THE MORAN FOUNDATION
PROGRESS REPORT FOR 2002-2003

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

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Of the several mouse models of IBD, the $G_{\alpha i2}$ deficient mouse may prove to be the most interesting since they spontaneously develop an ulcerative colitis-like pathology followed by adenocarcinoma characteristic of the human disease (1). The genetic background is critical, $G_{\alpha i2}^{-/-}$ 129/SvEv mice develop colitis whereas $G_{\alpha i2}^{-/-}$C57BL/6 mice remain healthy. We hypothesize that dendritic cell (DC) dysfunction contributes substantially to the development of inflammatory bowel disease in $G_{\alpha i2}^{-/-}$ mice and DCs from the different strains of mice may respond differently to microbial stimuli. DCs are the major antigen-presenting cells, but also function to regulate the immune response. We propose that disruption of the architecture of Peyer's patches and mislocalization of DCs subsets within the Peyer's patches may provide a basis for immune dysfunction leading to a loss of tolerance to intraluminal antigens and abnormal activation of the gut-associated lymphatic tissue (GALT) resulting in the development of colitis. Last year's proposal to the Moran Foundation was supported by the observation that diseased 129/SvEv $G_{\alpha i2}^{-/-}$ mice have fewer and more rudimentary Peyer's patches than wild type mice (2). The activation of DCs by pathogens through innate immune regulators such as the toll-like receptors coupled with signaling through G protein coupled chemokine networks may be the primary events that occur prior to the onset of uncontrolled inflammation in the gut. Differences in the penetrance of disease between 129/SvEv mice and C57BL/6 mice may reflect a different response to pathogens and different interactions between dendritic cells and T regulatory cells within the Peyer's patches. This work is ongoing and we are submitting another proposal in 2003 to follow up on the importance of the microbiota in contributing to disease in these mice.

It is not known whether the development of IBD in the $G_{\alpha i2}^{-/-}$ mice is dependent on microbial stimulation since the $G_{\alpha i2}^{-/-}$ mouse has never been established in germ-free conditions. We would like to test this by re-deriving the 129 $G_{\alpha i2}^{-/-}$ mouse, which demonstrates 100% penetrance of disease, in a gnotobiotic facility. If the $G_{\alpha i2}^{-/-}$ germ-free mice do not develop IBD, we will monoassociate the mice with specific bacteria and test lysates of specific microbes (antigens) and pathogenic molecules to identify factors that initiate the onset of IBD in these animals. In addition, we will test whether adoptive cell transfer of isolated antigen pulsed dendritic cell subsets from normal mice into $G_{\alpha i2}^{-/-}$ mice can induce tolerance to defined luminal antigens or, alternatively, accelerate disease.

SUMMARY OF DATA

We are examining cytokine production by splenic dendritic cells (DCs) isolated from *wt* and $G_{\alpha i2}^{-/-}$ 129/SvEv and C57BL/6 mice. When splenic DCs are incubated for 18 hours with IL-4, GM-CSF with and without LPS or CpG, we find that DCs from 129/SvEv $G_{\alpha i2}^{-/-}$ mice have an attenuated production of IL-10 in response to LPS or CpGs stimulation compared to wt 129/SvEv, wt C57BL/6, or $G_{\alpha i2}^{-/-}$ C57BL/6 mice (Fig.1). This suggests a reduced capacity of 129/SvEv $G_{\alpha i2}^{-/-}$ DCs to maintain tolerance. We also observe a significant decrease in both IL-12p70 and IL-6 in response to either low LPS or CpG in the 129Sv/Ev strain relative to the C57BL/6. This is consistent with our hypothesis of a defect in toll-like receptor signaling in these cells.

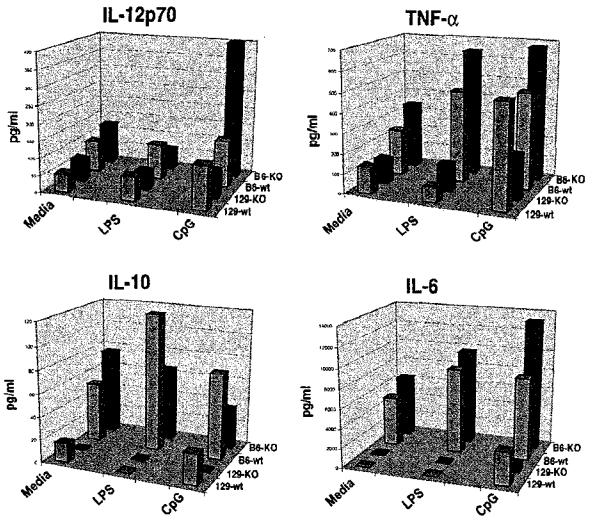


Fig.1: Cytokne profiles of enriched splenic DCs from wt and ko mice.

Comparing DCs from mesenteric lymph nodes (MLN) of wt and $G_{\alpha i2}^{-/-}$ 129/SvEv mice, we find major differences in expression of the co-stimulatory molecule, B7-2, a potent activator of T, B and NK-cells (3). Normal mice have more CD11c+/CD11b+/B7-2+ cells (mature phenotype) than the $G_{\alpha i2}^{-/-}$ mice (Fig. 2). Conversely, the $G_{\alpha i2}^{-/-}$ mice have more CD11c+CD11b-DEC-205+ cells than the normal mice. These observations are

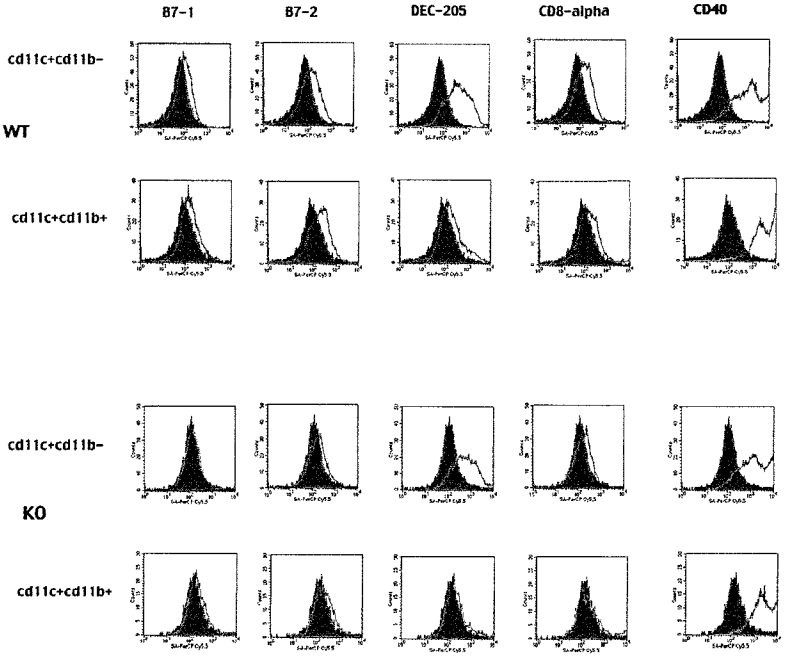


Fig.2: FACS of MLN DCs.

consistent with the hypothesis that the $G_{\alpha i2}^{-/-}$ DCs are less mature and can more readily take up antigen for presentation to T-cells and B-cells. The DCs isolated from MLN's produce detectable IFN-γ and we have observed that the $G_{\alpha i2}^{-/-}$ mouse produces significantly more of the Th1 cytokine than the wild-type mouse. The most compelling difference we have seen between the MLN/DC of the wild-type mouse vs. the $G_{\alpha i2}^{-/-}$ mouse is the production of IL-10 (Fig.3). Freshly isolated DCs from the MLN of wild

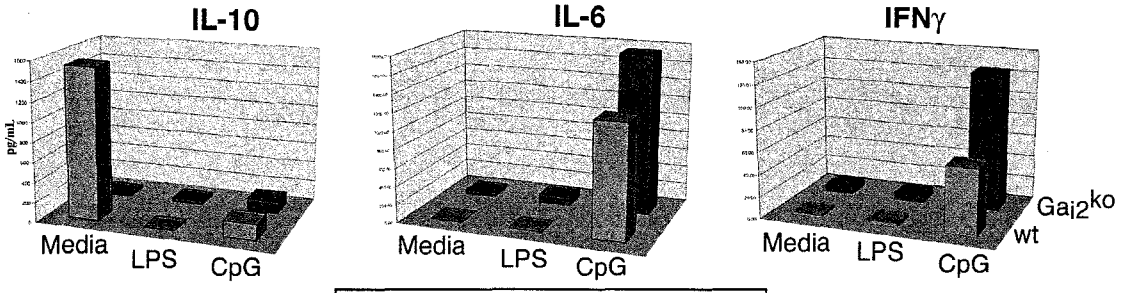


Fig.3: Cytokine profile of MLN DC enriched from wt and ko mice.

type 129SV/ev mice produce significant amounts of IL-10. In preliminary experiments we have found that the DCs isolated from the spleens or the MLN of Gai2-/- mice stimulate naïve T cell proliferation in an allogeneic interaction to a greater extent than the

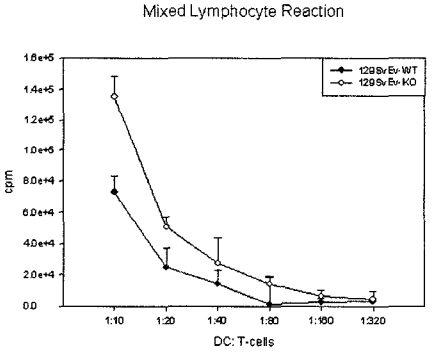
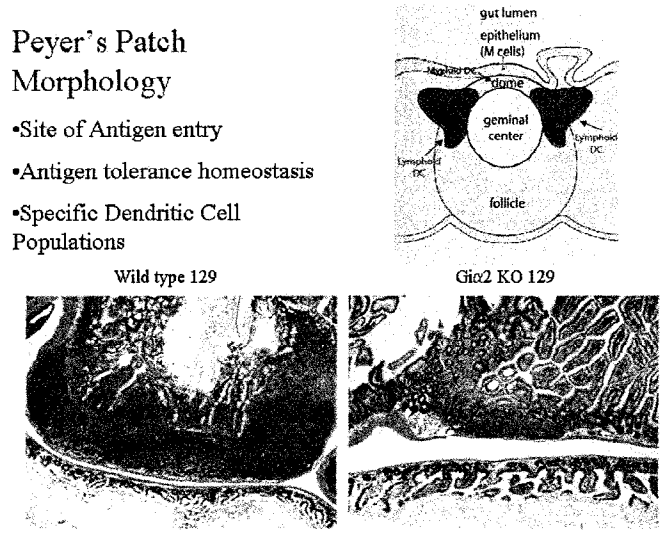


Fig.4: MLR of wt DCs and ko DCs with allogeneic T-

DCs from WT mice (Fig.4).

$G_{\alpha i2}^{-/-}$ mice maintained in our facility have only 2-3 very small Peyer's patches (PPs) compared to wild type having 6-7. Since both CD8- and CD8+ DC cells have been correlated with induction of peripheral T-cell tolerance, the distribution and quantity of these cells within the PP of mice undergoing an inflammatory response may be different than in the normal animal (Fig.5). We will use enhanced double-label immunofluorescence techniques to compare subsets of DCs and lymphocytes in PPs from normal and $G_{\alpha i2}^{-/-}$ mice. We will also enrich for DCs from the PP using a density gradient

and analyze for cytokine production in response to LPS and CpG activation using an intracellular cytokine staining method with flow cytometry. Recently we have had success in isolating DCs from the PPs.



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PP control

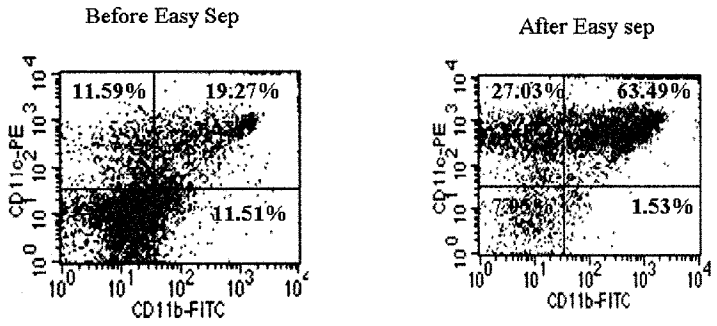

Fig.5: PP DC enriched by Optiprep (left) and CD11c beads (right).

We have recently rederived the $G_{\alpha i2}$ deficient mouse in SPF conditions and on 3 different background strains of mice. Each strain has been back-crossed to the original strain background at least 4 times to insure genetic homogeneity among the littermates. We have successfully worked out conditions for enriching for dendritic cells and are now in the process of preparing DNA free RNA for use in microarray analysis to compare the DCs from the various strains of mice.

We are now poised to rederive the mice in germ free conditions to test the effects of the absence of intestinal flora on the onset of disease, an experiment that has never been attempted before. The mice will be re-derived in a germ-free environment to determine the importance of the flora to disease progression and monoassociated with various well characterized enteric commensal bacterial species shown to induce disease in other murine models.

PRESENTATIONS OF WORK

The week of April 1, 2003 I was invited to present my data in a workshop at the Keystone Symposia "The Regulation of Mucosal Inflammation". The title of my talk was "Functional Differences in Dendritic Cells in Gai2 Deficient Mice".

While attending the Digestive Disease Week Conference, May 2003, I presented a poster entitled: "Functional Differences in Dendritic Cell in Gai2 Deficient Mice that develop IBD" that included the data presented in this update.

REFERENCES

1. Rudolph, U., M.J. Finegold, S.S. Rich, G.R. Harriman, Y. Srinivasan, P. Brabet, G. Boulay, A. Bradley, and L. Birnbaumer. 1995. Ulcerative colitis and adenocarcinoma of the colon in G alpha i2-deficient mice. *Nat Genet* 10:143-150.
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3. Bluestone, J.A. 1995. New perspectives of CD28-B7-mediated T cell costimulation. *Immunity* 2:555-559.