Bridging Lived Experience and Research: Developing Bone Marrow-Derived Mast Cells to Study Mast Cell Activation Syndrome

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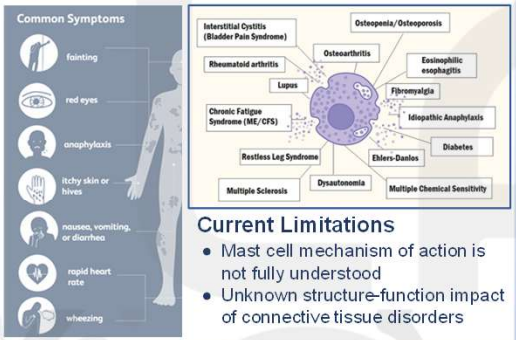
Introduction

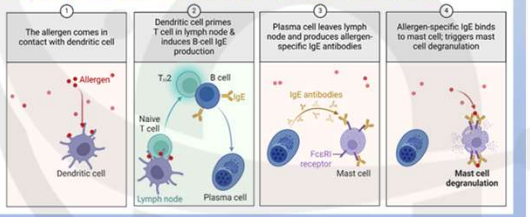
Mast cell activation syndrome (MCAS) refers to a group of disorders that occurs when there is an overproduction or excessive degranulation of mast cells. Often occurring with comorbid conditions involving connective tissues and is believed to effect **1 in every 10,000 to 20,000** people.[1]



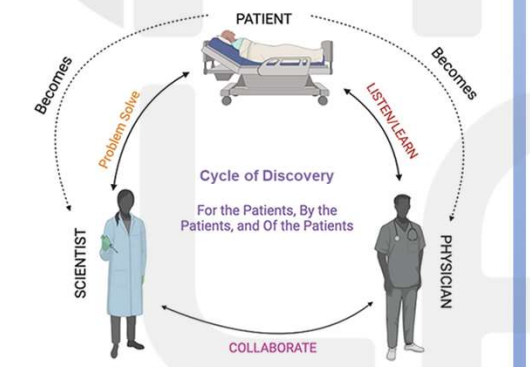
Current Limitations

- Mast cell mechanism of action is not fully understood
- Unknown structure-function impact of connective tissue disorders

Activation of Mast Cells *in vivo*

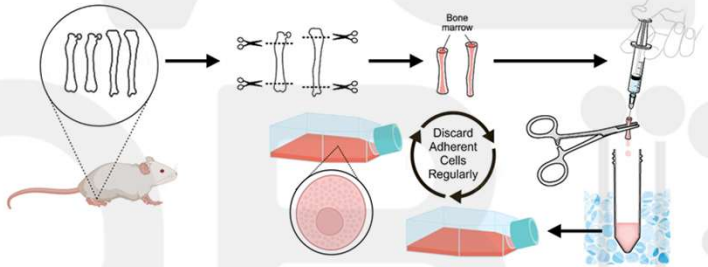


Patient-Scientist Model[3]



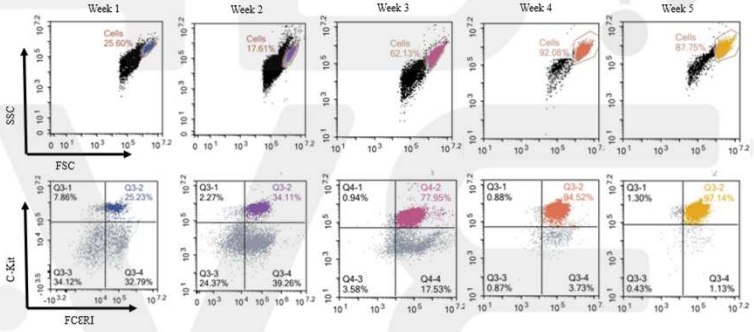
Developing Mast Cells *in vitro*

Differentiating stem cells from mouse bone marrow to create a pure mast cell culture.



A

Figure 1: Flow Cytometry of Maturing Mast Cells Over Time



B

Maturing Mast Cells Over Time

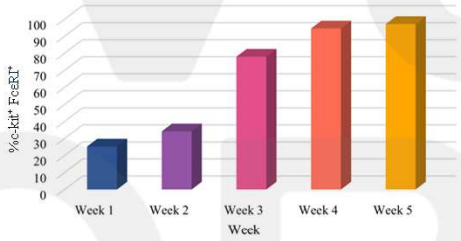
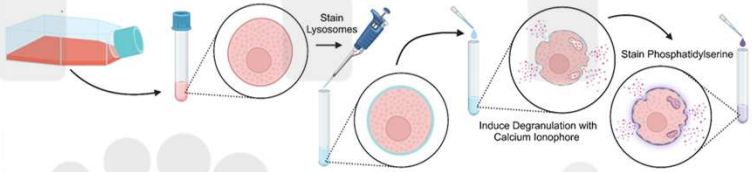


Figure 1: Over 5 weeks of *in vitro* culture, mast cells differentiate to 97.14% purity. Bone marrow cells were isolated from C57Bl/6 mice were cultured in media with IL-3. Fed and passaged every 3-4 days. Each week, a sample was collected and stained for c-kit and FcεRI with double positive indicating maturity. Cells were then analyzed by flow cytometry. (**A**) Shows FSC/SSC gate (top) and c-kit and FcεRI gating (bottom) within the "cells" population. (**B**) Graph represents the increase in c-kit+FcεRI+ over each week.

- For weeks 1-4, cells were passaged with feeding every 3-4 days to discard adherent cells.
- By Week 4 cells were passaged as needed with feedings ~1x a week.
- Once ideal purity is reached the model can be used for several weeks.
- By Week 9 we observed cell growth greatly diminished with increased debris and cell death.

Inducing Degranulation with Ca^+ Ionophore A23187



A

Figure 2: Flow Cytometry of Ca^+ Induced Degranulation Over Time

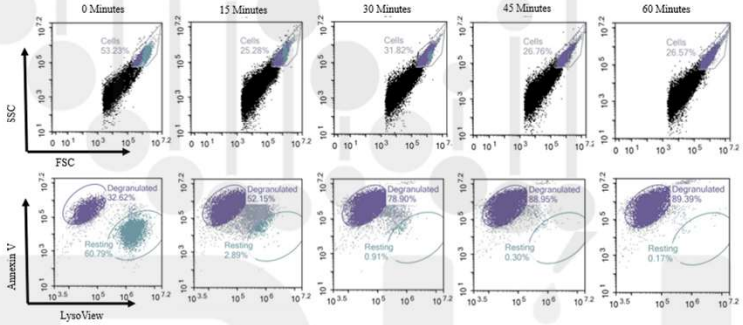
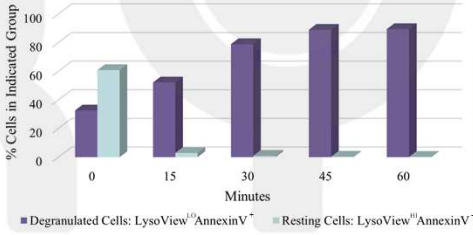


Figure 2: Mast cell degranulation induced using calcium ionophore A23187. Mast cells were first stained with LysoView to mark lysosomes then treated with A23187 for indicated time. Following this, the cells in each group were stained with Annexin V for phosphatidylserine (PS). Degranulated cells have low LysoView staining and high expression of PS. (**A**) Representative flow plots in each time point with gating used for graph in (**B**). Blue are resting cells and purple bars are degranulated cells.

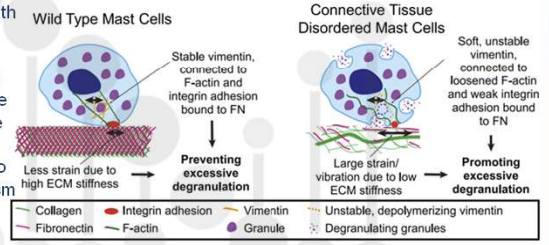
B

Ca^+ Induced Degranulation Over Time



Future Work - The Role of Connective Tissue

MCAS often co-occurs with connective tissue disorders. Therefore, creating *in vitro* models allow exploration into the interplay between these two conditions. Furthermore, we need to understand the mechanism that causes such phenotypes.[5]



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