Background: Dengue virus, an RNA virus of the genus flavivirus, is the world's most common arbovirus. Infection with dengue virus can cause a vascular leakage syndrome associated with up to 20% mortality in untreated populations. Even uncomplicated disease is associated with significant morbidity in endemic areas. Epidemiologic studies have implicated serial exposure to different, antigenically distinct serotypes (serotypes 1-4) as a major risk factor for development of vascular leakage, thus suggesting an immunologic component in the pathogenesis of vascular leakage. No animal model exists that convincingly replicates this phenomenon. As such, studies with human cells are critical for insights into disease pathogenesis.

In endemic countries, the burden of disease is primarily in pediatric populations and therefore the volume of blood available is small (typically 3-4ccs per blood draw). For investigations into T-cell responses, the lack of clinical specimens can be quite limiting. Most T-cell assays are functional in nature, typically looking at the downstream signals (e.g., cytokine production or cell lysis) of T-cell receptor binding to cognate antigen; these assay require high volumes of cells (between 1E5-1E6 cells). For reference, 1cc of whole blood generally contains 1E6 peripheral blood mononuclear cells (PBMC) of which between 40-60% are T-cells. Therefore, we sought to non-specifically expand T-cells derived from clinical PBMC ex-vivo with the goal of a developing a high throughput method for screening T-cell responses to specific dengue viral antigens.

One concern with ex-vivo expansion of T-cells is the introduction of bias into the expanded population. We intend to test the cells using a variety of both molecular, phenotypic, and functional tools to demonstrate that the cells are not perturbed by our technique.

Methods: PBMCs from normal donors were isolated using a Ficoll gradient. Commercially available 50nm beads (Miltenyi) coated with anti-biotin were incubated with biotinylated anti-CD2,anti-CD3, and anti-CD28 monoclonal antibodies. The beads were then admixed with PBMCs at a ratio of 2:1. Cells were maintained in RPMI medium plus 10% FBS supplemented with recombinant IL-2 at a concentration of 100IU/mL. Cells were counted on alternate days using a 1% trypan blue dye exclusion method. On days 7, 14, and 21, aliquots of cells were taken for RNA extraction. Flow cytometry was performed on Day 14 and day 45.

Results: We were able to expand T-cells in vitro by three orders of magnitude. There was an inversion in the normal CD4:CD8 ratio, although the functional significance of this inversion is unknown. Cells continued to expand in culture for up to 45 days, although they did require restimulation with beads after roughly 14 days.

Conclusions: Ex-vivo expansion using commercially available beads is a viable method for expanding PBMCs. The functional characterization of this non-specifically expanded T-cell population is ongoing. We intend to ensure that the expanded T-cell population is not biased via several methods: 1.) response to known widely distributed antigens 2.) in depth analysis of the T-cell receptor (TCR) repertoire via TCR spectratyping, and 3.) phenotypic characterization using flow cytometry.