.

Kinashi, T., Aker, M., Sokolovsky-Eisenberg,, M., Grabovsky, V., Tanaka, C., Shamri, R., Feigelson, S., Etzioni, A. and Alon, R. (2004) LAD-III, a leukocyte adhesion deficiency syndrome associated with defective Rap1 activation and impaired stabilization of integrin bonds. Blood, 103, 1033-1036.

Schwartz, U.S., and Alon, R. (2004) L-selectin-mediated leukocyte tethering in shear flow is controlled by multiple contacts and cytoskeletal anchorage facilitating fast rebinding events. Proc. Natl. Acad. Sci. U.S.A. 101, 6940-6945.

Cinamon, G., Shinder, V., Shamri, R. and Alon, R. (2004) Chemoattractant signals and β2 integrin occupancy at apical endothelial contacts combine with shear stress signals to promote transendothelial neutrophil migration. J. Immunol. 173, 7282-7291.

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) α4β1-dependent adhesion strengthening under mechanical strain is regulated by paxillin association with the α4 cytoplasmic domain J. Cell. Biol. 171, 1073-1084.

Acknowledgements

Our current research is supported by ISF, GIF, Minerva, and the 6th EU framework program (MAIN). RA is the incumbent of the Linda Jacobs Chair in Immune and Stem Cell Research.