Award Number:  W81XWH-10-1-0721

TITLE: HARNESING GPR17 BIOLOGY FOR TREATING Demyelinating Disease

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REPORT DATE: OCTOBER 2012

TYPE OF REPORT: FINAL

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT:

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HARNESSING GPR17 BIOLOGY FOR TREATING DEMYELINATING DISEASE

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14. ABSTRACT

The overarching hypothesis of this project was that GPR17 signaling results in blockade of remyelination in neuroinflammatory lesions. We thus predicted that GPR17 could serve as an important target for promoting remyelination in these lesions. The specific aims of this study were: (1) To delineate the role of GPR17 in murine models of demyelinating diseases; and (2) To test the therapeutic potential for GPR17 agonists and antagonists in two models of multiple sclerosis. Our studies demonstrate that GPR17-deficient mice developed less severe disease and recovered faster from paralysis. Moreover, these mice showed reduced CNS-targeted pathogenic immune responses. These results provide us a strong basis to pursue drug-based treatment for this disease in the future.

15. SUBJECT TERMS
EAE, GPR17, remyelination
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INTRODUCTION

Multiple sclerosis (MS) is an inflammatory, demyelinating disorder of the central nervous system (CNS) affecting over 400,000 individuals in the US. Although the etiology of the disease is unknown, it is believed to be an autoimmune disorder with an involvement of T-cell due to the characteristic pathology, T-cells in the CNS and modulation of clinical disease by immunotherapeutic agents (1). However, it is increasingly becoming clear that in addition to immunomodulation, therapeutic approaches aimed at promoting remyelination is essential for better management of the disease (2). Olig1, a bHLH transcription factor, promotes oligodendrocyte maturation and is required for myelin repair (3, 4). We have shown that one of the genes regulated by Olig1 is G-protein coupled receptor 17 (GPR17). GPR17 opposes the action of Olig1 thereby negatively regulating the differentiation of oligodendrocyte precursor cells (OPCs) into mature oligodendrocytes (5). The signaling pathways involving GPR17 which culminates in the prevention of OPCs is not well understood. In our attempt to understand these pathways, we have observed that GPR17 expression is restricted to oligodendrocyte lineage cells and is down regulated during myelination and adulthood. Using transgenic mice, we also observed that sustained expression of GPR17 causes myelination defects whereas knockout of GPR17 leads to early onset of myelination during development. Interestingly, we observed that GPR17 is upregulated in the inflammatory demyelinating lesions in experimental autoimmune encephalomyelitis (EAE) a mouse model of MS. Further analysis revealed that GPR17 is upregulated in MS plaques as compared to the white matter from non-neurological donor samples and normal appearing white matter from MS donors. These data suggest that GPR17 regulates the transition from immature to myelinating oligodendrocytes and hence may serve as a potential therapeutic target for CNS myelin repair. This study was mainly aimed at understanding biology of GPR17 in MS with an emphasis on developing novel combination therapeutic approaches for MS.

BODY

This was a two-year study focused on the following two aims:

1. To delineate the role of GPR17 in murine models of demyelinating diseases.
2. To test the therapeutic potential for GPR17 agonists and antagonists in two models of MS.

As outlined in the statement of work (SOW), Dr. Karandikar’s laboratory was responsible for experiments addressing the EAE model of MS. Below, we outline our research findings according to the tasks outlined in the SOW.

Task 1. Test the prediction that loss of GPR17 will diminish demyelinating pathology.

Study EAE in WT and GPR17-KO mice

To evaluate EAE in GPR17-KO mice we obtained breeding mice (4 generations backcrossed to C57BL/6) from Dr. Richard Lu’s laboratory and initiated generation of GPR17-KO mice. The breeding strategy involved breeding GPR17−/− males with GPR17+/− females. Mice were routinely genotyped, using primers shown in Figure 1.
Once mice were obtained, we initiated the studies of EAE in WT and GPR17-KO mice. For this, we induced active EAE by immunizing GPR17-KO mice and WT-C57BL/6J mice with myelin oligodendrocyte glycoprotein (MOG) derived peptide-MOG$_{35-55}$. We observed a significant difference in the disease severity between the two groups with GPR17-KO mice demonstrating lower disease grades (Figure 2). Interestingly, these mice also showed faster and enhanced recovery from the paralysis. These data suggest demonstrate an important role for GPR17 in EAE pathogenesis, supporting our underlying hypothesis.
We observed a decreased proliferation of CD4+ T-cells from GPR17-KO mice when compared to WT mice (Fig 3). This can be attributed to the reduced disease severity or can be a direct effect on the CD4+ T-cell functioning. Since CD4+ T-cells are the main auto aggressors in EAE, a suboptimal CD4+ T-cell response may prove to be beneficial in disease management. There was no significant difference observed in the proliferation of CD8+ T-cells. We are presently evaluating the effect of GPR17-deletion on immune responses by addressing T-cell functioning in terms of proliferation, cytokine production and disease induction.

![Figure 3. CFSE-based lymphoproliferation assay.](image)

**Figure 3. CFSE-based lymphoproliferation assay.** Spleens from MOG35-55 immunized GPR17-/- and WT were harvested and cells were harvested and stained with CFSE. Subsequently, 1x10^6 cells were added to FACS tubes and MOG35-55 was added at various concentrations. Staphylococcal enterotoxin B (SEB) was added at a concentration of 1 μg/ml as positive control for proliferation and OVA323-339 peptide was used as peptide control. The cells were cultured in a CO₂ incubator for 5 days following which they were surface stained for CD4, CD8 and TCRβ. CFSE-dilution was monitored by flow cytometry and the data was analyzed using FlowJo software. Live cells were gated on the basis of forward and side scatter profile following which T-cells were gated based on TCRβ expression. X-axis corresponds to CFSE intensity while Y-axis represents CD8. The upper panel corresponds to proliferation of T-cells isolated from WT mice and lowers one to GPR17-/- cells. The red arrows indicate the percent proliferation of CD4+ T-cells in response to MOG35-55.

**Task 2. Test therapeutic potential for GPR17 antagonists.**

Since GPR17-/- demonstrated significantly lower disease severity, we hypothesized that blocking the receptor would have a similar effect and hence can be a potential therapeutic approach for MS. To test this, we utilized the GPR17 antagonist, montelukast [already FDA-approved for other conditions], to study its effects on EAE. The disease severity of mice treated with montelukast was compared with the control group (treated with vehicle). As shown in Figure 4, mice receiving montelukast demonstrated a slight but significantly lower disease severity. However, when we attempted the converse by injecting mice with an agonist such as UDP-Glucose, we did not see an increase in the disease severity.

Optimization of montelukast dosing and use of alternative agonists are being attempted to address these concerns.
Figure 4. Effect of GPR17 antagonist and agonist on EAE severity. EAE was induced by immunizing mice with 200 ug of MOG35-55/CFA emulsion. 200 ng of Pertussis toxin was administered on day 0 and day 2. Disease severity was graded as described above. (A) 10 mg/kg of Montelukast in 10% ethanol was administered on day -1 and on alternate days for the course of the experiment. (B) 200 mg/kg of UDP-glucose was administered every other day starting day -1.

Task 3. Test role of GPR17 during GA therapy.
Task 3 turned out to be overambitious for a 2-year project. Based on results from Tasks 1 and 2, we are planning to develop a longer-term grant application to seek funding for testing and developing combination therapy for this disease.

KEY RESEARCH ACCOMPLISHMENTS
• Evaluated EAE in GPR17-KO mice in comparison to WT-mice and observed a lower disease severity in GPR17-KO mice. This establishes a role of GPR17 in EAE pathogenesis/recovery.
• Demonstrated that CD4+ T-cells from GPR17-KO respond less efficiently to MOG35-55.
• Evaluated the effect of GPR17 agonist and antagonist on EAE severity and demonstrated that GPR17 antagonist treatment decreases EAE. There is need to refine the dosing and use alternative reagents to evaluate the best course of therapy.

REPORTABLE OUTCOMES
None thus far.

CONCLUSION
Our initial EAE studies carried out in GPR17-KO mice have demonstrated that GPR17 plays an important role in EAE pathology. In addition to a possible role in remyelination as seen by a lower disease severity (Fig 2), deletion of GPR17 also appeared to dampen the autoreactive CD4+ T-cell responses (Fig 3). Furthermore, mice treated with GPR17 antagonist demonstrated a lower disease severity. These data have encouraged us to pursue our study with the goal of developing a novel combination therapy. This goal was overambitious for a 2-year project, but we plan to develop grant applications to seek funding for these studies in the future.
BIBLIOGRAPHY AND PERSONNEL

No Publications/abstracts thus far.

Personnel Supported by this award:
Venkatesh Kashi, PhD, Postdoctoral Fellow
Wallace Baldwin, BS, research Assistant

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