Investigating the role of sphingosine-1-phosphate receptor 1 endocytosis in regulating signaling induced by spingosine-1-phosphate

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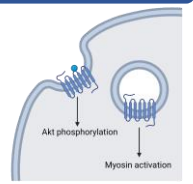
BACKGROUND

- Binding of signaling lipid, sphingosine-1-phosphate (S1P) to the T lymphocyte surface receptor, sphingosine-1-phosphate receptor 1 (S1PR1) regulates T cell entry into circulation
- S1P binding to S1PR1 induces early signaling events, including Akt phosphorylation. Binding also induces rapid receptor internalization.
- During S1P-mediated T cell migration, cells first extend actin-rich lamellipodia then transition to pressure-driven bleb-based migration, induced by myosin activity.
- Myosin activation, which correlates temporally with bleb-based motility, occurs in a delayed manner when S1PR1 internalization is well underway.

HYPOTHESIS

- Intracellular signaling in response to S1P occurs in a bifurcated manner. Initial signaling from the plasma membrane leads to Akt phosphorylation, while subsequent signaling from the endosome is responsible for myosin activation.



RESULTS

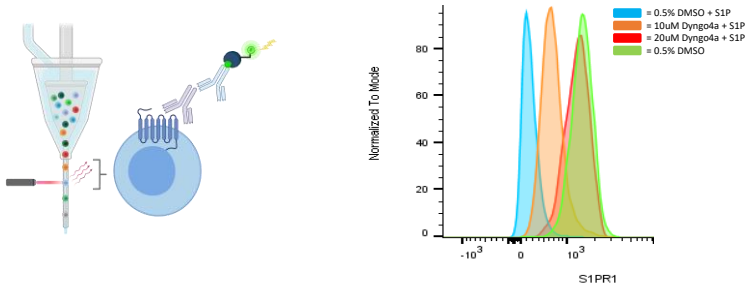


Figure 1. **Dynamin 2 inhibitor, Dyngo4a, effectively blocks S1PR1 internalization upon S1P binding**. Naïve CD4+ T cells were incubated with either 0.5% DMSO, 10 uM Dyngo4a, or 20 uM of Dyngo4a for 20 minutes before exposure to 10 nM of S1P for 20 minutes. Cells were fixed, stained for S1PR1, and analyzed by flow cytometry.

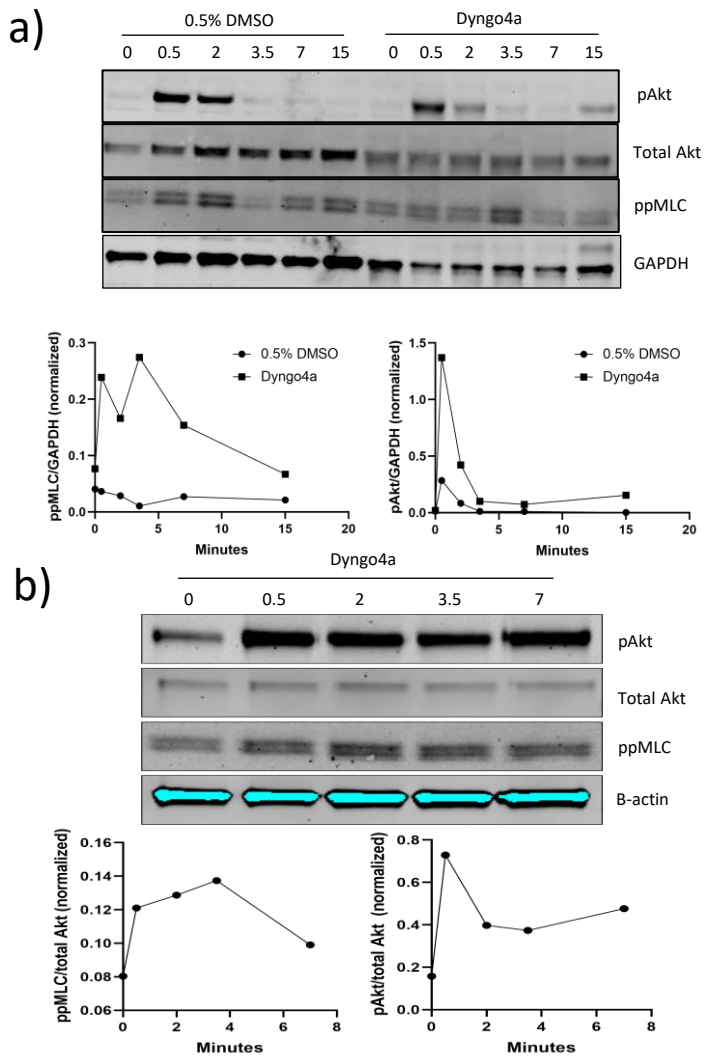


Figure 2: **Inhibition of endocytosis increases myosin activation, while Akt phosphorylation is sometimes prolonged. (a)** Naïve CD4+ T cells were incubated with 20 uM of Dyngo4a or 0.5% DMSO for 20 minutes before stimulation with 10 nM of S1P for 0, 0.5, 2, 3.5, 7, or 15 minutes. Cells were lysed, then immunoblotted for phospho-MLC (ppMLC; Thr18+Ser19), GAPDH, phospho-AKT (pAKT; Ser473), and total AKT (n=2). **(b)** Naïve CD4+ T cells were incubated with 20 uM of Dyngo4a for 20 minutes before stimulation with 10 nM of S1P for 0, 0.5, 2, 3.5, or 7 minutes. Cells were lysed, the immunoblotted for phospho-MLC (ppMLC; Thr18+Ser19), b-actin, phospho-AKT (pAKT; Ser473), and total AKT (n=2).

CONCLUSION

- Inhibition of S1PR1 endocytosis upon S1P binding sustains Akt phosphorylation and increases myosin activation.
- Surface localization of S1PR1 may be needed for Akt phosphorylation and myosin activation.
- Contrary to my hypothesis, S1PR1 does not need to traffic to endosomes in order to phosphorylate myosin.

FURTHER DIRECTIONS

- Further studies are needed to investigate the mechanism responsible for delayed myosin activation.
- We plan to test the role of Rac1 and RhoA antagonism on lamellipodia formation and myosin phosphorylation, respectively.

ACKNOWLEDGMENTS

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