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

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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 a second submission of an NIH R21 application in November, and an application to the Broad foundation within the next few weeks.

The $9833 received from the Moran foundation in 2004-2005 was used to purchase reagents and supplies needed to conduct a comparison of dendritic cell subsets between the Gαi2 deficient 129SvEv strain of mice that are susceptible to IBD and the Gai2 deficient C57/BL6 strain of mice that are resistant to IBD. The funds were spent to purchase reagents for Luminex cytokine assays, antibodies for cell staining and FACS analysis, tissue culture reagents, magnetic beads to enrich for dendritic cells and mice for *in vivo* experiments. With the help of a post-doctoral associate, Ryan Pena, these experiments led to the discovery of a significant difference in inflammatory cytokine production by a specific subset of dendritic cells derived from the bone marrow of mice. It is of great interest that dendritic cells from B6 strain of mice can produce the anti-inflammatory cytokine IL-10 even when the Gαi2 gene is missing (see Figure 1).

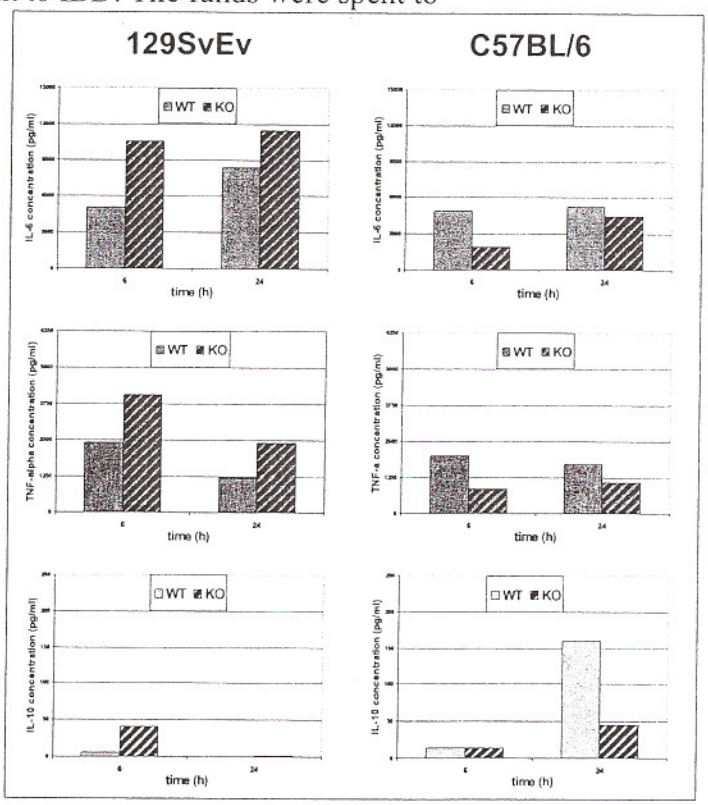


FIG.1 Cytokine production by sort purified DCS following

The preliminary data from these experiments was presented at a Keystone Symposia and published as an abstract in Gastroenterology (see attached copy of published abstract). We hypothesize that the difference in anti-inflammatory signaling may compensate for downstream signaling events that are defective in the Gαi2 deficient animal. Preliminary studies indicate that one of the defective signaling pathways in the Gαi2 mouse may involve the regulation of the MAP kinase p38 (Figure 2). After stimulation with CpG, the wild-type dendritic cells show a transient activation of p38 MAP kinase while the Gαi2 deficient dendritic cells show a continual activation of p38. This data is currently being prepared for publication.

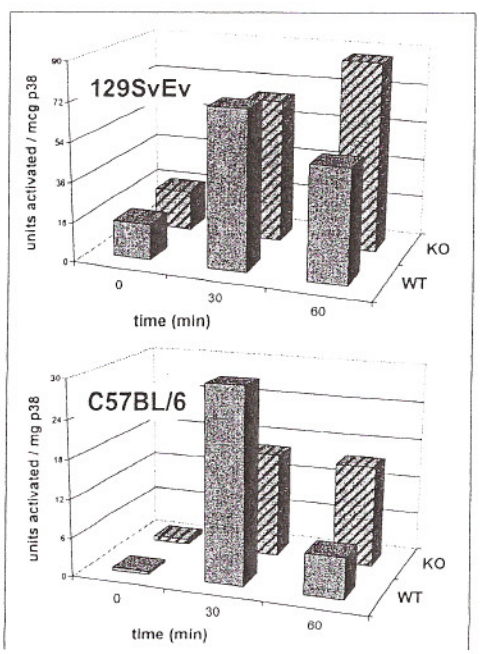


FIG. 2. Activation of p38 MAP kinase in wild type and Gαi2 deficient dendritic cells after stimulation of toll receptor 9 with CpG.

As described in Aim 1 of our 2004 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 using both immunohistochemistry and 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. To quantitate the numbers of dendritic cells in the compartments we are developing techniques to isolate viable dendritic cells from the mouse intestine. Also, it is important to determine if the DCs from the $G\alpha i2^{-/-}$ mice capable of presenting antigens and activating T-cells appropriately. To complete this study for a publication we are preparing to do a set of *in vivo* experiments proposed in our next application to the Moran Foundation.

In our 2004 Moran application we proposed to use fluorescent bio-particles injected into the gut lumen, and follow the uptake and transport of particles by DCs in the PPs of $G\alpha i2^{-/-}$ or wild-type mice during activation. The bioparticles proved to be very difficult to assess *in vivo* since there is a lot of fluorescent background in the normal mouse intestine. We then focused our efforts on a complete analysis on dendritic cell localization and T and B cells in the mouse intestinal and colon tissue during the onset of IBD in the Gai2 deficient mice. We have found a significant increase in follicles known as isolated lymphoid follicles (ILF) within the colon of the Gαi2 deficient mice. These enlarged follicles appear before the disease progresses beyond stage 1 IBD. The localization of the dendritic cells within the follicles in Gαi2 deficient mice appears less organized and randomly distributed compared to the dome like localization of dendritic cells in the small follicles of normal mice and in the Peyer's patches of a normal mouse (Figure 3 and 4, Appendix 1). In the future we will perfect a technique of assaying for cytokine production *in situ* within the follicles to determine if the inflammatory cytokines and chemokines are being made early in these 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. Using funds from our Moran account we have been able to work out a method to isolate over 10^7 purified dendritic cells from bone marrow cultures to use in chemotaxis studies. In our current application to Moran foundation we will propose to look at a multiple array of chemokines to determine which chemokine receptor is actually linked with Gαi2. If we can determine which chemokine receptor specifically is involved and which signaling pathway through toll receptors is disrupted we may gain significant insight into possible ways to treat the Gαi2 mouse and cure their IBD. This treatment may then be applied to some forms of IBD in humans.

Moran funding from 2002 and 2003 helped support the development of pure strains of the $G\alpha i2^{-/-}$ mice on different genetic backgrounds (129, B6, Balb/c). During this period we developed techniques in the lab for analyzing dendritic cell function and in situ localization. The funding from 2004-2005 continued to support the maintainence of our

animal colony as well as to purchase reagents to use in enriching dendritic cell from bone marrow cultures and mucosal tissues.

The Moran funds have allowed us to purify DC subsets by FACs sorting prior to RNA extraction for cDNA microarray studies needed to support our NIH grant application. We have performed fluorescent sorting of bone marrow-derived DC cultures from each strain of mice using a a Dakocytomation Moflo fluorescent cell sorter available to us at the Texas Children's Hospital Oncology group. So far, these experiments appear to be feasible now that 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.

The funding from the Moran Foundation has been instrumental in helping the lab get this project started and prepare for some publications that will hopefully lead to funding from the NIH. At least two new publications will be submitted within the next few months that used funds from the Moran Foundation. Thanks to the Moran Foundation we now have many of the tools in place that we need to help us understand these disease process and develop a research program worthy of NIH funding.

APPENDIX 1

COMPARISON OF CD11C+ (RED) DENDRITIC CELLS IN THE MOUSE INTESTINE

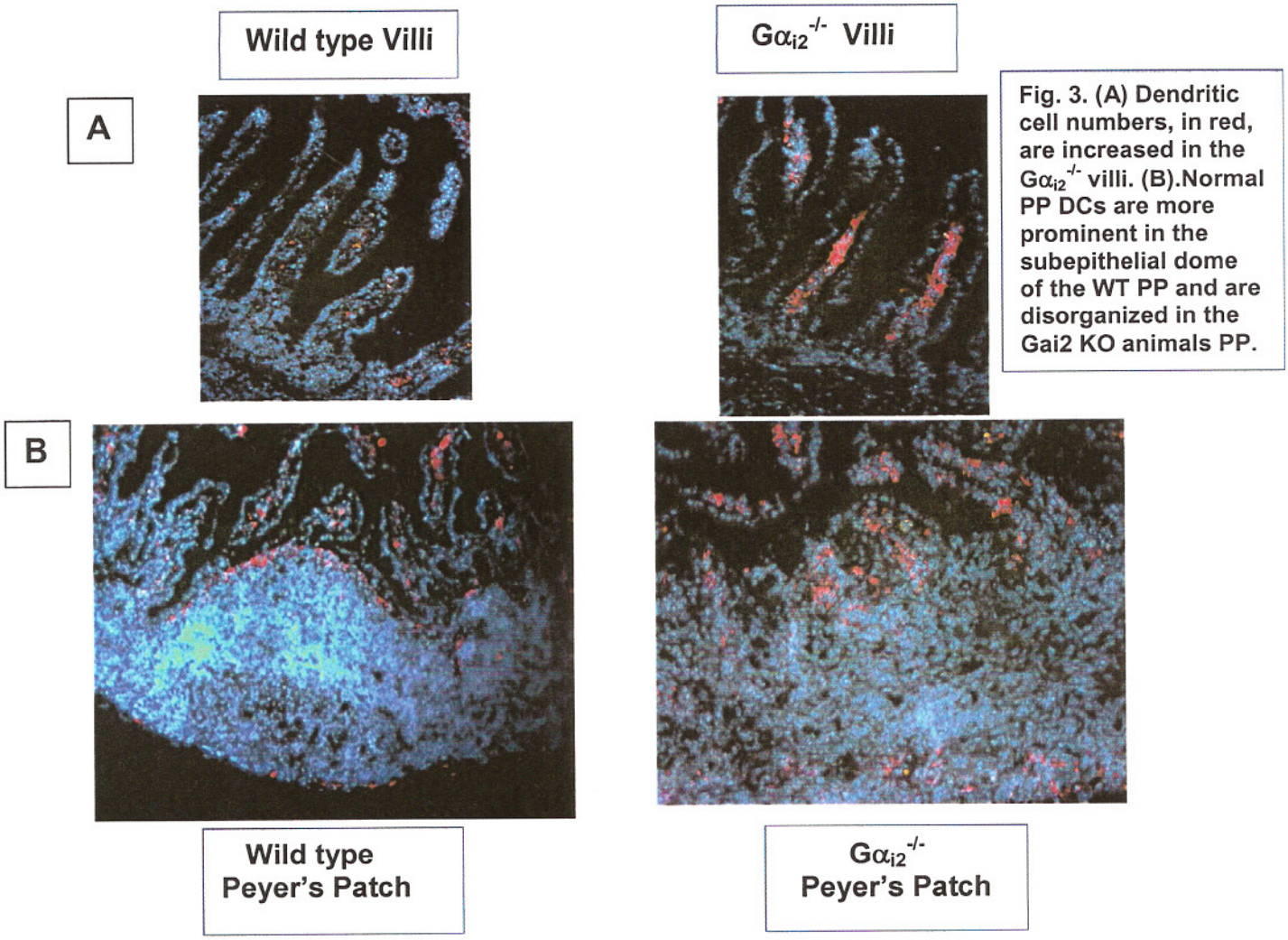
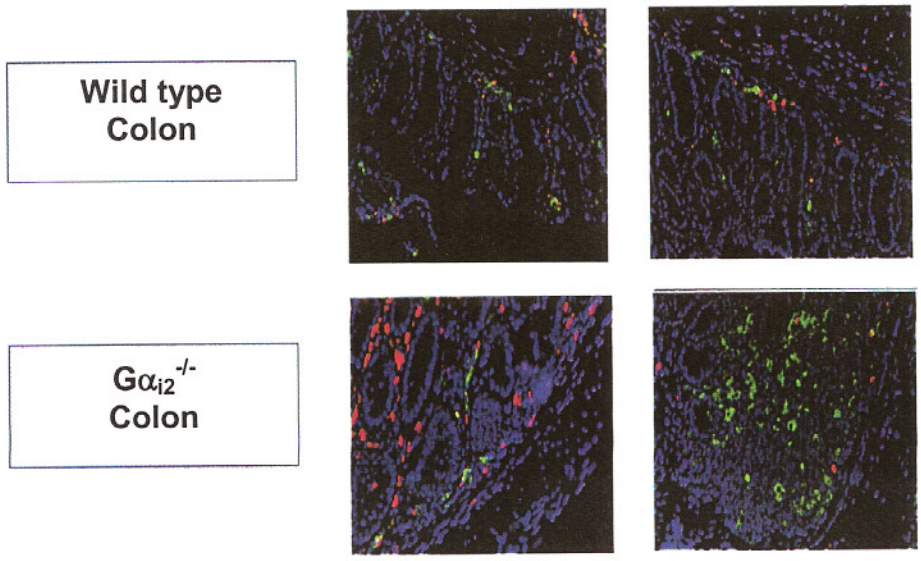


Fig. 3. (A) Dendritic cell numbers, in red, are increased in the $G\alpha_{i2}^{-/-}$ villi. (B).Normal PP DCs are more prominent in the subepithelial dome of the WT PP and are disorganized in the Gai2 KO animals PP.

Fig. 4. DENDRICTIC CELLS CD11C+ (GREEN) AND CD11B+ MACROPAHGES (RED)



Abstract: Pena JA, Chen L, Coskun Z, Finegold MJ, Thompson-Snipes L. IL-12 and IL-10 Cytokine Imbalance in Dendritic Cell Sub-types are Associated with Varying Susceptibility in Two Inbred Mouse Strains in the Gαi2-deficient Model of IBD. Gastroenterology, 128 (4): A209-A209, Suppl. 2, 2005

The G_i alpha protein subunit type-2 ($G\alpha_i2$)-deficient mouse develops inflammatory bowel disease (IBD), with disease severity influenced by mouse strain. $G\alpha_i2^{-/-}$ mice on a 129SvEv background develop severe IBD, while mice on a C57BL/6 background are fairly resistant the disease. Since dendritic cells (DCs) are key inducers and regulators of innate immune function, we hypothesized that in $G\alpha_i2^{-/-}$ mice, DCs suffer functional defects relating to the appropriate recognition of lumenal bacteria which may play a role in the pathogenesis of inflammatory bowel disease (IBD) in this mouse model. We reared incipient congenic $G\alpha_i2^{-/-}$ mice by backcrossing 129xB6 $G\alpha_i2^{-/-}$ hybrids onto 129SvEv (129) or C57BL/6 (B6) to the 5^{th} generation (N5). Since the CdcS1 locus of B6 mice imparts resistance to IBD, we screened N5 mice for homozygosity of 129 or B6 CdcS1 alleles by microsatellite mapping. Bone marrow monoblasts were cultured in the presence of GM-CSF for 5-7 days. Bone marrow-derived DCs were then sorted according to CD11b and CD11c expression following flow cytometry. Three DC subtypes were isolated: CD11b-CD11c+, CD11b+^{LO} CD11c+ or CD11b+^{HI} CD11c+ as well as macrophages (CD11b+ CD11c-). DCs were stimulated with bacterial CpG-DNA or LPS and production of IL-6, -10, -12 (p40), -17, TNF-α, IP-10, KC, and IFN-γ was measured using flow-cytometric bead analyses (Luminex). IL-17 and IFN-γ were undetectable, while IL-6, IL-12 (p40), IP-10 and KC were all produced in DC culture supernatants. IL-12 is highly produced by stimulated CD11b+^{LO} CD11c+ and CD11b+^{HI} CD11c+ DCs from wild-type (WT) B6 mice compared to the same DC subsets from $G\alpha_i2^{-/-}$ B6 mice. However, these DCs from $G\alpha_i2^{-/-}$ and WT B6 mice also produced elevated levels of IL-10 in response to CpG-DNA. In contrast, IL-12 production is increased only in CD11b+^{HI} CD11c+ DCs from $G\alpha_i2^{-/-}$ 129 compared to WT 129 counterparts. Interestingly, a negligible amount of IL-10 is produced by double positive DCs from $G\alpha_i2^{-/-}$ and WT 129 mice. DC subsets have discrete immune responses and may contribute differentially to the development of IBD in $G\alpha_i2^{-/-}$ mice. Furthermore, in 129 mice, the imbalance in IL-12 and IL-10 expression may partially account for increased susceptibility to IBD, whereas increased IL-12 coupled with regulatory IL-10 production leads to observed resistance in B6 mice.

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