

## Sample P&F research proposal

### Project #1 Proposal

TITLE: T-cell specific activation of  $\beta$ -catenin induces IL-17 mediated inflammatory bowel disease and dysplasia.

INVESTIGATOR: **Fotini Gounari, Ph.D.** (Immunology)

TH17 biased T-cells have been implicated in the induction of inflammatory diseases such as ulcerative colitis and Crohn's disease. Although the precise events for commitment to the TH17 lineage have not been clarified, both TCR and cytokine signaling (IL6 and TGF- $\beta$ ) are needed. Surprisingly Fox-P3 positive cells are more prone than naïve CD4 T cells to TH17 conversion, during which expression of Foxp3 is characteristically shut down in exchange for upregulation of ROR $\gamma$ T the transcriptional hallmark of TH17. It is not clear how the TGF- $\beta$  stimulation of Smad3 and Smad4 and the IL6 stimulation of the Jak/STAT signaling pathways synergize with TCR signaling to commit T-cells to TH17. Furthermore it is not known if commitment to TH17 is exclusively a peripheral event or is thymically selected. Since intraepithelial lymphocytes obtain their mucosal homing properties in the course of thymic selection, it is reasonable to expect that at least a subpopulation of TH17 committed T cells are thymically committed to this lineage. The wnt/ $\beta$ -catenin pathway is activated upon TCR signaling and modulates the outcome of thymic selection. Moreover T-cell specific activation of  $\beta$ -catenin predisposes mice to inflammatory bowel disease (IBD) and dysplasia. The gut microenvironment of these mice has elevated levels of IL-17, IL-6 and TNF- $\alpha$  and, CD4 T-cells show increased propensity to produce IL-17. On the basis of these findings we postulate that chronic stabilization of  $\beta$ -catenin causes activation of T-cells and production of IL-17, leading to inflammation and colon cancer through its impact on TCR signaling and/or cytokine signaling pathways. We propose to define the contribution of thymic versus peripheral T-cell specific stabilization of  $\beta$ -catenin to the observed pathology. Furthermore, we propose that  $\beta$ -catenin signaling is essential for TH17 commitment. We will test this by monitoring  $\beta$ -catenin signaling in lymphocytes that are committed in vitro or in vivo to TH17 lineage, and by interrupting  $\beta$ -catenin signaling in "to be" TH17 lymphocytes.

### **Project # 1 Progress Report**

TITLE: T-cell specific activation of  $\beta$ -catenin induces IL-17 mediated inflammatory bowel disease and dysplasia.

INVESTIGATOR(S): Fotini Gounari Ph.D.,

FUNDING PERIOD: 12/1/09-11/30/10

ELIGIBILITY: New Investigator

**PROGRESS REPORT:** We were supported from a pilot and feasibility grant from the

DDRCC to determine the role of  $\beta$ -catenin/Tcf-1 signaling in IL17 mediated Inflammatory Bowel Disease (IBD) and dysplasia. In the course of the studies we obtained evidence that  $\beta$ -catenin/Tcf-1 signaling regulate the balance between proinflammatory Th17 and anti-inflammatory Treg cells. The balance between Treg and Th17 cells is important for the pathogenesis of IBD and IL17 expressing Tregs have been recently reported to expand in Crohn's disease patients. This is consistent with suggestions that Tregs are endowed with plasticity and can undergo lineage reprogramming in response to environmental queues. The *in vivo* scarcity of unstable Tregs and lack of suitable animal models for investigating the consequences of their reprogramming complicate the study of Treg plasticity. Our novel finding is that Treg subsets with inherent plasticity expand in a mouse model of intestinal inflammation and polyposis in which  $\beta$ -catenin is stabilized in T-cells. This leads to expansion of Treg subsets that exhibit intermediate levels of Foxp3 (Foxp3<sup>int</sup>) and are able to express IL17. These mice are predisposed to Th17 intestinal inflammation that has unique histological similarities to Crohn's colitis and eventually to polyposis. The relevance of this pathway is underscored by the finding that Tcf-1 binds to the promoter of ROR  $\gamma$ 1 and regulates its expression depending on availability of  $\beta$ -catenin. This model enables investigation of the role of  $\beta$ -catenin/Tcf-1 in the expansion of a naturally occurring pro-inflammatory population of Tregs that is under-represented in *wt* mice, and of the resulting pathological consequences. We are in the process of preparing these findings for publication.

GRANTS: The pilot funding enabled us to obtain preliminary data to support two grant applications to the NIH and to CCFA with the objective of determining the molecular mechanisms that control the balance between Treg and Th17 lineage differentiation and how their deregulation generates pathogenic T-cells and induces inflammation and colorectal cancer.

Core Facilities Used: Host-Microbe Core

HONORS/AWARDS: none

ABSTRACTS: none

PUBLICATIONS: none

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