Inhibiting Degranulation in Immune Cell Signaling Pathways

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Background

According to the World Health Organization, 40% of the world's population have sensitivity to allergies and this number is only climbing. Existing treatments are only able to mitigate symptoms after an immune response has already occurred creating a potential to develop new treatment options for allergies by preventing this response from occurring.

Mast cells are the primary biological executors of the allergic response, and they are responsible for releasing inflammatory molecules into the bloodstream in response to an allergen being present in the body in a process called degranulation. These are generally responsible for common allergic symptoms like itchiness, runny nose, sneezing, and dry throat that have become hallmarks of the allergic response. By inhibiting molecules in this pathway and thus preventing degranulation from occurring, we hope to shine light on an alternative, preferred method of allergy treatment for the millions of people afflicted with them every day.

Research Question

Can we inhibit the release of chemical mediators from mast cells via degranulation and disrupt the allergic response by use of inhibitors?

Hypothesis: If known inhibitors of small molecules in the mast cell signaling pathway are applied to mast cell culture, then degranulation will be reduced in vitro.

Cell Cultures

- Mast cells are cells filled with allergic mediator granules that are found in connective tissue and release histamine and other substances during inflammatory and allergic reactions.
- Basophils are circulating white blood cells that function similarly to mast cells. We selected MC9 mouse mast cells and RBL-2H3 basophil cells to model the immune response.

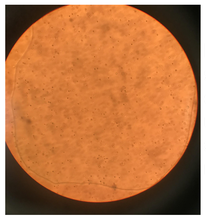


Figure 1. MC9 suspension cell culture viewed under microscope at 400X magnification.

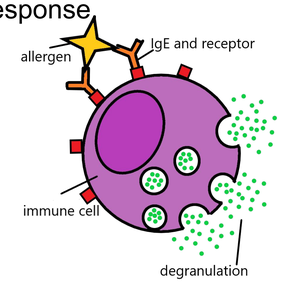
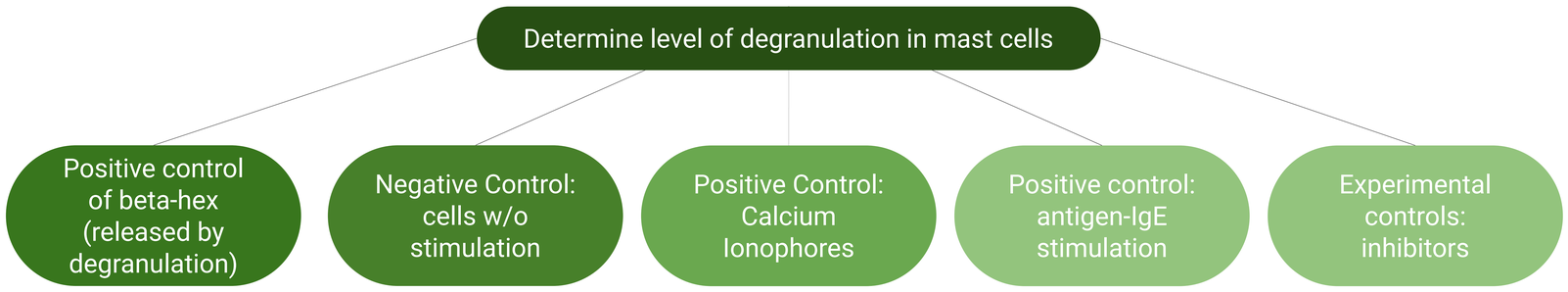


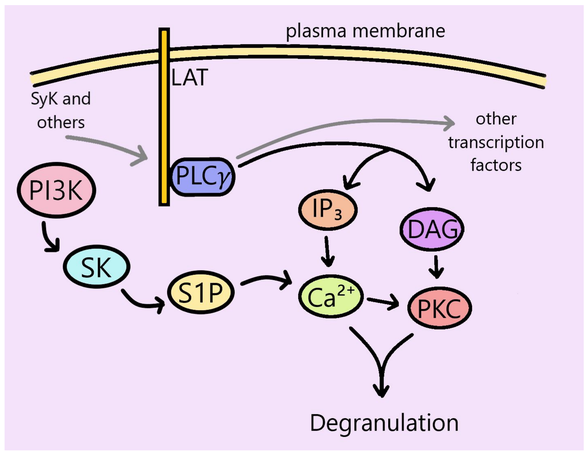
Figure 2. Overview of antigen-IgE mediated process of degranulation in mast cells

Research Design



Inhibitors

Figure 3. Signaling pathway in mast cells resulting in degranulation, highlighting small molecules of interest



- SHIP1 - SHIP1 inhibits immune receptor signaling through hydrolysis of the PI3K product phosphatidylinositol 3,4,5-trisphosphate,
- DMS - competitive inhibitor of SK (Sphingosine Kinase), previously tested in tested in leukemia, fibroblasts, and pheochromocytoma cells
- JTE013 - antagonist of $S1P_2$ (Sphingosine-1-Phosphate Receptor-2), shown to possibly alter histamine release or mast cell degranulation.
- IC84114: selective PI3K (Phosphoinositide 3-kinase) p110δ inhibitor was shown to inhibit the antigen-mediated mast cell activation and enhancement of degradulation

Current Results

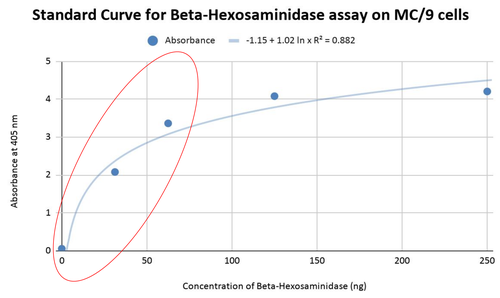


Figure 4. Beta-hexosaminidase (beta-hex) is found in mast cell granules and its release can be used to measure the amount of degranulation. In the positive control, we added varying concentrations (0-250 ng) of beta-hex to 1.5 mg/mL pNAG substrate in citrate buffer to determine the upper limit of detection by this assay. This positive control resulted in a linear standard curve in the concentration ranges between 0 and 100 ng, so we will be testing in this concentration range for future assays.

Future Research Goals

- Repetition of assay using a more concentrations on the lower end of the range to develop a linear fit for standard curve.
- Continue culturing MC9 and RBL-2H3 cell cultures
- Conduct ELISA assay on both cell lines to find optimal degranulation
- Introduce inhibitors and measure any changes in degranulation

Acknowledgements and Citations

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