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**Abstract Title:** O-glycan sialylation regulates bone marrow thrombopoiesis through interferon-secreting plasmacytoid dendritic cells

Circulating platelet count, an indicator of health and disease, has been linked with platelet glycosylation. Loss of sialic acid during platelet circulation leads to their recognition by the hepatic receptors/lectins, specifically the Ashwell-Morell receptor and the macrophage αMβ2 integrin, prompting their clearance. The role of lectins and glycans in regulation of bone marrow (BM) thrombopoiesis is understudied.
We investigated the role for BM plasmacytoid dendritic cells, major producers of Type 1 interferon (IFN-I), in regulating thrombopoiesis of aberrantly sialylated megakaryocytes (MKs). The TF-antigen is a cryptic disaccharide on O-glycans usually covered by a sialic acid moiety added by the sialyltransferase ST3Gal1. To investigate the role of the TF-antigen in thrombopoiesis, we generated ST3Gal1MK-KO mice (PF4-Cre) that display increased TF-antigen specifically in MKs.
ST3Gal1MK-KO mice developed significant thrombocytopenia, but had normal platelet half-life, suggesting that the TF-antigen affected BM thrombopoiesis. In vitro MK maturation and proplatelet production from primary ST3Gal1MK-KO mouse BM cells were normal, pointing to extrinsic factors in the BM environment affecting thrombopoiesis. Immunofluorescence stain of the ST3Gal1MK-KO BM revealed proplatelet structures positive for GPIbα+ and the TF-antigen, being infiltrated by mononuclear cells resembling lymphocytes.
Platelet counts of ST3Gal1MK-KO mice were restored to wild-type levels by crossing ST3Gal1MK-KO mice with Jak3KO mice that have impaired of lymphoid cell development. BM immunostaining showed infiltration of ST3Gal1MK-KO MKs by immune cells, marked by CD4+. Bulk RNAseq of CD4+ cells in ST3Gal1MK-KO BM confirmed a population bias for interferon-releasing plasmacytoid dendritic cells, a cell type regulated by unique sialic acid binding lectins (Siglecs). Inhibition of IFN-I activity, by a blocking receptor antibody, recovered platelet counts in ST3Gal1MK-KO mice to wild-type levels.
Together, the data shows that recognition of MK O-glycan sialylation by plasmacytoid dendritic cells, likely through inhibitory Siglecs G and H, regulates thrombopoiesis through IFN-I secretion.