Movement within and movement beyond
Synaptotagmin-mediated vesicle fusion during chemotaxis

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Leukocyte chemotaxis plays an essential role in generating and delivering immune responses and is a critical component of inflammation. In order to identify novel genes and pathways important for regulating chemotaxis, we performed an RNAi-based screen and identified several genes involved with vesicle movement and fusion as mediators of chemotaxis. Our recently published data1 show that during chemotaxis vesicle trafficking proteins are required for lysosome fusion, uropod release and efficient directed cell migration.

Leukocyte chemotaxis is an essential process that directs the immune system and drives inflammatory responses.2 The processes whereby an extracellular chemical signal induces a program that results in morphological changes to the cell and induces chemotaxis remain incompletely understood. Since the earliest days of the study of chemotaxis, a number of investigators have found clues suggesting that membrane flow and vesicle trafficking play roles in the movement of cells toward a chemotactic stimulus. As early as 1962 it was suggested that cell migration involves a membrane cycle.3 Data emerged suggesting that the plasma membrane flowed toward the rear of the migrating cell.4-6 More recently, it was suggested the membrane actually flowed toward the front of the migrating cell.7 This later hypothesis was supported by the finding that recycling transferrin receptors were routed to the plasma membrane of the leading edge lamellodia.8 Finally, a potential role for lysosome fusion with the uropod has been suggested.9-12 Despite these observations, a functional correlation of vesicle trafficking in chemotaxis had not been established.

We performed an RNAi-based genetic screen to identify novel regulators of CXCR4-mediated lymphocyte chemotaxis. In the screen, we found two genes in the synaptotagmin family of calcium sensing vesicle fusion proteins. Synaptotagmin-2 (Syt2) was identified as a negative regulator of lymphocyte chemotaxis and Synaptotagmin-like protein-5 (SytL5) was identified as a positive regulator of chemotaxis. Lymphocytes in which SYT2 expression was reduced using RNAi demonstrated enhanced migration in a CXCL12 gradient. In contrast, cells in which SYTL5 mRNA expression was reduced demonstrated decreased migration in a CXCL12 gradient. The knock-down of these genes had similar effects in the migration of a monocyte cell line to a different chemokine, CCL2.

In order to determine the extent of the role of synaptotagmin family member proteins in chemotaxis, we extended the screen to the remaining synaptotagmin and synaptotagmin-like family member genes and identified Synaptotagmin-7 (Syt7) as encoding a positive regulator of chemotaxis. The identification of SYT7 provided potential insight into the mechanistic role of these proteins in chemotaxis as it is expressed on the lysosomal membrane of many different cell types and has been shown to be important in lysosome-cell membrane fusion as well as in membrane repair. Additionally, an SYT7-deficient mouse line was available to facilitate further experiments to analyze the function of SYT7 in chemotaxis. Lymphocytes and neutrophils obtained from SYT7-deficient mice demonstrated decreased chemotaxis in response to chemokine gradients. By analyzing cell migration tracks of cells migrating in a Zigmond chemotaxis chamber, we found that the cells lacking SYT7 had both a defect in migration velocity, as well as in migration direction. In an in vivo model of monosodium urate (MSU) crystal induced gout-like inflammation, we found that SYT7-deficient female mice demonstrated decreased neutrophil recruitment into an experimentally produced airpouch containing MSU crystals. The decreased neutrophil recruitment was not due to decreased IL-1 or neutrophil-active chemokine release as the airpouches contained similar amounts of these cytokines. These results confirmed and extended the hypothesis that synaptotagmin family member proteins have a role in chemotaxis.

In addition to synaptotagmin family members, the process of vesicle trafficking within the cell also requires SNARE proteins. Indeed, a role for the SYT7 interacting SNARE protein, VAMP7,
confocal micrographs of podocytes, large cells of renal origin that express CXCR3, show that podocytes express the lysosomal marker LAMP-2 on their surface following stimulation with the CXCR3 ligand CXCL11. These data support the hypothesis that chemokine-mediated calcium influx induces the fusion of lysosomes with the cell membrane in a SYT7-dependent manner. To further investigate the role of lysosomes in chemotaxis, we analyzed the migration of lysotracker-stained wild-type and Syt7−/− lymphocytes in a CXCL10 gradient in a Dunn chamber. We found that lysotracker accumulated in the uropods of Syt7−/− cells. Additionally, these cells were adherent to the fibronectin-coated substrate at their uropods. Taken together, these data suggested that Syt7-mediated fusion of lysosomes is required for efficient uropod release during chemotaxis.

During the 1970s, chemoattractant stimulation of neutrophils was shown to induce lysosomal enzyme release and a role for lysosomal fusion was speculated to play a role in neutrophil adhesion. However, it was difficult to identify a direct role of lysosomal enzyme release or to separate lysosome membrane fusion with that of enzyme release in chemotaxis.

We speculate that the migration and fusion of lysosomes is required to deliver cargo during chemotaxis that allows uropod release. It is possible that the fusion of lysosomes is required to deliver a specific protein required for uropod release. Alternatively, the cargo may be lipids required for membrane cycling during chemotaxis or for the delivery of a specific lipid or protein needed in the chemotaxis signaling pathway. Finally, it is possible that the contents of the lysosome, perhaps enzymes, are required on the outside of the cell to facilitate the release of uropods from the extracellular matrix. The identification of these factors is of great interest.

While our data specifically implicate a role for lysosomal trafficking and fusion in chemotaxis, it is likely that the flow of other vesicles and membrane is important in directed cell migration. As the interaction of SYT7 and VAMP7 has also been shown to be important for lysosome fusion with the cell membrane and SYT2 has been shown to be a negative regulator of lysosomal exocytosis, we investigated whether lysosome fusion was important for chemotaxis. Using FACS analysis, we found that chemoattractant stimulation of lymphocytes and neutrophils resulted in the expression of the lysosomal membrane protein LAMP-1 (CD107a) on the cell surface. Expression of LAMP-1 was dependent on the expression of SYT7 as well as the release of intracellular calcium. Further, has been shown in epithelial cell migration. As the interaction of SYT7 and VAMP7 has also been shown to be important for lysosome fusion with the cell membrane and SYT2 has been shown to be a negative regulator of lysosomal exocytosis, we investigated whether lysosome fusion was important for chemotaxis. Using FACS analysis, we found that chemoattractant stimulation of lymphocytes and neutrophils resulted in the expression of the lysosomal membrane protein LAMP-1 (CD107a) on the cell surface. Expression of LAMP-1 was dependent on the expression of SYT7 as well as the release of intracellular calcium. Further,
These data support a role for the trafficking of other types of vesicles in addition to lysosomes during chemotaxis. Future studies are needed to determine the roles of vesicle trafficking in regulating chemotaxis. We have presented experiments specifically demonstrating a role for lysosome or lysosome-like vesicle fusion in uropod release during chemotaxis (Fig. 2). However, it is likely that there will be many more roles for vesicle trafficking in chemotaxis. For example, we have found that the knock-down of additional Rab family member proteins not specifically associated with lysosomal movement also reduced chemotaxis in lymphocytes. It is likely that vesicle trafficking is essential for delivering lipid membrane to the cell surface as the cell shape changes. Additionally, chemokine receptor recycling during chemotaxis depends on vesicle trafficking and SYT3. Specific cargo, including integrins and other receptors, are likely to be delivered to the leading and trailing edges by vesicles. It is likely that the movement of vesicles within the cell is essential for many aspects of cell movement beyond the cell.

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