HYPOTHESIS AND SPECIFIC AIMS:

The transcription factor FOXP3 is critical to the regulation of numerous debilitating human immune-mediated diseases, the prevalence of which together affect over 8.5 million people (1 in 31 U.S. residents). In Inflammatory Bowel Disease (IBD) chronic intestinal inflammation indicates aberrant in vivo FOXP3+ T regulatory (Treg) cell function (1). Similarly, proinflammatory signals in vitro impair Treg function (2). Our lab was the first to characterize the essential role for the histone methyltransferase (HMT) EZH2 in the epigenetic regulation of FOXP3 (3). Recent published work extended our observations indicating a key role for EZH2 in FOXP3 repressor function (4); however the regulation and biological impact of the FOXP3-EZH2 pathway to IBD is unknown. This knowledge is important given the apparent loss of function of Treg cells in inflammation.

Our long-term goal is to dissect epigenetic mechanisms regulating Treg cellular differentiation and function, particularly within the setting of GI inflammatory diseases; as these discoveries will facilitate design of human cell therapy trials for IBD. Consequently, the objective of this grant is to characterize the role for the epigenetic regulator EZH2 in Treg suppressive function. These investigations are strongly supported by preliminary data demonstrating that: 1) EZH2 is required for Treg suppressive function; 2) IL6 signaling leads to phosphorylation and inhibition of EZH2; 3) lymphocytes isolated from the intestine of IBD patients demonstrate activation of IL6-induced gene networks and loss of EZH2 HMT function; and 4) conditional knockout of EZH2 in FOXP3+ T cells leads to in vivo immune dysfunction. Based upon these compelling data we propose the CENTRAL HYPOTHESIS that EZH2 plays a critical role in the homeostasis of Treg cells, and the disruption of EZH2 function by inflammatory signaling pathways contributes to IBD. Our rationale is that identification of the mechanism(s) to restore Treg suppressive function in the setting of intestinal inflammation will offer new therapeutic opportunities within the field of IBD. Our specific aims will test the following hypotheses:

**Aim 1:** Repression of immunoregulatory gene networks by FOXP3 requires the formation of a complex between this transcription factor and EZH2.

**Aim 2:** Inflammatory stimuli, such as IL6 lead to EZH2 phosphorylation and thereby disrupt the enzymatic activity of this epigenomic regulator.

**Aim 3:** Inhibition of the IL6 to EZH2 signaling pathway permits sustained Treg suppressive function in the setting of intestinal inflammation.

Upon conclusion, we will understand the role for EZH2 in Treg loss of function in the setting of active inflammation. This discovery will stimulate new areas for experimental therapeutics in human chronic inflammatory diseases. Our environment in the Epigenetic and Chromatin Dynamics Laboratory combined with the Department of Immunology at the Mayo Clinic makes us uniquely qualified to pursue this objective given the extensive collective experience of histone methyltransferase biology, proinflammatory signaling networks, and FOXP3 gene regulation.