

Editorial: **Cytohesin-1, on the tail of two integrins**

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In this issue of the *Journal of Leukocyte Biology*, Bourgoin and colleagues [1] report that cytohesin-1 functions to restrain fMLF-induced activation of the $\beta 2$ integrin Mac-1 in PMNLs. This is in stark contrast to the role of cytohesin-1 in the activation of LFA-1, a different $\beta 2$ integrin.

The $\beta 2$ integrins are heterodimers composed of a common β chain ($\beta 2$ or CD18) and one of four α chains. The four members of the family include $\alpha L\beta 2$ (LFA-1, CD11a/CD18), $\alpha M\beta 2$ (Mac-1, CD11b/CD18 or complement receptor 3), $\alpha X\beta 2$ (CD11c/CD18), and $\alpha D\beta 2$ (CD11d/CD18). LFA-1 and Mac-1 bind the adhesion molecule ICAM-1 expressed by vascular endothelium but participate in distinct steps of leukocyte recruitment [2]. Other ligands for Mac-1 include fibrinogen and complement protein fragment C3bi.

Integrin avidity for ligands is dynamically regulated. Activation can occur through changes in conformation, which increase the intrinsic affinity of the integrin-ligand bond, or through increased valency, which refers to an increase in the number of simultaneous integrin-ligand adhesive interactions. Activation is mediated by “inside-out” signaling events that result in the binding of regulatory proteins to the cyto-

plasmic domains of integrins. Regulation of integrin affinity for ligand is essential for a variety of cellular functions. Chemokines activate integrins by triggering a complex signaling network downstream of GPCRs, and this is a key step in the arrest of rolling leukocytes and the recruitment of specific leukocytes from the blood. A number of proteins that interact with and regulate the functions of integrins have been identified. Yet, our understanding of the molecular mechanisms that regulate integrin activities under physiologic conditions relevant to migration is still limited.

Cytohesin-1 was discovered in a yeast two-hybrid screen as a binding partner for the $\beta 2$ integrin cytoplasmic tail [3]. It is one of four mammalian cytohesins that share a similar domain organization and are GEFs. All family members contain an amino terminal CC domain, a central Sec7 domain, and a carboxy-terminal PH domain (reviewed in ref. [4]). The CC domain is involved in homodimerization. The Sec7 domain contains the highly conserved GEF activity for Arf GTPases and binds to the $\beta 2$ cytoplasmic tail. The PH domain mediates association with the cell membrane through binding to (a PI3K metabolite) PIP₃, Arf6 and three Arf-related GTPases, Arl4, Arl4c, and Arl4d [4, 5]. The Arf GTPases belong to the Ras superfamily and were named based on their capacity to act as cofactors for cholera toxin-catalyzed ADP-ribosylation of heterotrimeric G proteins. They interact with cell membranes through myristoylation at their amino termini, and their

primary function is the regulation of vesicular trafficking pathways. Arfs also activate PLD and some lipid kinases. In addition to their GEF activity, cytohesins are involved in vesicular transport and organization of the actin cytoskeleton and bind to $\beta 2$ integrins. Cytohesin-1 binds to a membrane-proximal region of the $\beta 2$ chain via the Sec7 domain, but the precise mechanism of LFA-1 activation is not understood (reviewed in ref. [4]). In T cells, the GEF activity of cytohesin-1 is not required for expression of the high-affinity epitope of LFA-1, but activation of Arf-GTPases is important for adhesion, cell spreading, and migration [6, 7]. LFA-1 activation is dependent on PI3K and requires an intact cytohesin-1 PH domain, thus implying that the membrane localization of cytohesin in response to the production of PIP₃ is critical for the $\beta 2$ integrin/cytohesin-1 interaction. It was demonstrated recently that activation of Arf6 by cytohesin-1 and association with the plasma membrane, independent of PI3K activation, are important for trans-endothelial migration [5].

Much of the evidence for the role of cytohesin-1 in $\beta 2$ integrin activation comes from studies using lymphocytes or lymphocyte cell lines, and focused on the LFA-1 integrin. Overexpression of full-length or GEF-inactive cytohesin-1 induced LFA-1 activation epitopes in T

Abbreviations: Arf=ADP-ribosylation factor, CC=coiled-coil, GEF=guanine nucleotide exchange factor, GPCR=G-protein coupled receptor, Mac-1=macrophage antigen 1, PH=pleckstrin homology, PIP₃=phosphatidylinositol 3,4,5-trisphosphate, PMNL=PMN leukocyte, siRNA=small interfering RNA

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cell lines [6]. Chemokine-triggered arrest of T cell lines to TNF- α -activated HUVECs was also augmented by cytohesin-1 overexpression but did not require the GEF activity of cytohesin-1 [7]. However, adhesion under static conditions and migration requires GEF activity [6, 7]. A recent study demonstrated that cytohesin-1 regulates the GTP loading of RhoA in DCs [8]. Activation of LFA-1 by chemokines and adhesion to ICAM-1 under flow were dependent on PI3K, cytohesin-1, and RhoA [8]. Similarly, LFA-1-mediated arrest of monocytic cells (Mono Mac 6 cell line) under conditions of physiological flow required the integrin-binding function of cytohesin-1 but not its GEF activity, whereas adhesion under static conditions and chemotaxis requires the GEF activity [7].

One other study directly investigated the role of cytohesin-1 in Mac-1 activation and function [9]. Treatment of the THP-1 monocytic cell line with a mycobacterial glycolipid (lipoarabinomannan) induced PI3K-dependent association of PIP₃ with cytohesin-1 and colocalization of cytohesin with Mac-1 [9]. Furthermore, siRNA knockdown of cytohesin-1 inhibited Mac-1-dependent phagocytosis of mycobacteria [9]. Recently, it was demonstrated that cytohesin-1 and Arf6 are involved in the functional PMNL response to fMLF stimulation [10]. The current study by the same group is the first to investigate the role of cytohesins in regulation of neutrophil β 2 integrin functions. As seen in other systems, GPCR signaling induced an association of cytohesin with neutrophil β 2 integrins, which could be abrogated by inhibition of cytohesin (SecinH3 in PMNL or siRNA silencing in PLB-985 cells). However, in contrast to the previous studies, this association was inhibitory in nature; an increase in cytohesin association restrained the activation of Mac-1 and reduced Mac-1-dependent functions, including adhesion, migration, and phagocytosis.

As the β 2 chain is common to LFA-1 and Mac-1, it would be expected that cytohesin binding would have similar effects on the functions of both integrins. However, the results presented in the current study suggest divergent effects of cytohesin-1 on Mac-1 and LFA-1 activity (Fig. 1). Perhaps this is

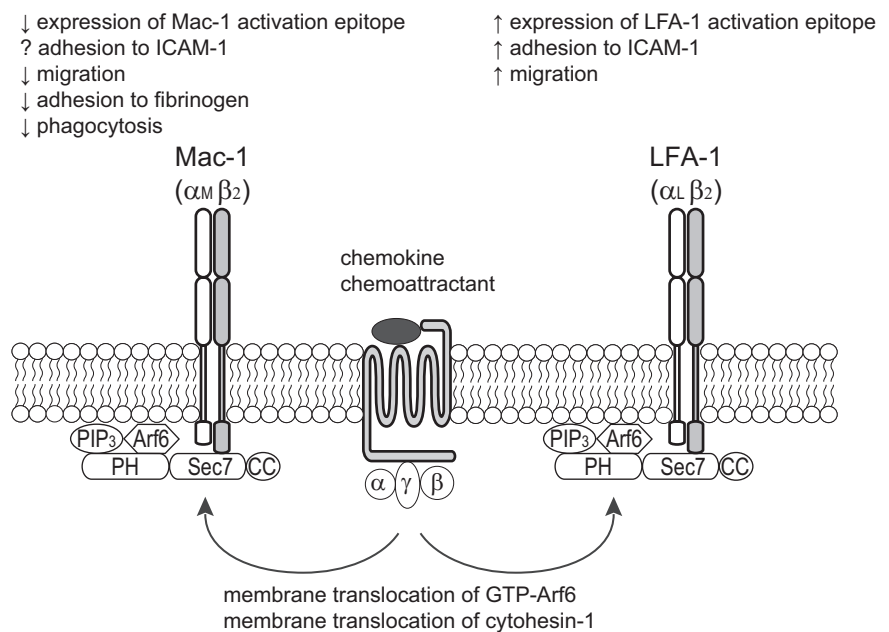


Figure 1. Divergent responses of α L β 2 and α M β 2 integrins to chemokine/chemoattractant-induced cytohesin-1 activation. GPCR signaling induces the activation of cytohesin-1 and the recruitment of GTP-bound Arf6 and cytohesin-1 to the plasma membrane, where cytohesin-1 interacts with β 2 integrins via the Sec7 domain. Binding of cytohesin-1 to the β 2 cytoplasmic tail of α L β 2 induces activation of this integrin and stimulates α L β 2-mediated adhesion to ICAM-1 and migration. In contrast, cytohesin-1 association with the β 2 cytoplasmic tail of α M β 2 inhibits activation, adhesion to fibrinogen, migration, and phagocytosis.

not surprising, as despite binding to the same ligand (ICAM-1), LFA-1 and Mac-1 have different subcellular localization and functions in the recruitment of leukocytes. On unactivated PMNL, LFA-1 is expressed primarily on the cell surface, whereas Mac-1 is abundant in intracellular granules and vesicles. Upon stimulation with inflammatory mediators, secretory vesicles translocate to the plasma membrane to increase Mac-1 expression six- to tenfold [11]. Chemokine-stimulated slow rolling and arrest are mediated by LFA-1, whereas intraluminal crawling of neutrophils and monocytes to sites of transmigration is mediated by Mac-1/ICAM-1-dependent interactions [12]. Thus, these two integrins play distinct roles during leukocyte recruitment to sites of inflammation, despite sharing a common β chain. The current study investigated interactions of Mac-1 with fibrinogen, a ligand for Mac-1, but not LFA-1 [1]. It would be interesting to determine whether divergent roles for cytohesin are also

observed for interactions with ICAM-1, a ligand common to both integrins.

The molecular mechanisms involved in divergent regulation of LFA-1 and Mac-1 integrins by cytohesin-1 have yet to be determined, and indeed, the current findings require confirmation in other leukocyte types. Perhaps it is naïve to assume that all integrins or even those that share a common β (or α) chain are activated in an identical manner. This study highlights the fact that not only might there be cell-type specific differences in activation of integrins, but also, even within the same cell, closely related integrins may be regulated independently.

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