THE MORAN FOUNDATION
Progress Report August 2004

TITLE: The Role of Tolerogenic Dendritic Cells within the Intestinal Mucosal Immune System in Induction of Inflammatory Bowel Disease

INVESTIGATOR/DEPARTMENT:

LuAnn Thompson-Snipes, Ph.D.
Department of Pathology
Baylor College of Medicine
Texas Children's Hospital, Rm. AB190.11

LuAnn Thompson-Snipes, Ph.D.

Moran Update August 2004

The project entitled "The Role of Tolerogenic Dendritic Cells within the Intestinal Mucosal Immune System in Induction of Inflammatory Bowel Disease" is still ongoing in our laboratory. We are preparing for an NIH RO1 application within the next few months, and an application to the Crohns and Colitis foundation in early 2005. The $8500 received from the Moran foundation in 2003-2004 was used to purchase reagents and supplies needed to conduct immunofluorescent staining, phagocytosis assays, chemotaxis assays, and cytokine assays of dendritic cell populations and immunofluorescent and cytokine assays of tissues obtained from the $G\alpha i2^{-/-}$ mice and compare with age/sex matched wild-type controls. Our preliminary results from the analysis of DCs in the small intestine of $G\alpha i2^{-/-}$ mice were published as an abstract at the 12th International Congress of Immunology and 4th Conference of FOCIS in July 2004 (see attached).

As described in Aim 1 of our 2003 Moran application, we have quantitatively and qualitatively compared subpopulations of dendritic cells in the gut mucosa of the wild-type mice compared with $G\alpha i2^{-/-}$ mice by immunofluorescence. These studies revealed major disruption in the architecture of the dendritic cells in the gut-associated lymphoid tissue (GALT) of $G\alpha i2^{-/-}$ mice. Architectural features of DCs in the $G\alpha i2^{-/-}$ mice include their apparent random distribution in lymphoid structures and increased numbers of DCs in the intestinal villi. These features can be seen in Figure 1, which compares the distribution of $CD11c^{+}$and $CD11b^{+/-}$ DCs in the GALT (including Peyers Patches; PPs) in $G\alpha i2^{-/-}$ mice and wild-type mice. One possible explanation for the abnormal distribution of the DCs in the $G\alpha i2^{-/-}$ mice that they have an abnormal response to chemokines that are required for DC homing in the GALT. Also, it is important to determine if the DCs from the $G\alpha i2^{-/-}$ mice capable of presenting antigens and activating T-cells appropriately.

We are beginning to use 3-color confocal technology to further look at the upregulation of co-stimulatory molecules, which are required to stimulate T cell proliferation during antigen presentation, on DCs from the GALT of $G\alpha i2^{-/-}$ mice and wild-type mice. The in situ studies will reinforce our findings obtained by FACS analysis of mucosal DCs from $G\alpha i2^{-/-}$ mice showing increased expression of these co-stimulatory molecules as compared to DCs from wild-type mice. This data will be ready for publication as soon as we obtain confocal images to verify the dual fluorescence in these DC populations. An example of preliminary confocal imaging of DCs using our current procedures for labeling cells is shown in Figure 2.

Our current application for Moran funding includes a proposal to look at antigen uptake (phagocytosis) and chemotaxis by DCs in the $G\alpha i2^{-/-}$ mouse. To assess the functional attributes of DCs, we used primary cultures of bone marrow derived DCs prepared by culturing bone marrow suspension cultures for 6-8 days with murine GM-CSF. With each bone marrow culture, we assessed DC function in 2 different assays. In our preliminary studies, using classical phagocytosis assays, we have determined that the $G\alpha i2^{-/-}$ DCs are not deficient in antigen uptake (data not shown). However, *in vitro* they do not respond normally to the chemokine MIP-3β (Figure 3), which may explain why we have observed a more random distribution of CD11c+ cells within the PP of the $G\alpha i2^{-/-}$ mice (Figure 1).

The results obtained from these experiments will further support the importance of mucosal DC function in the IBD mouse model and will help strengthen our NIH grant proposal.

Moran funding from 2002 and 2003 has helped support the development of pure strains of the $G\alpha i2^{-/-}$ mice on different genetic backgrounds (129, B6, Balb/c). Within the past 6 months we have been able to obtain enough $G\alpha i2^{-/-}$ mice from all three strains to begin to analyze cytokine expression in the gut mucosa. The 129 and Balb/c mice both are susceptible to the IBD when they are deficient in Gαi2 while the C57/BL6 strain is resistant. We have confirmed this in our lines of mice we have breeding at the TMF facility at Baylor.

The Moran funds from 2003 have allowed us to purify DC subsets by FACs sorting prior to RNA extraction for cDNA microarray studies. We have performed preliminary fluorescent sorting of bone marrow-derived DC cultures from each strain of mice. Further progress will require longer sort times to achieve enough cells for the isolation of RNA for microarray studies. So far, these experiments appear to be feasible once our mouse colonies have been expanded to give us at least 4-6 age/sex matched mice. However, we may have to pool samples of cells and RNA from different groups of sex/age matched animals obtained at different times in order to obtain enough RNA.

Fig. 1

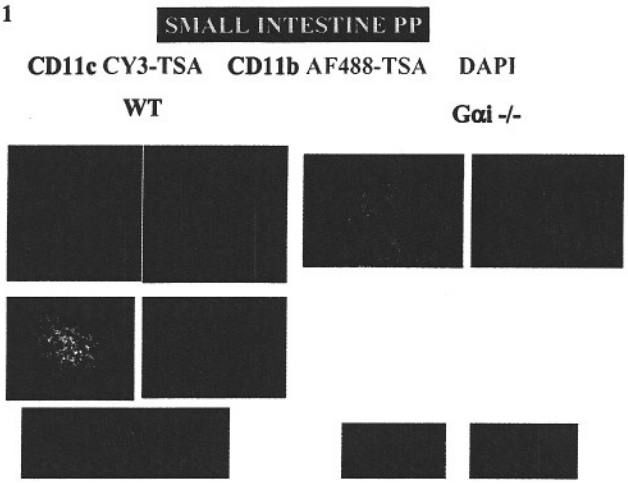

Figure 1. Small intestines from wild-type (wt) and Gai2-/- mice were frozen in OCT and 8 micron sections fixed in acetone and stained with antibodies to CD11c (DC specific) and CD11b (macrophages and some DCs). The antibodies were detected with Cy3 and AF488 respectively. Note the organized DCs in the subepithelial dome of the Peyer's Patch in the wt mice and the disarray of DCs in the $G\alpha i2^{-/-}$ mice.

Fig. 2

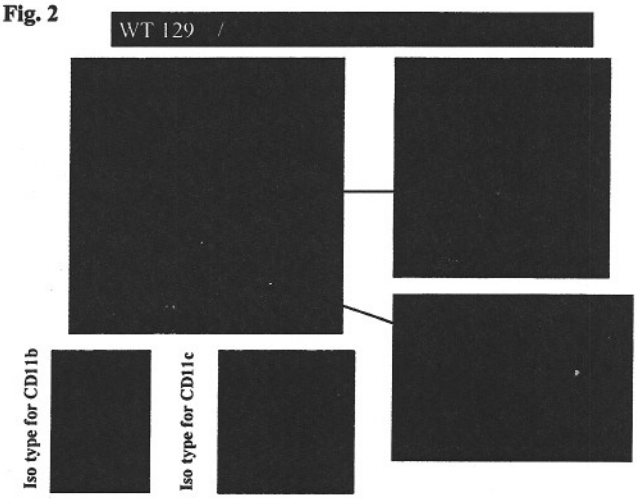

Figure 2. Confocal imaging of DCs in the intestinal villi of a wild-type mouse. Using the same antibodies as in Figure 1 we can detect a few double positive staining cells as shown in the upper right panel with distinctive dendrite features. A single double positive cell is found in the villi shown in the lower right panel.

Fig. 3 **$G\alpha i_2$ KO BMDCs are uncoupled from CCR7**

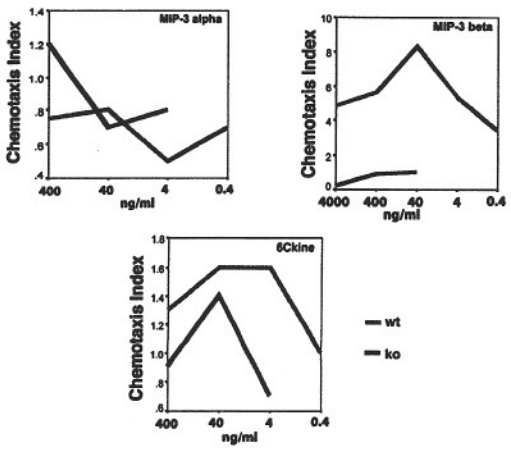

Figure 3. Chemotaxis assays of BMDC cultures. Comparing bone marrow derived dendritic cells from wt vs. Gai2 KO mice we have found a defect in the ability of the KO cells to respond to MIP-3β in a standard chemotaxis assay. Migration to MIP-3α and 6Ckine appear to be close to normal.