Leukocyte chemotaxis: from lysosomes to motility

Gabriela Constantin & Carlo Laudanna

Chemoattractants direct the extravasation of leukocytes to the site of immune response. New data highlight the role of synaptotagmins and Rab proteins in leukocyte chemotaxis.

Leukocyte extravasation is the *primum movens* of the immune response[1]. Chemoattractants regulate leukocyte extravasation by controlling integrin activation and chemotaxis. The central role of chemotaxis in immune-system homeostasis has prompted a constant flow of studies in the past. In this issue of *Nature Immunology*, Colvin and colleagues describe a series of elegant experiments that demonstrate the role of synaptotagmins and Rab small GTPases in the regulation of leukocyte migration[2]. Their new findings indicate that three members of the synaptotagmin family of transmembrane proteins, SYT2, SYT7 and SYTL5 (synaptotagmin-like protein 5), and two members of the Rab small GTPases family, Rab3a and Rab27a, are regulators of intracellular vesicular traffic and exocytosis that leads to surface delivery of endomembranes, which promotes chemotaxis. The study by Colvin and colleagues provides a novel, somehow unexpected, addition to the understanding of the leukocyte chemotactic process and definitively expands the view of leukocyte motility by highlighting the importance of lysosome exocytosis and surface delivery of lipid membranes during cell migration.

Leukocytes are the most dynamic cells in the body, able to be in the right place at the right time. Leukocytes fulfill their duties by means of a unique ability to adhere to blood vessels and the extracellular matrix and to move toward gradients of chemoattractants, which leads to tissue microenvironmental repositioning. Random and directional motility, called 'chemokinesis' and 'chemotaxis', respectively, are crucial to several leukocyte activities, including crawling on the endothelium after arrest, diapedesis and directional movement regulated by chemokine gradients, and allowing accumulation in critical sites of tissues inflammation and antigen presentation. Much effort has been expended in elucidating the intracellular signaling machinery that is triggered by chemoattractants and controls directional motility. It is now established that complex signaling events, including the movement of intracellular calcium, Rho small GTPases, and specific protein and lipid kinases, control the various aspects of chemotaxis, including integrin triggering, cytoskeleton dynamics and sensing of gradients of chemotactic factors[3,4]. However, despite exciting progress, the journey is still not over and many details are still missing.

Chemotaxis is an ensemble of cell activities that are precisely orchestrated in space and time and consist of highly dynamic cell shape rearrangements that lead to the acquisition of a polarized phenotype with a clear distinction between the leading edge, called the 'lamellipodia', and the trailing edge, called the 'uropod', accompanied by rapid adhesion–de-adhesion cycling and directional movement. During chemotaxis, continuous cytoskeleton remodeling leads to incessant changes in cell shape; this in turn must be accompanied by continuous remodeling of the plasma membrane with the purpose of generating new transient polarized adhesive contacts so the cell can follow the gradient. Given the fast dynamics of surface remodeling, this phenomenon probably needs rapid mobilization of intracellular stores of preformed layers of lipid membranes to allow extension of cell surface toward the gradient. However, although the mechanisms that generate the asymmetrical signaling events that lead to gradient sensing and directional motility have been studied and at least partially elucidated, the importance of membrane remodeling and the signaling mechanisms that control it have until now remained elusive.

In the new work published here, Colvin and colleagues provide a novel and convincing answer to this topic[2]. The authors start from a rather general perspective looking for genes previously unknown to be related to chemotaxis regulation. By applying high-throughput small interfering RNA (siRNA)-based genetic screening, they identify SYT2, SYT7 and SYTL5 as regulators of leukocyte chemotaxis. To further support the idea of a role for lysosome exocytosis in leukocyte chemotaxis, Colvin and colleagues investigate the involvement of Rab small GTPases, whose role in intracellular vesicle traffic and exocytosis is well known[5]. They find that Rab3a and Rab27a are also critically involved in the regulation of lymphocyte chemotaxis. Importantly, they show that interference with synaptotagmin or Rab activity does not alter the activation of Rho small GTPases or polymerization of the actin cytoskeleton; this further supports the idea that the involvement of synaptotagmins and Rab proteins in chemotaxis regulation is at the level of intracellular vesicle traffic. On the basis of that finding, Colvin and colleagues propose a model for the regulation of chemokine-induced chemotaxis that involves activation of a signaling module consisting of calcium, synaptotagmins and Rab proteins globally devoted to the control of vesicle trafficking and endomembrane surface delivery and fusion essential to the cell-shape remodeling necessary for the overall dynamics of leukocyte chemotaxis (**Fig. 1**).

Synaptotagmins are a family of proteins that act as calcium sensors for calcium-triggered vesicle fusion, in particular of synaptic vesicles in neurons[6]. Most of the 15 members of the synaptotagmin family are expressed in neurons, but studies have also shown synaptotagmin expression in leukocytes, which suggests that synaptotagmins are involved in leukocyte physiology. For example, chemokine-induced migration is much lower in T lymphocytes with impaired SYT3 function, apparently due to less recycling of the chemokine receptor CXCR4 during migration[7]. In addition, SYT7 is expressed on lytic granules in activated $CD8^+$ T cells and is required for full cytotoxicity under conditions in which exocytosis is triggered by the antigen-specific recognition of targets[8]. Moreover, the synaptotagmin-like proteins SYTL1 and SYTL2a, by interacting with Rab27a, localize to the plasma membrane of $CD8^+$ T cells and contribute to the formation of a docking complex, capturing secretory lysosomes at the immunological synapse[9].

The role of synaptotagmins in leukocyte chemotaxis identified by Colvin and colleagues definitively opens a wider horizon on the chemotactic process[2]. Indeed, although the occurrence of vesicle trafficking and exocytosis during chemotaxis has been known for several years, these new data demonstrate that synaptotagmin-mediated leukocyte exocytosis not only is relevant to enzyme secretion (or receptor recycling) but also is an integral part of the

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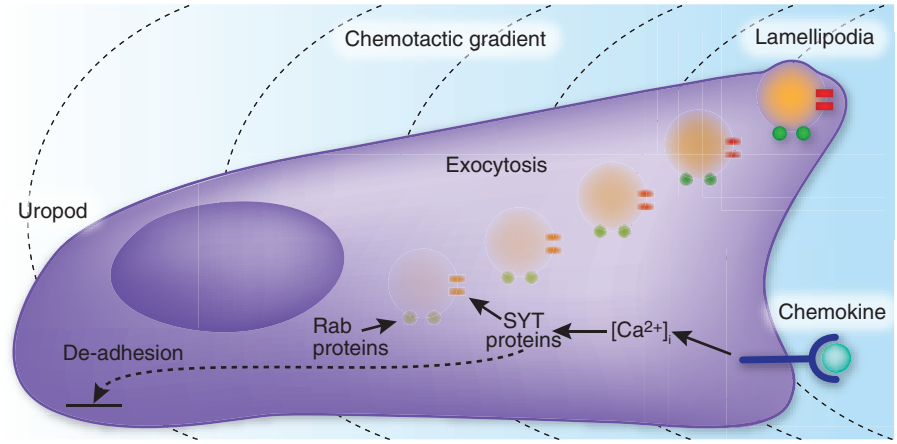

Figure 1 Leukocytes follow a chemotactic stimulus by sensing the gradient and generating a continuous cycling of adhesive events, cytoskeleton polymerization and plasma membrane remodeling. Colvin *et al.* now demonstrate the critical role of synaptotagmins (SYT proteins) and Rab small GTPases (Rab proteins) in controlling the permanent flow of preformed endomembranes to the plasma membrane, thus allowing continuous cell-shape changes. Chemokines trigger an increase in intracellular calcium ($[Ca^{2+}]_i$). This acts together with synaptotagmins and Rab small GTPases in controlling vesicle trafficking and exocytosis during chemotaxis. Exocytosis delivers to the cell surface the preformed layers of lipid membrane necessary for continuous plasma membrane turnover and extension toward the gradient. Synaptotagmins also participate in the adhesion–de-adhesion cycling that leads to uropod release.

chemotactic process itself by providing continuous delivery of preformed membrane layers critical to plasma membrane plasticity and that this trafficking of endomembranes critically regulates directional leukocyte motility.

The study raises questions that are surely important for future investigations. For example, the mechanism by which synaptotagmin-mediated vesicle fusion leads to rear release of uropods and chemotaxis is not known. Notably, to induce exocytosis, synaptotagmins interact with SNARE (soluble *N*-ethylmaleimide-sensitive factor attachment protein receptor) proteins, which have a key role in vesicle and plasma membrane fusion by perturbing lipid bilayers[6]. Thus, SNARE proteins should be explored as possible mediators of membrane dynamics that control leukocyte rear release of uropods and locomotion. Moreover, the role of lysosome exocytosis should be investigated under different conditions, with the aim of possibly identifying different combinations of synaptotagmins and Rab isoforms that regulate chemotaxis in distinct leukocyte subtypes, thus introducing into the model important elements of cell specificity. Furthermore, it will be very useful to test whether the model also holds true for chemokinesis, which is probably more relevant to other cell contexts, such as metastatic cancer cells. In this last perspective, the study by Colvin and colleagues[2] may prove useful for envisioning novel directions for therapy.

COMPETING FINANCIAL INTERESTS
The authors declare no competing financial interests.

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Give and take in the germinal center

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B cell–T cell interactions in germinal centers are needed to generate high-affinity antibodies. PD-1 signaling is now shown to influence the quality of germinal center responses.

A major outcome of the immune response is the production of high-affinity antibody-secreting plasma cells and memory B cells. This process requires direct contact between activated B cells and T cells in a specialized structure known as the germinal center (GC). The GC supports cross-talk between B cells and T cells that controls their survival, proliferation and differentiation. In this issue of *Nature Immunology*, Shlomchik and colleagues demonstrate that the interactions between PD ligands on B cells and PD-1 on T cells is required for the optimal output of the GC; that is, long-lived antibody-secreting plasma cells[1].

PD-1, originally identified as a marker of programmed cell death, has subsequently been shown to be an important negative regulator of immune activation by the development of florid autoimmunity in PD-1-deficient mice[2]. More recently, PD-1 has come to signify a state known as immune exhaustion, in which T cells, unable to clear a viral infection, relapse into a torpor, apparently unable to raise the barest of effector functions[2]. Remarkably, however, blocking access to PD-L1—a ligand for PD-1—revives the exhausted T cells[3], which indicates that their inactive state is actively maintained. It is now widely held that PD-1 engagement promotes and maintains a state of T cell unresponsiveness.

PD-1 also has an alter ego, with high expression on T cells present in GCs[4]—so much so that PD-1, along with the costimulatory molecule ICOS and chemokine receptor CXCR5, has become a key means of identifying the $CD4^+$ follicular helper T cell (T_{FH} cell) subset (**Fig. 1**). Work on T_{FH} cells has exploded in recent years, particularly after the discovery that this population secretes interleukin 4 (IL-4) and IL-21 and is required for GC development[4]. This last connection is somewhat circular, as it has been clear for some time that there is an interdependence of the development of T_{FH}

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