Progress report on:

# The Regulation of Mpz/P0 gene expression.

For the Moran Foundation

Submitted by:
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Major Goals for Research supported by the Moran Foundation in 2005-200. Our main goals were to (1) determine whether the Mpz/P0 gene contains identifiable regulatory elements that are responsive to elevated cAMP, Pax3, and Egr2 in growth-arrested primary Schwann cells. Our second major goal (2) was to determine whether the effects of elevated cAMP on the Mpz/P0 gene regulation in growth-arrested primary Schwann cells are secondary to Sox10 and/or Egr2 activation and or Pax3 inactivation.

Brief Background. A number of candidate transcription factors and signaling pathways may directly regulate the activity of the Mpz/P0 gene, which encodes the major structural myelin protein in the peripheral nervous system. A number of missense and frameshift mutations have been identified in the Mpz/P0 gene, which cause a variety of hereditary neuropathies including Charcot-Marie-Tooth disease and Dejerine-Sottas syndrome that are characterized by loss of myelin. In addition, loss of one Mpz/P0 allele or overexpression of Mpz/P0 also cause hereditary neuropathies in mice, suggesting that altered myelin gene dosage is a cause of CMT. This hypothesis is also supported by studies of the PMP22 protein, another structural myelin protein in the PNS that is a major cause of hereditary neuropathies in humans. Thus, we are interested in how Mpz/P0 and PMP22 are regulated as an explanation and possible treatment for hereditary neuropathies, and as a basis for understanding axon/glial interactions that are required for myelination. Interestingly, mutations in two transcription factors, SOX10 and EGR2 also cause demyelinating peripheral neuropathies, perhaps via abnormal regulation of the Mpz/P0 and PMP22 genes.

We proposed to work backward from the Mpz/P0 gene to identify important signaling pathways involved in Mpz/P0 gene expression. Cyclic AMP (cAMP) has marked effects on the regulation of the Mpz/P0 gene, and has been proposed as an important mediator of axon-Schwann cell interactions leading to myelination. The transcription factors Egr2 and Pax3 can also regulate Mpz/P0 expression. Initially, we will examine the role of cAMP, Egr2, and Pax (in conjunction with Sox10) and map cis-regulatory elements in the Mpz/P0 gene that are responsive to these pathways/molecules by administering forskolin or cotransfecting the transcription factors with various Mpz/P0 luciferase deletion constructs in primary Schwann cells. We will also determine whether these factors work through independent pathways to regulate the Mpz/P0 gene. Initially, we will examine the role of cAMP, Protein kinase A (PKA), CREB, EGR2, and Pax3 (in conjunction with SOX 10) and map cis-acting regulatory elements in the Mpz/P0 gene that are responsive to these pathways/molecules.

13 kb of the *Mpz*/P0 gene contains the regulatory elements necessary for *Mpz*-like expression. In our preliminary studies, we isolated bacterial artificial chromosomes containing the murine *Mpz*/P0 gene and contiguous sequences known to be required for efficient *Mpz*/P0 expression. We then engineered an *Mpz*/P0/*lacZ* gene/reporter construct by cloning *lacZ* (with a transcription termination/polyadenylation signal) in frame with the start codon for *Mpz*/P0 thereby creating a +7, -6 kb *Mpz*/P0/*lacZ* transgene. We then generated transgenic mice from this transgene using standard pronuclear injection. Our preliminary results indicate that a –7, +6 kb *Mpz*/P0/*lacZ* construct (3/3 lines) strongly targets expression of $\beta$-galactosidase to myelinating Schwann cells in transgenic mice. The β-galactosidase expression promoted by the –7, +6 kb *Mpz*/P0/*lacZ* construct appears to be appropriately upregulated during development, down-regulated following focal nerve injury, and upregulated during the remyelination associated with nerve regeneration.

Interspecies sequence comparisons for the *Mpz*/P0 gene.
After masking repetitive sequences in human, rat, and mouse *Mpz*/P0 genomic sequences with RepeatMasker, we performed interspecies sequence comparisons using PipMaker, (Schwartz,

et al., 2000) and Vista (Mayor, et al., 2000) gene and found 5 regions (designated A-E) of intermediate homology (approximately 75% homology over 100 bp) of which 3 have relatively high homology (>75% identity over 100 bp) across all three species in noncoding, non-repetitive elements of the *Mpz/P0* gene spanning over 8 kb from 2 kb upstream of the first coding exon (exon 1) to 1 kb downstream of penultimate exon, exon 6. Most of the conserved noncoding regions in the *Mpz*/P0 gene are downstream of the first coding exon, which for *Mpz*/P0 is exon 1. Putative SOX10 binding sites have been identified in conserved noncoding region B in the *Mpz*/P0 gene, immediately adjacent to the proximal promoter region upstream of exon 1(proximal 600 bp 5' to exon 1). The proximal 1-2 kb of the *Mpz*/P0 gene (containing both SOX10 binding sites) is inefficient as a promoter for driving transgene expression to myelinating Schwann cells in transgenic mice.

Hypothesis: *Mpz*/P0 gene contains an enhancer from +1 to +3 kb

We suspect that the downstream portion of the *Mpz*/P0 gene has enhancer activity for several reasons. First, our results confirm the results of Feltri et al, (1999) who identified the importance of regulatory elements in the 3' portion of the *Mpz*/P0 gene using a +7, –6 kb *Mpz*/P0 transgene. The *Mpz*/P0/*lacZ* transgene produced by Fetri et al, however, was not really suitable for promoter analysis. We have engineered a series of deletion mutants eliminating both 3' and 5' elements to define the minimal portion of the *Mpz*/P0 gene required for efficient reporter gene expression in myelinating Schwann cells. We found that a –5, +3 kb *Mpz*/P0/*lacZ* (2/3 lines) construct, but not a –5, +1 *Mpz*/P0/*lacZ* construct (0/9 lines), targets high levels of $\beta$-galactosidase to myelinating Schwann cells in transgenic mice. We conclude that there are important cis-regulatory elements from +1 to +3 kb of the *Mpz*/P0 gene. Interestingly, this noncoding region contains significant sequence homology (> 86% identity over 100 bases) that is shared among the human, rat, and mouse *Mpz*/P0 genes. Finally, we created a transgene using the Hsp68 minimal promoter driving the lacZ reporter gene to test whether the addition of the 0, +3 kb of the Mpz/P0 gene might have targeting and enhancer activity. We found strong b-galactosidase expression from this transgene in dorsal root ganglion neurons.

To summarize the interspecies sequence comparisons and the the Mpz/P0 promoter studies in transgenic mice, we find strong evidence that the proximal 0.8 kb (-0.8 kb to +1 bp) has poor autonomous promoter activity but strong sequence conservation. There is a distal +1 to +3 kb element that is also highly conserved and may have autonomous enhancer activity in transgenic mice. Thus, we have examined these regions in more detail in transient transfection studies.

The proximal 1.1 Mpz/P0/luciferase construct contains a functional TATA box and Sox10 binding sites. We have created a number of Mpz/luciferase reporter constructs to test the activity of various portions of the Mpz/P0 gene in Schwann cell lines. Examination of the sequence of the proximal Mpz/P0 promoter revealed the presence of a possible downstream promoter element (DPE) and a putative TATA box. We mutation both of these sites and found little effect of altering the DPE, but mutating the TATA box reduced the activity of a 1.1 kb Mpz/P0/luciferase construct by about 50%. We also mutated two Sox10 binding sites in the 1.1 kb Mpz/P0/luciferace construct and found that mutating both of these sites (Sox10 sites B,C) reduced the activity to the Mpz/luciferase construct to less than 10% of control (non-mutated) - 1.1 kb Mpz/P0/luciferase construct in Schwann cells.

We then tested whether the 0, +3 Mpz/P0/luciferase had endogenous promoter activity in Schwann cells. We focused on the +0.9, +2.1 Mpz/luciferase gene (since the size of the construct affects transfection efficiency), and found that this region also had autonomous ability to activate luciferase transcription from a minimal promoter in Schwann and N2A neuronal cells, but not in HEK cells. We then combined the -1.1, 0 kb and the +0.9, +2.1 kb constructs and

found that their effects on luciferase expression were somewhat additive, though this was offset somewhat by the size of the combined constructs.

Sox10 transactivates the proximal (-1.1 kb) Mpz/P0 promoter. When we tested the transactivating activity of Sox10 on the -1.1 kb Mpz/P0/luc, +0.9 -+2 kb Mpz/P0/luc, and the combined -1.1; +0.9 +2 kb Mpz/P0/luc, we found that Sox10 only had a effect on the -1.1 and the combined reporter construct, but not the +0.9, +2 Mpz/P0/luc alone. An even then, the effect was only observed in the neuronal N2A cells, presumably due to high endogenous Sox10 in the RT4 Schwann cell line. That Sox10 is constitutively expressed in the Schwann cell lines is supported by the fact that mutating the Sox10 sites had a strong effect on Mpz/P0/luc expression in these cells.

Egr2 transactivates the distal Mpz/P0 (+0.9, 2 kb) promoter. Similar studies using Egr2 as a transactivator showed that overexpression of Egr2 had no effect on luciferase expression driven from the proximal -1.1 kb Mpz/P0/luc construct, but did activate luciferase transcription from the +0.9 - +2 kb Mpz/P0/luc, and the combined -1.1 and +0.9 - +2 kb Mpz/P0/luc constructs in both Schwann cell and neuronal cell lines. Mutating the Sox10 binding sites (in the -1.1 to 0 kb Mpz/P0) reduced the activity of Egr2 on the combined -1.1 kb and +0.9 - +2 kb Mpz/P0/luc construct approximately 10 fold. Nonetheless, the overexpression of Egr2 was still was able to stimulate the luciferase activity of the combined -1.1 kb and +0.9 - +2 kb Mpz/P0/luc construct containing the mutated Sox10 sites an additional 10-fold. Thus, the combined effects of Sox10, acting on the proximal -1.1 kb, and Egr2, acting on the distal +0.9 to +2 kb, appear to account for an up to 100-fold activation of the Mpz/P0 gene.

Pax3, acting on proximal -1.1 kb Mpz/P0, inhibits the transactivation effects of both Egr2 and Sox10 on the Mpz/P0 gene. We found that overexpression of Pax3 inhibited expression from the -1.1 kb and the combined -1.1 kb and +0.9 to 2 kb Mpz/P0/luciferase constructs, but not the +0.9 to +2 kb Mpz/P0/luc constructs indicating that Pax3 is a transcriptional repressor of Mpz and likely has a binding site in the proximal -1.1 kb Mpz/P0 promoter. We have generated a series of overlapping fragments, approximately 100 bp each, of the -1.1 kb Mpz/P0 promoter for electrophoretic mobility shift assays (EMSA) to identify the Pax3 binding site. Interestingly, increasing Sox10 expression inhibits the repressor activity of Pax3 and visa versa suggesting the possibility that they might work through the same DNA binding sites or via the same transcription regulation complex.

We have identified separable activities of Sox10, Egr2, and Pax3 on the Mpz/P0 promotor. We are now in a position to examine putative signal transduction pathways involving these three factors in primary Schwann cells. Towards this goal, we have generated or obtained recombinant adenoviruses expressing Sox10, DN-Sox10, Egr2, DN-Egr2, Pax-3, constutively active Pax3 (Pax3-Engrailed fusion), Creb, KCREB, and DN-AKT. In addition, from the literature, we have identified ligands including IGF-1, IL6-IL6R, forskolin, and the neurotrophins that are known to regulate myelin protein (Mpz/P0 or Pmp22) expression in Schwann cells or in the PNS. Thus, it should be relatively straightforward to use these reagents, pharmacological inhibitors, and the the Sox10, Egr2, and Pax3 assays to identify signal transduction pathways that regulate myelination.

This work has been selected for a platform presentation at the upcoming International Association of Neuropathology meeting in San Francisco (2006) and we are writing up this work combining the transgenic mouse studies and the cell culture studies on Mpz/P0 expression for publication.

THE MORAN FOUNDATION
REQUEST FOR FINANCIAL SUPPORT 2005

TITLE: The Role of Dendritic Cells in the Induction of Inflammatory Bowel Disease

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Moran Update July 2006

The project entitled "The Role of Dendritic Cells in the Induction of Inflammatory Bowel Disease" is still ongoing in our laboratory and is still active. We have submitted a grant to the Crohn's and Colitis Foundation and will submit an RO1 grant in early 2007 after the publication of our papers. The following is an update of our research supported by the Moran Foundation. One abstract was submitted and accepted for presentation at the Annual Meeting of the American Association of Immunologists in May 2006 (see attached abstract and title).

Gαi2-/- Mouse model of IBD.

We are investigating the Gαi2 knockout (-/-) mouse as a model for identifying immune mechanisms involved in IBD [1]. In this model, susceptible strains of Gαi2 $^{-/-}$ mice (129/SvEv and BALB/c) develop colitis with 100% penetrance and approximately one third develop colorectal adenocarcinoma [2] while the C57/B6 strain of mice is relatively resistant to disease. Gαi2 is the inhibitory subunit for a family of heterotrimeric GTP-binding proteins involved in the transmembrane signal transduction of over 500 G-protein coupled receptors [3]. Despite the widespread expression of Gαi2, ablation of the inhibitory Gαi2 subunit leads to development of colitis as the only major phenotype in susceptible strains of mice. Abnormal function of the gut-associated lymphoid tissue in the Gαi2 mice has been ascribed to a variety of immune mechanisms. Gαi2 deficient T cells or B cells alone cannot adoptively transfer disease into a normal animal ([4], [5] [6]). However, it is clear that both B cells and T cells within the Gαi2 -/- mouse have some degree of abnormality that may collectively contribute to the disease. The major hypothesis we are testing, however, is that loss of Gαi2 activity in dendritic cells (DCs) precursors causes abnormal DC localization and trafficking, abnormal cytokine production, and/or abnormal antigen recognition of enteric flora within the T-cells in the gastrointestinal tract and susceptibility to colitis.

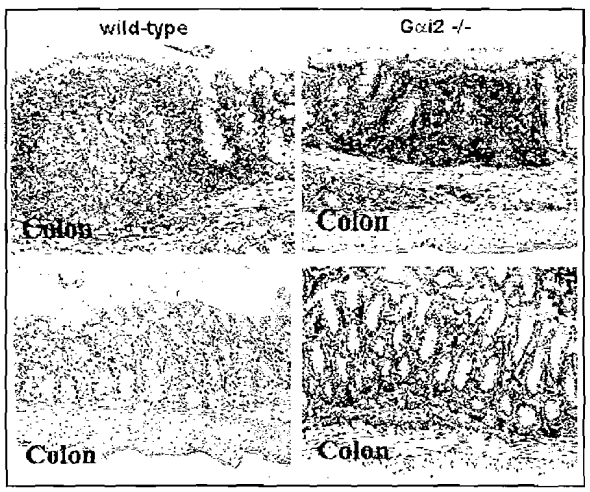


Fig.1: Immunohistochemical staining of CD11c in colon (brown) counterstained with hematoxylin.

Dendritic cells in the GALT

One way in which DCs can affect the immune response in the GALT is by modulating the T-cell response. Normally, the DCs in the GALT maintain a population of regulatory T-cells producing anti-inflammatory cytokines, such as IL-10 or TGF-β, in response to specific commensal flora antigens [7]. DCs orchestrate the activation of T-cells through cognate interactions of cell surface molecules on both the DCs and the T-cells such as CD40/CD40L, CD86/CD28, OX40L/OX40 and modulate the immune response through the production of specific cytokines. However, during the development of IBD, for reasons that are not yet clear, the immune response to commensal flora changes and T-cells producing inflammatory cytokines such as IFNγ predominate. We have focused our

studies on whether DCs from Gαi2 -/- mice have the capacity to perform their major function in the GALT, namely, to present commensal bacterial antigens to normal T-cells and activate an appropriate cytokine response.

Several studies suggest that DCs, as immune regulatory cells, are critically important in the etiology of IBD. In the adoptive transfer models of IBD, in which $CD45RB^{hi}$ T-cells are transferred into SCID mice, clusters of host DCs have been observed surrounding the donor T-cells early during the development of the inflammation [8]. There is an increase in DCs in the mesenteric lymph nodes and an increase in a marker of DC activation (OX40L) in this model [8] consistent with a role for DCs in the development of inflammation leading to colitis [9]. In the Gαi2 -/- model of IBD, we have detected increases in DCs in the lamina propria (LP) of the colon compared to wild-type mice, even before the onset of colitis and before there are large numbers of T-cells present (Figure 1), suggesting that there is an intrinsic difference in DC function in the Gαi2 -/- mouse. Further support for a role of DCs in IBD comes from studies where blocking cognate interactions of T-cells and DCs *in vivo* by treatment with OX40L monoclonal antibodies were shown to ameliorate colitis in the SCID adoptive transfer model of IBD[8]. DCs producing the pro-inflammatory cytokines IL-12 and IL-6 have been found at sites of inflammation in human IBD [10], which supports our hypothesis that DCs can promote a pro-inflammatory response. In our preliminary studies, we find an increase in the spontaneous secretion of inflammatory cytokines IFNγ, IL-6 and IL-12p40 in Gαi2 -/- colon organ cultures relative to normal 129SvEvTac colon cultures (Figure 2), perhaps related to the increased number of DCs that we have observed in the LP of Gαi2 deficient mice. Furthermore, the increase in IL-6 production seen in the colon cultures from the KO mice could be directly contributed by DCs in the colon. As shown in Figure 3 we have also observed a significant increase in the secretion of IL-6 by Gαi2 -/- DCs. Thus, it is plausible that DC dysfunction could contribute to the development of IBD in the Gαi2 -/- mouse. We have proposed in our CCFA grant to test this hypothesis directly by blocking Gαi2 function specifically in DCs in genetically engineered mice.

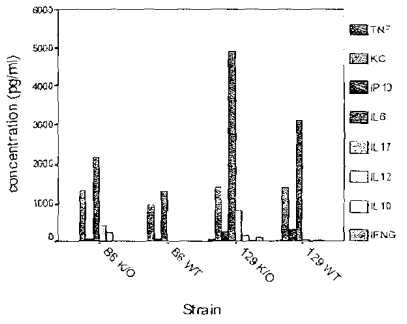


Fig. 2: Cytokine analysis from wild-type (WT) and Gai2-/- (KO) colon "organ" cultures by

There are two major subtypes of DCs, CD11c+/CD11b+ and CD11c+/CD11b-, that may modulate the T-cell response in the GALT. In addition, several subpopulations of these two DC subtypes have been described based on the presence or absence of CD8a and the level of CD11c expression (hi or low). Further analysis of DC subtypes isolated from the PPs of mice has revealed that they differ not only in the expression of surface markers, but also, the subsets of DCs apparently differ functionally as assessed by their cytokine expression profiles [11 12] (see table 2). In addition, subsets of DCs reside in

Table 2

| Dendritic Cell Population | Region of the PP | Chemokine Receptor | Cytokine expression |
|---|---|---|---|
| $CD11c^{+}CD11b^{+}CD8\alpha^{-}$ | SED | CCR6 | IL-10 |
| $CD11c^{+}CD11b^{-}CD8\alpha^{+}$ | Interfollicular Region | CCR7 | IL-12p70 |
| $CD11c^{+}CD11b^{-}CD8\alpha^{-}$ | SED Interfollicular Region | CCR7 CCR6 | IL-10 IL-12p70 |

specific microdomains within the PP [11]. The precise positioning and trafficking of specific DC subsets, and their ability to produce specific cytokines within the microenvironment of the GALT most likely plays an important role in regulation of T-cells and the immune response.

Our preliminary studies have shown that Gαi2 deficient DCs have a greater propensity to produce inflammatory cytokines than normal DCs. Interestingly, specific phenotypic subsets of DCs are responsible for the greatest differences. In 4 separate experiments, we sorted bone marrow derived DCs (BMDCs) according to their expression of CD11c and CD11b and reproducibly found significant increases in inflammatory cytokine expression by Gαi2 -/- DCs. The most significant differences occurred when cells were stimulated with the TLR9 ligand, CpG, for 24 hours. The population of Gαi2 -/- $CD11c^{hi}CD11b^{hi}$ DCs exhibited a 20,000 fold increase in IL-12p40 and IL-6 secretion upon CpG stimulation as assessed by Luminex protein multiplex immunoassay. Similarly, CpG stimulated the production of the chemokine, KC (the mouse homologue to IL-8), more than 12,000-fold and TNFa levels increased over 3000-fold from the same $CD11c^{hi}CD11b^{hi}$ Gαi2 deficient DC subpopulation as compared to the analogous population sorted from wild type 129SvEv BMDC cells. We did not detect IL-10 or IFNg production by any of the subtypes of wild-type or Gαi2 -/- BMDCs (see Figure 3).

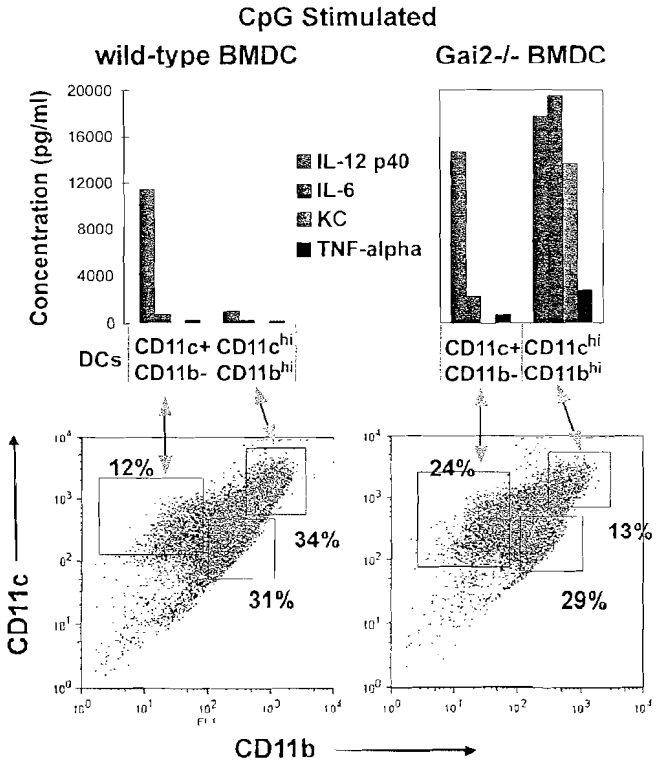


Fig. 3: Purified subsets of BMDCs were stimulated with CpG and assayed for their production of cytokines by Luminex.

These levels of cytokine expression would be expected to have a significant impact on the resident T-cells and the development of the immune response if they were produced by the DCs we have observed in the GALT of the Gαi2 -/- mouse as seen in Fig 1. Our DC specific ablation of Gαi2 function will allow us to determine whether this defective cytokine production by Gαi2 -/- $CD11c^{hi}CD11b^{hi}$ DCs is cell autonomous.

There are four niches for DCs within the GALT that may initiate the development of immune recognition of the microbial compartment: (1) the lamina propria (LP) within the small intestinal villi and the colon, composed of T-cells, B-cells, macrophages and several subsets of dendritic cells (DCs); (2) primary lymphoid structures, cryptopatches (CP), located near the base of the intestinal crypts and containing small clusters of cells (generally less than 1000) composed of 70% lin-c-kit+ cells and 30% CD11c+ DCs lining the periphery including some CD3+ T-cells [13 14]; (3) secondary lymphoid structures, Peyer's patches (PP), located in the small intestine directly under the epithelial lining composed of organized germinal centers of B-cells, organized T-cell areas, and three subsets of CD11c+ DCs localized to the subepithelial dome and interfollicular T-cell area [12]; (4) and isolated lymphoid follicles (ILF), composed of B220+ B-cells, T-cells, IL7R+/c-kit+ cells and CD11c+ DCs [15 16]. All of these are potential sites for antigen processing and the formation of an adaptive immune response to microbial, food or self-antigens within the gastrointestinal tract. A defect in any of these GALT immune cells, including DCs, could lead to a loss of the normal regulatory mechanisms that keep the mucosal immune response to normal enteric flora in check. We have examined the lymphoid follicles in the GALT in a number of Gαi2 -/- and control 129SvEv mice by immunohistochemistry. Unlike the organized sub-epithelial localization of the DCs in the 129SvEv mice, the Gαi2 -/- mice at 2 months, 4 months and 6 months consistently shows a random distribution of CD11c+ cells within the GALT (Figure 4). In addition, when we stain for DCs for CD11c+ and CD11b+ by double-label

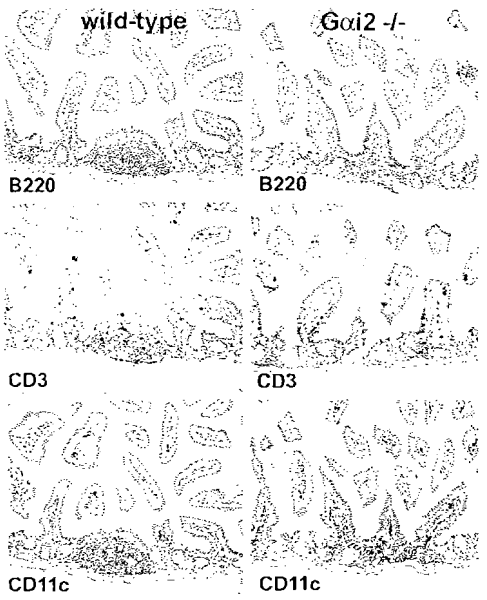


Fig. 4: Paraffin section immunohistochemistry of adjacent sections of wild-type and G□i2 deficient small intestinal follicles stained for B220, CD3 and CD11c stained with DAB (brown) and counterstained with hematoxylin.

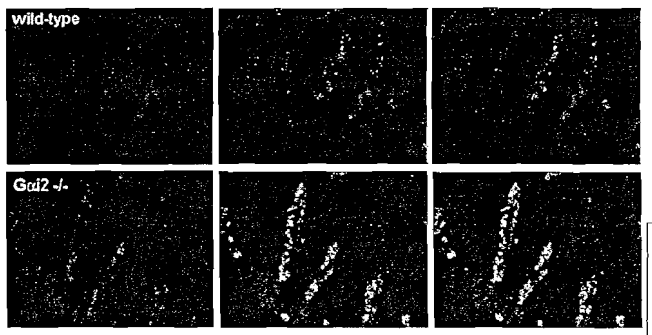


Fig. 5: Multi-label immunofluoresence staining of small intestinal villi showing increased LP DCs in Gαi2 -/- mice stained for CD11c (green), CD11b (red) and counterstained with DAPI (blue).

immunofluorescence, we find that an increase in the numbers of CD11c+ DCs in the lamina propria (LP) of the small intestinal villi of the Gαi2 -/- mouse (Figure 5). So far, the largest qualitative differences that we have observed by comparing the Gαi2 -/- with the wt129SvEvTac mice is the increased density of DCs in the cecum of the mutant mice (Figure 6).

Distinct DC lineages housed within the various niches of the muscosal gastrointestinal compartment have been proposed to have different roles in maintaining recognition,

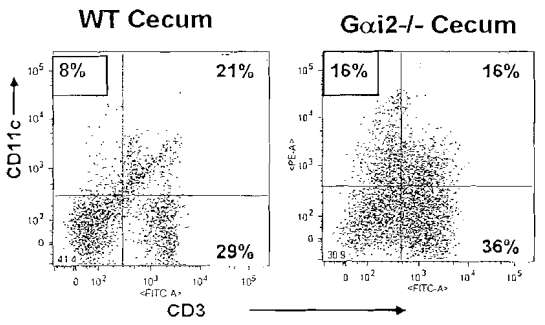


Fig.6: FACS analysis of mononuclear cells from cecum showing a two-fold increase in the DCs in the cecum of Gαi2 -/- mice.

tolerance, and protection from the enteric flora, food antigens, pathogens and self-antigens [17 18 19 20]. Elegant studies by Salazar-Gonzalez et al have recently shown that T-cell activation in the PP is regulated by specific subsets of DCs during a pathogen challenge that are recruited to the PP sub-epithelial dome only when the pathogen is present[19]. How specific DC subsets contribute to maintaining an innate as well as an adaptive immune response to commensal flora is not known. However, the cellular interactions that take place between the microbiota, epithelial barrier and the gastrointestinal associated lymphoid tissue are clearly important, but complex. We plan to continue our characterization of mislocalization of DC in the Gαi2 deficient mice by quantitatively determining the distribution of the various DC subsets as a function of age, which we would like to correlate with the anatomic location of colitis initiation in the Gαi2 -/- and DC-specific Gnai2 deficient mice. The cellular event(s) that initiate and promote inflammation are important targets for immune therapy for IBD.

Gαi2 coupled receptors may be important for DC function

Clearly, the lack of Gαi2 in the Gαi2 mouse uncouples a subset of G-protein coupled receptors (GPCRs), which provides the likely basis for the development of IBD in this model. Although GPCRs are involved in many pathways, for this application it is noteworthy that Gαi2 -associated GPCRs are involved in critical pathways of DC maturation and function, such as chemotaxis, the production of cytokines, and inflammatory responses induced by adenine nucleotides (adenosine), histamine, and LPA. Adenosine, which binds to a GPCR (2PY11), synergizes with low concentrations of TLR ligands (LPS) to induce DCs to produce pro-IL-12 as well as IL-10 [21]; histamine produced by mast cells can interact with closely apposed immature DCs via $H_2$ receptors and change their production of chemokines depending upon the microenvironment [22]; and, lysophosphatidic acid (LPA) is emerging as a very important mediator of DC chemotaxis [23 24 25]. LPA is produced by both epithelial cells and DCs and, intriguingly, DCs express functional LPA receptors [24]. At this time, it is not known which GPCR(s)

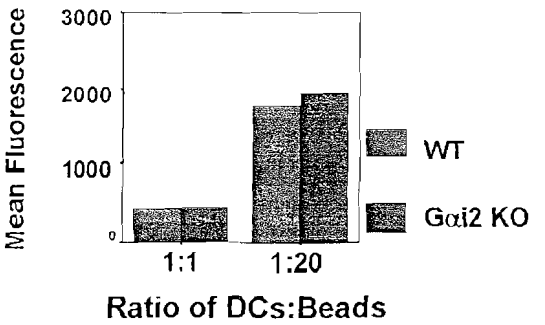


Fig. 7: Comparison of capacity of WT and Gai2 -/- BMDCs for phagocytosis of fluorescent beads in vitro.

contribute to the pathology in the Gαi2 -/- model. Although this is beyond the scope of this application, we are actively evaluating candidate GPCRs for aberrant function in Gαi2 deficient DCs.

DCs are the primary cells that deliver both soluble and particulate antigens to the T-cells within the MLN [26]. This process likely depends on the proper localization of appropriate DC subsets, phagocytosis and processing of antigen, migration of DCs to appropriate sites for the presentation of antigen and the stimulation of T-cells. How this leads to an adaptive immune response to food and commensal antigens while maintaining tolerance to these same antigens has been a major subject of investigation in mucosal immunology. Since phagocytic pathways are G-protein linked, we investigated whether Gαi2 deficient BMDCs might have a primary defect in phagocytosis that might limit antigen uptake. We found Gαi2 -/- BMDCs to be competent at phagocytosing fluorescent bioparticles (Figure 7) in three separate experiments. Recently, Worbs et al demonstrated that MLN is critical for the induction of oral tolerance. Furthermore, the maintenance of this oral tolerance required the function of chemokine receptor, CCR7, for chemotaxis and trafficking of the antigen-loaded DCs to the T-cell regions of the MLN [27]. Studies using pertussis toxin sensitivity of chemotaxis has linked Gai signaling with many of the chemokine receptors [28]. Lack of Gαi2, therefore, may inhibit the migration of the DC to the MLN and change the normal mechanisms involved in delivery of commensal and food antigens to the MLN. This could be one of the DC defects in the Gαi2 -/- mouse that leads to loss of a balanced anti-inflammatory environment in the intestinal tissue. In this proposal, we will determine whether Gαi2 -/- GALT DCs have the capacity to take up particulate antigens from the gut lumen, process and shuttle them to the MLN where cecal antigens are presented for T-cell activation in order to identify a possible basis for the IBD in the Gαi2 mouse and whether these functions are also deficient in the DC-specific Gαi2 deficient mouse models.

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Presentation at AAI 2006

**Thompson-Snipes, L.**, Bustos, K., Tatevian, N., Coskun, Z. Isolated Lymphoid Follicles in the G$\alpha$i2 $^{-/-}$Mouse: A Model of IBD. J. Immun., 176(7):S228, Suppl. 1, 2006.

**ISOLATED LYMPHOID FOLLICLES IN THE G$\alpha$i2 $^{-/-}$MOUSE: A MODEL OF IBD**

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Inflammatory bowel disease (IBD) is a chronic inflammation of the gastrointestinal tract involving an inappropriate immune response to environmental factors in genetically predisposed individuals. Peyer patches (PP), cryptopatches and isolated lymphoid follicles (ILFs), represent the primary sites for uptake and presentation of intestinal antigens. ILFs, composed of B220+ lymphocytes and an organized dendritic cell network with germinal centers, form after birth in response to commensal flora. We examined ILFs in the G$\alpha$i2-/- mouse model of IBD. G$\alpha i2^{-/-}$ mice lack PP, however, ILFs do form within 4 months of age. We have analyzed DCs within the gut mucosa of the G$\alpha i2^{-/-}$ mouse and found significantly more DCs and ILFs within the intestine, cecum and colon of the G$\alpha i2^{-/-}$ mice. Cryosections of the GI tract of 2,4, and 6 month old female 129Sv/Ev wild type and G$\alpha i2^{-/-}$ were fixed in 2% paraformaldehyde and CD11c, B220 or CD3 expression checked by immunohistochemistry. The number, and distribution of follicles in the intestine, cecum and colon were determined by microscopy and the relative size of the follicles was measured using ImagePro Plus. G$\alpha i2^{-/-}$ mice have significantly more medium sized ILFs containing CD11c+ dendritic cells, B220+ B cells and T-cells in the distal intestine, cecum and colon than normal mice. The DCs in the

Gαi2-/- ILFs are scattered suggesting abnormal migration. Since DCs are the major antigen presenting cell, this could lead to a loss of normal immune reactivity to intra luminal antigens in the follicles and abnormal activation of the gut associated lymphoid tissue resulting in the development of colitis. Supported by a Pilot Feasibility Award from the Texas Gulf Coast DDC Grant #P30 DK5623803/05 and the Moran Foundation.

## Gonzalez, Sylvia Yvonne

**From:** Thompson-Snipes, LuAnn [lxthomp1@TexasChildrensHospital.org]
**Sent:** Monday, July 31, 2006 12:50 PM
**To:** Gonzalez, Sylvia Yvonne
**Subject:** re: Moran update

**Follow Up Flag:** Follow up
**Flag Status:** Red

**Attachments:** Thompson_Snipes Moran update_2006.doc

Thompson_Snipes Moran update_2...

Dear Sylvia,

My Moran project is still ongoing. I have attached a file describing our progress thus far including a presentation given in Boston at the recent AAI meeting that acknowledges funding from Moran.

Thanks,
LuAnn
<<Thompson_Snipes Moran update_2006.doc>> LuAnn Thompson-Snipes

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