**Specific Aims**

Marginal Zone (MZ) B cell deficiency, characterized by a decreased number or impaired function of this subtype of splenic B lymphocytes, is clinically linked to reduced IgM titers and heightened susceptibility to sepsis and mortality associated with encapsulated bacterial infections. Impairment in MZ B cell function and localization also correlates with autoimmune pathologies. Currently, there are few therapeutics to treat MZ B cell deficiency besides managing symptoms. Despite the importance of MZ B cells, many aspects of their ontogeny, homeostasis, and clonal regulation remain obscure. MZ B cell deficiency is thought to result from defects in these processes, caused by genetic mutations, autoimmune diseases, viral infections, or even certain medications. Thus, understanding MZ B cell homeostasis is critical for identifying new treatments. The **central aim** of this proposal is to quantitatively map the developmental trajectories of marginal zone B cells and to dissect the mechanisms that maintain their numbers and clonal diversity, throughout life.

Strategically positioned as the gatekeepers between the circulation and the immune system, splenic MZ B cells form a frontline of defense against blood-borne pathogens. MZ B cells mediate early protective responses against diverse T-independent and T-dependent antigens, by employing strategies that blur the boundary between innate and adaptive immunity. The establishment and maintenance of MZ B cells in their splenic niche are determined by a complex set of rules that regulate cell division, the influx of new bone marrow (BM) derived cells, death, and onward differentiation. Mathematical models, when tightly coupled with experiments, are a natural tool for resolving the dynamics of such complex systems. Here we propose to combine custom-built mathematical models and experimental strategies to understand the rules controlling MZ B cell ontogeny and maintenance across the lifespan.

**Aim 1: Quantify the steady-state dynamics of MZ B cells – modeling the kinetics of their renewal and replacement throughout life.** The mechanisms regulating MZ B cell numbers are unknown, and their lineage relationships to other B cell subsets are unclear. In this Aim, we will combine mathematical models and an experimental fate-mapping system to quantify MZ B cell dynamics in healthy mice. We will use a BM chimera system to reveal the developmental progression and kinetics of infiltration of new (donor-derived) cells into the intact peripheral B cell compartments of congenic recipient mice, while measuring self-renewal using Ki67 expression. Using a Bayesian inference framework, we will then fit an array of models to these data to identify the immediate precursor(s) of MZ B cell and to quantify their division and turnover in detail. We will also identify any quorum sensing (density-dependent division or loss), kinetic heterogeneity, or variation in their dynamics with either host or cell age. The latter will reveal the rules of replacement within the MZ B cell compartment.

**Aim 2: Dissect the mechanisms underlying the establishment of the MZ B cell niche in neonates.** The processes that establish and regulate lymphocyte populations change with age. Specifically, we recently found evidence for distinct dynamics of FO B cells in neonates and adults and hypothesize that similar mechanisms act on the MZ B cell pool. We will test this hypothesis using a novel reporter mouse strain that allows us to track changes in pool size, the extent of proliferation, and the frequency of recent BM emigrants among MZ B cells. We will extend the models employed in Aim 1 to identify the rules governing the establishment of the MZ B cell niche in early life and formulate them as PDE systems to quantify variation in cells’ ability to persist as a function of their residence time.

**Aim 3: Define and model MZ B cell fate-determination during immune responses.** Immune activation triggers dramatic changes in the lymphatic environment. How and to what extent activation-related cross-talk modulates cell-fate decisions and the response kinetics of mature B cells, is poorly understood. Based on...(?), we hypothesize that the interplay of B cell receptor and Notch2 mediated signals skews B cell differentiation toward the generation of antigen-specific MZ B cells upon immune activation. We will use a dynamical modeling strategy to map B cell differentiation pathways during immune responses, using novel mouse strains expressing an antigen-inducible reporter gene and B cell-specific mutations in Notch2. We will extend our analysis to infer phylogenetic trees of antigen-specific clones as they diversify during a response, by building a computational pipeline to study single-cell immune repertoire and transcriptomic profiles of activated B cells.

The results of this study could lead to the discovery of therapeutic targets for the restoration of MZ B cell numbers in splenectomized patients, infants, and the elderly, who are vulnerable to blood-borne pathogens. This study may also support the development of interventions against MZ B cell linked autoimmune pathologies and highlight the B cell stages that are most permissive to malignant transformations.