Published in final edited form as:

Sci Signal. ; 5(235): pe33. doi:10.1126/scisignal.2003383.

Tyrosine Kinases EnAbling Adaptor Molecules for Chemokine-Induced Rap1 Activation in T Cells

Laurent P. Malherbe* and Demin Wang*

Blood Center of Wisconsin, Blood Research Institute, Milwaukee, WI 53226, USA

Abstract

Chemokines regulate T cell trafficking into secondary lymphoid organs and migration across endothelial cells in response to inflammatory signals. The small guanosine triphosphatase Rap1 is a critical regulator of chemokine signaling in T cells, but how chemokines activate Rap1 has been unclear. A study showed that Abl family tyrosine kinases were essential for chemokine-induced Rap1 activation, T cell polarization, and migration. Abl family kinases promoted Rap1 activation by phosphorylating the adaptor protein human enhancer of filamentation 1 (HEF1), thus establishing a critical Abl-HEF1-Rap1 signaling axis for chemokine-induced T cell migration.

Chemokine receptors on the surface of T cells play a critical role in regulating T cell trafficking to secondary lymphoid organs and to sites of inflammation. Chemokine signaling results in rapid conformational changes in integrin receptors that enable cells to adhere to vascular barriers (1). It also contributes to lymphocyte migration by activating intracellular signaling pathways that result in cytoskeletal reorganization and polarization of the cell, to form a leading edge and a tail (uropod) (2, 3). T cell polarization in response to chemokine signaling is a prerequisite for efficient migration, but the molecular pathways leading to polarization have not been fully elucidated. Through a combination of biochemical analysis and inhibition of Abl family kinases by inhibitors and gene deletions, Gu *et al.* (4) demonstrated that Abl family kinases were activated downstream of CXC chemokine receptor 4 (CXCR4) and CC chemokine receptor 7 (CCR7), two chemokine receptors constitutively expressed by T cells that regulate T cell migration along gradients of the chemokines CXCL12 [also known as stromal cell–derived factor 1 α (SDF-1 α)] and (C-C motif) ligand 21 (CCL21). The authors showed that T cells deficient in Abl and Arg exhibited impaired T cell polarization and migration in response to either of these two chemokines, defective homing to lymph nodes, and impaired migration to sites of inflammation *in vivo*.

The Abl family nonreceptor tyrosine kinases, Abl (Abl1) and Abl-related gene (Arg, also known as Abl2), have been implicated in regulating cell shape and motility in several cell types (5, 6). A unique feature that distinguishes these kinases from other nonreceptor tyrosine kinases is the presence of an F-actin–binding domain. In T cells, Abl family kinases are involved in the regulation of actin reorganization at the immunological synapse (7) and T cell receptor (TCR)–mediated integrin activation (6). T cells deficient in both Abl and Arg

*Corresponding author. laurent.malherbe@bcw.edu (L.P.M.); demin.wang@bcw.edu (D.W.).

show profound defects in development, proliferation, and cytokine production (8). A previous study suggested that Abl plays a role in chemokine-induced T cell migration, but the mechanistic link between Abl and chemokine signaling has not been described (7).

Cellular responses to chemokines are mediated by heterotrimeric guanine nucleotide-binding protein (G protein)-coupled receptors (GPCRs) that are characterized by seven transmembrane domains. Upon activation, a conformational change in the receptor's cytoplasmic region promotes the exchange of guanosine diphosphate (GDP) for guanosine triphosphate (GTP) on the $G\alpha$ subunit and subsequent dissociation of the $G\alpha$ and $G\beta\gamma$ subunits (9, 10). The resulting GTP- $G\alpha$ and $G\beta\gamma$ activate multiple downstream signaling pathways and their corresponding biological responses, including cell migration (2, 3, 11). Chemokines can activate the phospholipase C (PLC), phosphatidylinositol 3-kinase (PI3K), Ras, or Rho family of small guanosine triphosphatases (GTPases), or mitogen-activated protein kinase (MAPK) pathways (12). The activation of Rho and Rac are critical for actin polymerization (13, 14) and cell migration (15).

Rap1, a member of the Ras family of small GTPases, is an important regulator of T cell integrin activation, cell polarization, and migration in response to chemokines (16, 17). Rap1 cycles between an active GTP-bound form and an inactive GDP-bound form. There are several effectors downstream of the active GTP-bound form of Rap1, such as protein kinase D (PKD), regulator for cell adhesion and polarization enriched in lymphoid tissues (RAPL), and RAP1-interacting adaptor protein (RIAM). RAPL induces chemokine-mediated T cell polarization, which is characterized by the segregation of lymphocyte function-associated antigen-1 (LFA-1) to the leading edge and CD44 to the uropod of the migrating cell (18). Neither the signaling pathways involved in chemokine-induced activation of Rap1 nor the specific guanine nucleotide exchange factors (GEFs) for Rap1 have been fully investigated.

Chemokine-induced Rap1 activation in T cells requires the adaptor protein Cas and HEF1-associated signal transducer in hematopoietic cells (Chat-H) (19). Knockdown of Chat-H impairs chemokine-stimulated Rap1 activation and integrin-mediated adhesion (19). Chat-H-deficient T cells are also defective in trafficking to peripheral lymph nodes (19). Chat-H localization to the plasma membrane, association with its binding partner HEF1 [also known as CasL (Crk-associated substrate in lymphocytes) and NEDD9 (neural precursor cell expressed, developmentally down-regulated 9)], and Chat-H-mediated serine or threonine phosphorylation of HEF1 are all required for chemokine-induced T cell migration (19).

The HEF1 protein is implicated in the regulation of cell polarity, adhesion, motility, and invasion in multiple cell types (20). In lymphocytes, integrin, or TCR engagement induces HEF1 phosphorylation, which is mediated by the tyrosine kinases Fyn and Lck and is essential for T cell migration (21). Gu *et al.* demonstrated that Abl family kinases were activated by CXCL12 and CCL21, and that inhibition of Abl family kinases by the specific inhibitor STI571 or knockout of both Abl1 and Abl2 genes impaired chemokine-induced T cell chemotaxis, chemokinesis (random nonvectorial movement), polarization, and F-actin polymerization. Gu *et al.* discovered that Abl family kinases controlled the chemokine-induced tyrosine phosphorylation of HEF1 and that this was required for chemokine-induced

Rap1 activation and T cell migration. These findings demonstrate an important role for Abl family tyrosine kinases in GPCR signaling and establish the Abl-HEF1-Rap1 signaling axis as a critical pathway for regulating chemokine-induced T cell migration (Fig. 1).

Tyrosine kinases, such as Src, focal adhesion kinase (FAK), and proline-rich tyrosine kinase 2 (Pyk2) are activated by GPCRs (22–24). In addition, the epidermal growth factor receptor (EGFR) tyrosine kinase is transactivated by GPCRs (25). Src is activated by GPCRs through its association with β -arrestin, Gs and Gi, or GPCRs (26–28), whereas FAK activation by GPCRs is through G α 12- and G α 13-mediated Rho-dependent tyrosine phosphorylation (29). EGFR transactivation by GPCRs occurs through the release of EGFR ligand precursors by activation of ADAM (a disintegrin and metalloproteinase) (30). One important question arising from the findings of Gu *et al.* (4) is what is the molecular mechanism underlying the activation of Abl family kinases by chemokine receptors? It is tempting to speculate that GPCRs may activate Abl kinases in a manner similar to that for Src or FAK, namely, through β -arrestin, G protein subunits, or GPCRs directly (Fig. 1).

Rap1 activation by chemokines is a pertussis toxin-sensitive, Gi/o-dependent process (31). It will be interesting to determine whether the activation of Abl family kinases is controlled by a Gi-dependent or -independent pathway. Gu *et al.* have found that Lck is required for chemokine-induced Abl activation (4), connecting GPCRs to Abl. Further studies into the molecular mechanism by which Lck links chemokine receptors to Abl activation are warranted.

The mechanism by which phosphorylated HEF1 promotes Rap1 activation and the nature of the GEF involved in Rap1 activation also remain unclear. In B cells, the Rap1 GEF AND-34 associates with both of the docking molecules Chat-H and HEF1 (32). In T cells, TCR triggering activates a complex composed of Wiskott-Aldrich syndrome protein family verprolin-homologous protein 2 (WAVE2), actin-related protein 2/3 (arp 2/3), and Abl that associates with and phosphorylates the Rap1 GEF C3G in complex with CRKL (1, 33). Thus, we can envision that Abl family kinases induce the phosphorylation of HEF1 and, subsequently, tyrosine-phosphorylated HEF1 could serve as a platform to recruit Rap1-specific GEFs, ultimately leading to Rap1 activation (Fig. 1). Tyrosine-phosphorylated HEF1 may also directly or indirectly recruit Rac-specific GEFs (34) (Fig. 1). Activated Rap1 can both recruit the partitioning defective 3 (Par3)–partitioning defective 6 (Par6)–protein kinase (PKC ζ) complex and activate Cdc42 (35). Activated Cdc42 activates the Par3-Par6-PKC ζ complex, which in turn recruits the Rac GEF Tiam1 (T cell lymphoma invasion and metastasis 1), leading to Rac activation (35) (Fig. 1).

Ultimately, activated Rap1 coordinates with Rac activation to promote cell polarization and migration (Fig. 1). Further study of the detailed molecular mechanisms underlying chemokine-induced Abl and Rap1 activation will continue to reveal important insights into how chemokines activate immune signaling. Given the central importance of chemokines in T cell trafficking and migration to inflamed tissue, these studies will also be vital in advancing our understanding of pathogen sensing and autoimmunity.

References

1. Hogg N, Patzak I, Willenbrock F. The insider's guide to leukocyte integrin signalling and function. *Nat Rev Immunol.* 2011; 11:416–426. [PubMed: 21597477]
2. Thelen M, Stein JV. How chemokines invite leukocytes to dance. *Nat Immunol.* 2008; 9:953–959. [PubMed: 18711432]
3. Ward SG, Marelli-Berg FM. Mechanisms of chemokine and antigen-dependent T-lymphocyte navigation. *Biochem J.* 2009; 418:13–27. [PubMed: 19159344]
4. Gu JJ, Lavau CP, Pugacheva E, Soderblom EJ, Moseley MA, Pendergast AM. Abl family kinases modulate T cell-mediated inflammation and chemokine-induced migration through the adaptor HEF1 and the GTPase Rap1. *Sci Signal.* 2012; 5:ra51. [PubMed: 22810897]
5. Hernández SE, Krishnaswami M, Miller AL, Koleske AJ. How do Abl family kinases regulate cell shape and movement? *Trends Cell Biol.* 2004; 14:36–44. [PubMed: 14729179]
6. Gu JJ, Ryu JR, Pendergast AM. Abl tyrosine kinases in T-cell signaling. *Immunol Rev.* 2009; 228:170–183. [PubMed: 19290927]
7. Huang Y, Comiskey EO, Dupree RS, Li S, Koleske AJ, Burkhardt JK. The c-Abl tyrosine kinase regulates actin remodeling at the immune synapse. *Blood.* 2008; 112:111–119. [PubMed: 18305217]
8. Gu JJ, Zhang N, He YW, Koleske AJ, Pendergast AM. Defective T cell development and function in the absence of Abelson kinases. *J Immunol.* 2007; 179:7334–7343. [PubMed: 18025176]
9. Oldham WM, Van Eps N, Preininger AM, Hubbell WL, Hamm HE. Mapping allosteric connections from the receptor to the nucleotide-binding pocket of heterotrimeric G proteins. *Proc Natl Acad Sci USA.* 2007; 104:7927–7932. [PubMed: 17463080]
10. Johnston CA, Siderovski DP. Receptor-mediated activation of heterotrimeric G-proteins: current structural insights. *Mol Pharmacol.* 2007; 72:219–230. [PubMed: 17430994]
11. Ebert LM, Schaerli P, Moser B. Chemokine-mediated control of T cell traffic in lymphoid and peripheral tissues. *Mol Immunol.* 2005; 42:799–809. [PubMed: 15829268]
12. Kinashi T. Integrin regulation of lymphocyte trafficking: Lessons from structural and signaling studies. *Adv Immunol.* 2007; 93:185–227. [PubMed: 17383542]
13. Ridley AJ, Hall A. The small GTP-binding protein rho regulates the assembly of focal adhesions and actin stress fibers in response to growth factors. *Cell.* 1992; 70:389–399. [PubMed: 1643657]
14. Ridley AJ, Paterson HF, Johnston CL, Diekmann D, Hall A. The small GTP-binding protein rac regulates growth factor-induced membrane ruffling. *Cell.* 1992; 70:401–410. [PubMed: 1643658]
15. Faroudi M, Hons M, Zachacz A, Dumont C, Lyck R, Stein JV, Tybulewicz VL. Critical roles for Rac GTPases in T-cell migration to and within lymph nodes. *Blood.* 2010; 116:5536–5547. [PubMed: 20870900]
16. Stork PJ, Dillon TJ. Multiple roles of Rap1 in hematopoietic cells: Complementary versus antagonistic functions. *Blood.* 2005; 106:2952–2961. [PubMed: 16076873]
17. Kinashi T, Katagiri K. Regulation of lymphocyte adhesion and migration by the small GTPase Rap1 and its effector molecule, RAPL. *Immunol Lett.* 2004; 93:1–5. [PubMed: 15134891]
18. Katagiri K, Maeda A, Shimonaka M, Kinashi T. RAPL, a Rap1-binding molecule that mediates Rap1-induced adhesion through spatial regulation of LFA-1. *Nat Immunol.* 2003; 4:741–748. [PubMed: 12845325]
19. Regelman AG, Danzl NM, Wanjalla C, Alexandropoulos K. The hematopoietic isoform of Cas-HeF1-associated signal transducer regulates chemokine-induced inside-out signaling and T cell trafficking. *Immunity.* 2006; 25:907–918. [PubMed: 17174122]
20. O'Neill GM, Fashena SJ, Golemis EA. Integrin signalling: a new Cas(t) of characters enters the stage. *Trends Cell Biol.* 2000; 10:111–119. [PubMed: 10675905]
21. Kanda H, Mimura T, Hamasaki K, Yamamoto K, Yazaki Y, Hirai H, Nojima Y. Fyn and Lck tyrosine kinases regulate tyrosine phosphorylation of p105CasL, a member of the p130Cas docking protein family, in T-cell receptor-mediated signalling. *Immunology.* 1999; 97:56–61. [PubMed: 10447714]

22. Dikic I, Tokiwa G, Lev S, Courtneidge SA, Schlessinger J. A role for Pyk2 and Src in linking G-protein-coupled receptors with MAP kinase activation. *Nature*. 1996; 383:547–550. [PubMed: 8849729]
23. Rodríguez-Fernández JL, Rozengurt E. Bombesin, bradykinin, vasopressin, and phorbol esters rapidly and transiently activate Src family tyrosine kinases in Swiss 3T3 cells. Dissociation from tyrosine phosphorylation of p125 focal adhesion kinase. *J Biol Chem*. 1996; 271:27895–27901. [PubMed: 8910389]
24. Zachary I, Rozengurt E. Focal adhesion kinase (p125FAK): a point of convergence in the action of neuropeptides, integrins, and oncogenes. *Cell*. 1992; 71:891–894. [PubMed: 1458538]
25. Daub H, Weiss FU, Wallasch C, Ullrich A. Role of transactivation of the EGF receptor in signalling by G-protein-coupled receptors. *Nature*. 1996; 379:557–560. [PubMed: 8596637]
26. Luttrell LM, Ferguson SS, Daaka Y, Miller WE, Maudsley S, Della Rocca GJ, Lin F, Kawakatsu H, Owada K, Luttrell DK, Caron MG, Lefkowitz RJ. Beta-arrestin-dependent formation of beta2 adrenergic receptor-Src protein kinase complexes. *Science*. 1999; 283:655–661. [PubMed: 9924018]
27. Ma YC, Huang J, Ali S, Lowry W, Huang XY. Src tyrosine kinase is a novel direct effector of G proteins. *Cell*. 2000; 102:635–646. [PubMed: 11007482]
28. Cao W, Luttrell LM, Medvedev AV, Pierce KL, Daniel KW, Dixon TM, Lefkowitz RJ, Collins S. Direct binding of activated c-Src to the beta 3-adrenergic receptor is required for MAP kinase activation. *J Biol Chem*. 2000; 275:38131–38134. [PubMed: 11013230]
29. Needham LK, Rozengurt E. Galpha12 and Galpha13 stimulate Rho-dependent tyrosine phosphorylation of focal adhesion kinase, paxillin, and p130 Crk-associated substrate. *J Biol Chem*. 1998; 273:14626–14632. [PubMed: 9603980]
30. Gschwind A, Zwick E, Prenzel N, Leserer M, Ullrich A. Cell communication networks: Epidermal growth factor receptor transactivation as the paradigm for interreceptor signal transmission. *Oncogene*. 2001; 20:1594–1600. [PubMed: 11313906]
31. Shimonaka M, Katagiri K, Nakayama T, Fujita N, Tsuruo T, Yoshie O, Kinashi T. Rap1 translates chemokine signals to integrin activation, cell polarization, and motility across vascular endothelium under flow. *J Cell Biol*. 2003; 161:417–427. [PubMed: 12707305]
32. Cai D, Felekis KN, Near RI, O'Neill GM, van Seventer JM, Golemis EA, Lerner A. The GDP exchange factor AND-34 is expressed in B cells, associates with HEF1, and activates Cdc42. *J Immunol*. 2003; 170:969–978. [PubMed: 12517963]
33. Nolz JC, Nacusi LP, Segovis CM, Medeiros RB, Mitchell JS, Shimizu Y, Billadeau DD. The WAVE2 complex regulates T cell receptor signaling to integrins via Abl- and CrkL-C3G-mediated activation of Rap1. *J Cell Biol*. 2008; 182:1231–1244. [PubMed: 18809728]
34. Gu J, Sumida Y, Sanzen N, Sekiguchi K. Laminin-10/11 and fibronectin differentially regulate integrin-dependent Rho and Rac activation via p130(Cas)-CrkII-DOCK180 pathway. *J Biol Chem*. 2001; 276:27090–27097. [PubMed: 11369773]
35. Gérard A, Mertens AE, van der Kammen RA, Collard JG. The Par polarity complex regulates Rap1- and chemokine-induced T cell polarization. *J Cell Biol*. 2007; 176:863–875. [PubMed: 17353362]

Fig. 1.

Putative mechanism for chemokine-induced T cell polarization and migration through the Abl-HEF1-Rap1 signaling axis. Stromal cell-derived factor 1 α (SDF-1 α) binding to CXCR4 chemokine receptor 4 (CXCR4) on T cells leads to the activation of Abl family tyrosine kinases by the tyrosine kinase Lck. Abl phosphorylates the adaptor protein HEF1, which is localized to the membrane by its binding partner Chat-H. Phosphorylated HEF1 recruits Rac1 GEFs and Rap1 GEFs, which then activate Rac1 and Rap1, respectively. Activated Rap1 can also participate in Rac1 activation indirectly. Ultimately, activated Rap1 coordinates with Rac1-mediated F-actin polymerization to promote T cell polarization and migration.