ABSTRACT
Principal Investigator: Shiv Pillai
Grant Title: 9-*O*-acetyl sialic acid and Autoimmunity

Enhanced 9-*O*-acetylation of sialic acid on B lymphocytes was observed in patients with lupus. Marked enhancement of 9-O-acetylation of sialic acid was also observed specifically in B cells in the lupus prone MRL/+ mouse at a time when there are no signs of disease.

Objectives:

a) To determine the nature of the increased 9-*O*-acetylation of sialic acid on B cells in lupus prone mice and in lupus patients and to determine if this change alters B cell tolerance
b) To determine if the sialic acid *O*-acetyl transferase CasD1 could be a potential therapeutic target in lupus

Methods Used:

We have used flow cytometry, apoptosis assays, chemical treatment of cells with trypsin, *O*-sialoglycoprotease and methanol (to remove gangliosides). We have used CHE-FcD to measure 9-*O*-acetylation of sialic acid and treatement with a bovine coronavirus hemagglutinin esterase to remove *O*-acetyl moieties from sialic acid.

Results:

We observed that increased 9-*O*-acetylation of sialic acid on B cells occurs in the earliest B lineage cells and is maintained through B cell development in MRL/+ mice. We show that this post-synthetic change occurs primarily on gangliosides in MRL/+ B cells. In certain cell types the GD3 ganglioside has been shown to be required for apoptosis and 9-*O*-acetylated GD3prevents apoptosis. MRL/+ B cells are protected from B cell receptor mediated apoptosis. Enzymatic de-acetylation of 9-*O*-acetylated sialic acid results in MRL/+ B cells becoming susceptible to anti-IgM mediated apoptosis. These data suggest that the enhanced 9-*O*-acetylation of sialic acid on MRL/+ B cells occurs on gangliosides and may contribute to a break in tolerance by abrogating BCR mediated apoptosis. We have separately shown, using a CasD1 knockout mouse, that the 9-O-acetylation of sialic acid seen on B cells is dependent on CASD1. Overall these data argue that the enhanced 9-O-aectylation of sialic acid on B cells in autoimmune prone mice and in autoimmune individuals is of pathogenic significance and that a potential new therapy could involve the inhibition of CasD1 enzymatic activity.