



## 2010 Research Grant Program Winning Abstract

### B Cell Trafficking in Mucosal Immunology and Autoimmune Disease

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Manipulation of lymphocyte trafficking has been shown to safely alleviate symptoms of autoimmunity without severely immune-compromising patients. Unfortunately, no means are currently available to stably control lymphocyte circulation. Before the rational design of therapeutic drugs can occur, significant research focused on homing receptor regulation in lymphocyte lineages must occur. To this end, we have recently discovered the first transcription factor that regulates B lymphocyte trafficking. Gene-targeted mice with excised Kruppel-like factor 2 (Klf2) have aberrant B cell migration patterns due to altered expression of homing receptors. Surprisingly, these divergent homing patterns result in a gain-of-function phenotype in gene-targeted animals, including enhanced ability to clear blood-borne pathogens. These counterintuitive results raise the following question: why is the B cell compartment purposely restrained from responding to dangerous pathogens in wild-type animals? We hypothesize that restriction of B cell trafficking by Klf2 limits autoimmunity while ensuring immunologic tolerance. Therefore, we propose a series of innovative experiments to directly test this hypothesis. Results from this study will provide crucial information concerning the regulation of homing receptors and lymphocyte trafficking in human health and disease.

**Aim 1:** In the United States, more than 23 million people are affected by diabetes. Type 1 diabetes (T1D) is an autoimmune disease that results in the destruction of insulin-producing beta-cells in the pancreas. Since T and B cells are causal for T1D, most therapies revolve around the depletion of these lymphocyte populations. Unfortunately, this approach leaves patients susceptible to opportunistic infections due to the resulting immunosuppression. However, exciting new data suggests that manipulation of lymphocyte trafficking may be sufficient to alleviate symptoms of autoimmunity without globally suppressing the immune system. In this regard, we have recently discovered that Klf2 controls B cell migration and limits humoral immunity. Aberrantly trafficking B cells in Klf2 gene-targeted mice are exposed to self-antigens, which we speculate promotes autoimmunity. To test this hypothesis, we are examining T1D disease progression in wild-type and Klf2 gene-targeted animals that have been crossed onto a disease-susceptible background. Disease progression will be monitored by analysis of blood glucose levels and insulin-specific IgG production. Insulinitis will be examined by immunofluorescence microscopy following staining with antibodies directed against B220 and CD3 (BD Biosciences). Additional experiments will necessitate eight-color flow cytometric analysis of B lymphocytes using a panel of fluorochrome-conjugated BD Biosciences antibodies (IgM, IgD, B220, CD5, CD19, CD21, CD23, CD93/AA4.1). Data will be collected on a BD™ LSR II flow cytometer. Results from these studies will reveal the pathological significance of Klf2-dependent homing in B cell-mediated T1D. Should we find that aberrant B cell migration directly contributes to autoimmune disorders, this paradigm-shifting result will spearhead development of new drugs that combat autoimmunity at the level of lymphocyte trafficking.

**Aim 2:** Approximately three-quarters of all lymphocytes in a healthy individual are



contained within the mucosal immune system. This multifaceted complex provides protection from invading pathogens that cross thin-layered epithelial cell surfaces such as those found in the small intestine. In addition, the mucosal immune system associated with the gut is charged with the task of preventing unwarranted host responses against commensal bacteria. Cells comprising the gut-associated lymphoid tissue (GALT) have developed sophisticated strategies to execute these divergent functions, including targeted deployment of B1 B cells to the peritoneal cavity. B1 cells maintain symbiotic microbial homeostasis in the small intestine while simultaneously contributing to humoral immunity, including early responses against invading pathogens within the GALT. We hypothesize that B1 cell functions and their unique trafficking patterns are inextricably linked. In this regard, we have recently discovered that Klf2 is required for the accumulation of B1 cells in the peritoneal cavity. Therefore, our Klf2 gene-targeted animals provide an exciting platform to investigate how defects in B1 cell trafficking impact antibody production, pathogen clearance, and protection from GALT-associated disease. In this aim, expression of homing receptors will be examined by flow cytometric analysis and real-time PCR following *ex vivo* excision of Klf2 in peritoneal B1 cells sorted by fluorescent activated cell sorting (BD FACST<sup>™</sup>). Additionally, adoptive transfer of fluorescently labeled Klf2-deficient B1 cells or B1-specific B cell precursors will reveal lymphocyte migration patterns *in vivo*. To accomplish these goals, we will again rely upon eight-color flow cytometric analysis using BD reagents. These experiments will reveal how Klf2 controls B cell trafficking and the physiologic significance of this process in mucosal immunity. We emphasize that gut-associated mucosal surfaces are highly vulnerable to microbial assault, reinforcing the need to understand how immune functions carried out by B1 B cells are coordinated within this specialized environment.

The studies proposed here will reveal how B cell migration directed by Klf2 contributes to mucosal immunology and autoimmunity. Should we find that migratory defects in the B cell compartment promote autoimmunity, these results will significantly shift both basic research and clinical practice paradigms in the treatment of autoimmune disease.

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