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Optimizing Flow Cytometry Analysis of SMS2 and KDM6A in Germinal Center B cells in the Study of Female-Bias in Lupus Autoimmunity

Systemic Lupus Erythematosus (SLE) is a female-biased autoimmune disease driven by antinuclear autoantibodies. We reported sphingomyelin synthase 2 (SMS2), a plasma membrane lipid enzyme, regulates germinal center (GC) B cell tolerance, the key mechanism that prevents lupus autoimmunity. SMS2-regulated GC B cell tolerance is impaired in SLE due to a loss of expression of SMS2. The process by which SMS2 expression is lost in SLE remains unknown. Our group identified KDM6A, an X linked histone demethylase as a potential regulator of SMS2 expression. We aim to measure SMS2 and KDM6A protein expression in murine splenocytes on an individual cell level via flow cytometry. The intracellular and intranuclear locations of these proteins poses challenges for this type of study. The goal of this project is to develop a consistent flow cytometry protocol for measuring SMS2 and KDM6A in GC B cells.

A protocol for staining for SMS2 and KDM6A proteins was developed by optimizing the parameters: cell quantity, GC B cell surface antibodies, fixation time, volume of fixation solution and permeabilization buffer concentration. Primary antibodies for KDM6A and SMS2 and secondary staining of the primary antibodies were tested. Protocols were tested with SMS2 and KDM6A knockout cells. Cells were analyzed by flow cytometry (Novocyte).

The final protocol after optimization included surface staining for the GC B cells using the cocktail: B220-APC, GL7-FITC and FAS-PE. Cells were fixed and permeabilized at room temperature with Invitrogen kits before staining by SMS2 rabbit antibody (Invitrogen) or KDM6A rabbit antibody (BD) followed by anti-rabbit biotin IgG and Strep APC-Cy7 secondary antibodies. This yielded repeatable results for staining both proteins when compared to knockout mouse counterparts.

By optimizing staining protocols, we can reliably measure SMS2 and KDM6A protein levels from splenocytes. This is an essential step forward for future experiments for our research group.