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14. ABSTRACT

Many therapeutic strategies for SLE focus on the central role that autoantibody-producing B cells play in the pathology of this disorder. One general immunotherapeutic theme employs monoclonal antibodies (mAb) to interfere with, and/or deplete, B cells to ultimately reduce disease causing autoantibody levels. However, current strategies are inherently limited because they are not specific for the disease state. Thus, treatments that can specifically block autoantibody production without compromising B cell function are needed. In our application we presented preliminary evidence in an *in vivo* model of a related autoimmune disease (rheumatoid arthritis) that showed antibodies to RhoB, a small GTPase blocked autoantibody secretion without affecting the overall B cell repertoire. This data led us to propose the objective of this DOD-Discovery Award, to evaluate the ability of anti-RhoB antibodies to reduce levels of pathologic autoantibodies, ultimately attenuating the severity of symptoms in the MRL-lpr murine model of SLE. Our results demonstrate that dosing with an anti-RhoB mAb reduces serum anti-dsDNA titers in MRL-*lpr* mice. Interestingly, the two anti-RhoB Igs tested exhibited differing effects on renal disease pathology in the SLE model. One anti-RhoB antibody reduced renal inflammation while the other did not. We plan to seek new funding to extend these intriguing observations, and better understand the therapeutic potential of these novel anti-RhoB biologics.

15. SUBJECT TERMS

RhoB, animal model, antibody secretion, antibody therapy, monoclonal antibody, Systemic lupus erythematosus, autoantibodies.

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1. INTRODUCTION:

Systemic lupus erythematosus (SLE) affects approximately 300,000 to over a million individuals in the United States with a female gender bias of 90%. Many therapeutic strategies for SLE focus on the central role that autoantibody-producing B cells play in the pathology of this disorder. One general immunotherapeutic theme employs monoclonal antibodies (mAb) to interfere with and/or deplete B cells to ultimately reduce disease causing autoantibody levels. However, current strategies are inherently limited because they are not specific for the disease state. Thus, treatments that can specifically block autoantibody production without compromising B cell function remain lacking. Our work aims to address this therapeutic gap. The objective of our study is to evaluate the ability of anti-RhoB peptide antibodies to reduce levels of pathologic autoantibodies, ultimately attenuating the severity of symptoms in the MRL-*lpr* murine model of SLE. Our research strategy includes the determination of autoantibody levels over the course of disease progression in animals treated with therapeutic and control antibodies. Additionally, autoantibody levels will be compared to renal pathology to correlate autoantibody levels with attenuation of disease symptoms. This study will lay the groundwork for developing an innovative therapeutic strategy to improve the care of SLE patients, address important and timely scientific questions, as well as, focus on a major unmet medical need.

2. KEYWORDS:

RhoB, animal model, antibody secretion, antibody therapy, Systemic lupus erythematosus, autoantibodies.

3. OVERALL PROJECT SUMMARY:

Through the DOD-Discovery Award, we explored the potential of a novel immunotherapy for the treatment of SLE. Specifically, we targeted the small GTPase, RhoB using a preclinical animal model of SLE. The rationale for the study came from preliminary experiments showing that administration of an anti-RhoB antibody attenuated disease and lowered autoantibody levels in an animal model of autoimmune rheumatoid arthritis (RA).

Objective to complete in the award period of 18 months.

Evaluate the ability of anti-RhoB antibodies to attenuate the severity of symptom in the MRL/MpJ-Faslpr (abbrev. MRL-*lpr*) animal model of SLE.

Task I. Prepare anti-RhoB antibody. *Months 1-3*

Task II. Obtain approval for animal study. *Months 1-3*

Task III. Obtain MRL/MpJ-Faslpr female mice from approved vendor. *Month 3*

Task IV. Administer anti-RhoB antibody to MRL-*lpr* mice and monitor for the development of autoantibodies and proteinuria. Mice will be dosed regularly during the course of the experiment, approximately 6 months. We anticipate repeating the experiment to reach statistical significance. *Months 3-15*

Task V. Perform ELISA analyses to evaluate serum autoantibody levels. *Months 3-15*

Task VI. Final serum autoantibody levels will be determined, as well as the levels of cells actively secreting anti-chromatin antibodies by ELISpot from isolated spleens. Renal function will be determined by monitoring for proteinuria. Renal pathology will be scored for nephritis using histological kidney sections. *Months 10-16*

Task VII. Statistically analyze the data. *Months10-18*

Tasks I, II and III.

During the first 3 months of the award period, the first two tasks listed in the SOW were completed. During this time, we refined the methodology and began purification of the RhoB antibodies 7F7 and 9G5 (*Task I*). Also, we completed the approval process for our animal studies (*Task II*).

However, because our animal facility began treating all mice in the facility for fur mites in August of 2012, we did not bring in the first set of MRL-*lpr* mice until the facility was cleared in October 2012. The first set of mice was obtained in October of 2012, the second set in February 2013, and the third set in late October 2013 (*Task III*). The fur mite outbreak in the animal facility complicated the completion of the remaining tasks.

Tasks IV through VII.

The report on Tasks IV through VII has been written collectively because multiple sets of MRL-*lpr* mice were dosed with anti-RhoB antibodies and of the continuous nature of the experimental design. The design included dosing the MRL-*lpr* mice (Task IV), ELISA analysis of serum autoantibodies (Task V), analysis of anti-chromatin antibodies and renal pathology (Task VI), and statistically analysis of the data (Task VII). These tasks were done over the remaining time of the award (November, 2012 through June, 2014).

As stated in our July 2013 report, approximately 3 weeks after we had imported and begun treating the first set of MRL-*lpr* mice with our targeted therapy, fur mites were again found in the facility. During this second outbreak, all animals in the facility were fed ivermectin-containing food, and in our study, we continued to treat the MRL-*lpr* mice with our anti-RhoB antibodies. Analysis of the serum autoantibody levels from this group of mice did not show differences between the groups. However, we were concerned about the significance of the result from this first set of animals because ivermectin treatment has been shown to affect immune responses [1-3]. In these reports, changes were observed in altered disease development and antibody levels as compared to studies done prior to the detection of mites and subsequent ivermectin treatment.

In February 2013, we imported a second group of MRL-*lpr* mice after ivermectin treatment ended and sentinel screening was clear. The mice were divided into 3 treatment groups that received anti-RhoB antibody 7F7 or 9G5, or mouse IgG as a control. Dosing began at 4 weeks of age and continued one dose per week until the end of the experiment when the mice were 16 weeks of age. We assessed the development of serum autoantibody titers and renal pathology. The data suggested a trend towards a decrease in titers for anti-double stranded DNA antibodies in the groups treated with the anti-RhoB antibodies compared to control IgG, however, this trend did not reach statistical significance. Urine and kidney tissue from these mice was analyzed to assess renal nephritis. We did not observe a significant difference in urine protein levels in the treatment groups by 16 weeks of age, even though there was a decreasing trend in the group treated with antibody 9G5 (Figure 1, panel A). Interestingly, the two anti-RhoB Igs exhibited differing effects on disease pathology in the preclinical SLE model. RhoB mAb 9G5 appeared to attenuate renal inflammation while 7F7 did not (Figure 1B & C).

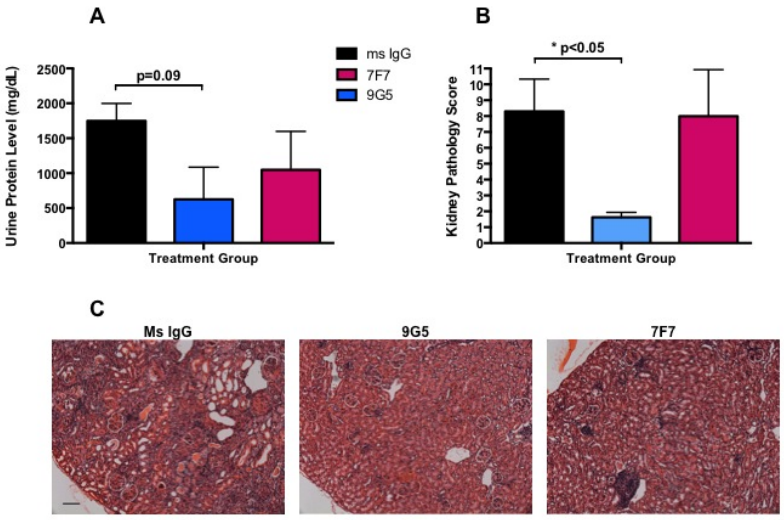

Figure 1: Effects of Anti-RhoB antibodies on SLE disease pathology by 16 weeks of age. Weekly doses (500 ug) of anti-RhoB IgG (7F7 or 9G5) or control mouse IgG were administered to MRL-*lpr* mice. Data show mean ± SEM. *A:* Urine protein levels. *B*: Extent of SLE associated glomerular nephritis in Ig treated MRL-*lpr* mice. The Kidney Pathology Score was determine by totaling scored histopathology on a scale of 0-3 using the following criteria: Glomerular cellularity, basement membrane thichening, thickened mesangium, adhesions to capsule, crescents and interstitial inflammation. *C*: Histologic hematoxalin and eosin staining of kidney sections from treated animals. Representative images are show; scale bar, 100um. ms IgG (N=5), 7F7 (N=5), 9G5 (N=5).

While we were dosing the second set of MRL-*lpr* mice, we learned that the two anti-RhoB antibodies showed different cross-reactivity for members of the Rho small GTPase family. We determined that 7F7 only recognized RhoB in elisa assays, while 9G5 recognized RhoB, RhoA and cdc42. Because 7F7 appeared specific for RhoB, we treated the third set of MRL-*lpr* mice with 7F7 and ms IgG. However, before importing more animals we obtained both Institutional and DOD approval to increase the number of animals on the respective protocols. Additional animals were needed because the first groups of MRL-*lpr* mice were treated for, and possibly had, fur mites.

After completing the administration of anti-RhoB 7F7 to the third set of mice, acquiring and analyzing the data, tasks IV through VII were complete. Analysis of autoantibodies after weekly administration of anti-RhoB 7F7 immunoglobulin (Ig) showed a significant reduction of serum anti-dsDNA titers (Figure 2, panel A) in MRL-*lpr* mice compared to mice dosed with mouse IgG. Antibody 7F7 did not have a therapeutic effect on renal disease in this preclinical SLE model (Figure 2, panel B). A possible explanation for this difference may be the specificity difference between the two antibodies. Antibody clone 7F7 is specific for RhoB, while 9G5 recognizes other family members, as well as, RhoB. We also examined the levels of serum rheumatoid factor and observed that the administration of anti-RhoB Ig did not alter titer levels (data not shown).

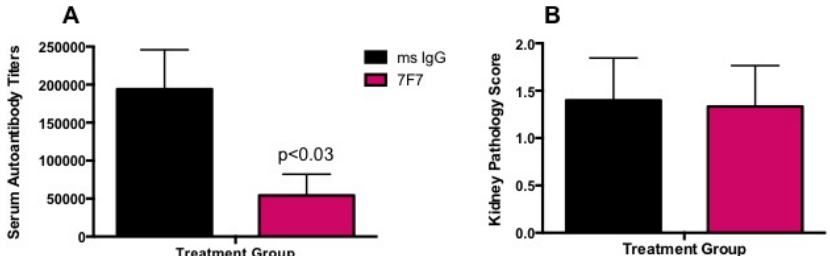

Figure 2: Effects of Anti-RhoB antibody 7F7 on SLE disease by 16 weeks of age. Weekly doses (500 ug) of anti-RhoB IgG (7F7) or control mouse IgG were administered to MRL-*lpr* mice. Data show mean ± SEM. *A:* Serum dsDNA autoantibody titers were determined by ELISA. The serum titer was defined as the reciprocal of the last dilution that gave an OD > 3× background. ms IgG (N=14), 7F7 (N=14). *B*: Extent of SLE associated glomerular nephritis in treated MRL-*lpr* mice. The Kidney Pathology Score was determine by totaling scored histopathology on a scale of 0-3 using the following criteria: Glomerular cellularity, crescents and adhesions to capsule, basement membrane thickening, thickened mesangium, tubule distortion and dilatation, interstitial inflammation, and blood vessel thickening. ms IgG (N=10), 7F7 (N=10).

We suggest that the impact of these results is significant to the understanding of autoimmunity. First,

these results have uncovered the importance of a previously unidentified mediator of autoantibody-mediated disease development. Second, our results provide intriguing justification to further explore the therapeutic potential of these novel anti-RhoB biologics to target the production of pathogenic autoantibodies. And finally, because of the differing effect that the RhoB Igs had on the development of glomerular nephritis, these results suggest that other Rho proteins may contribute to lupus-associated kidney disease, and that targeting other RhoGTPases may be beneficial. We hope to obtain future funding to further understand the role of RhoB in autoimmune disease.

4. KEY RESEARCH ACCOMPLISHMENTS:
The key research accomplishment was the observation that targeting the small GTPase, RhoB with a monoclonal antibody significantly decreased autoantibody levels in the MRL/lpr model of SLE. Additionally, data obtained with a monoclonal antibody that recognizes RhoB, RhoA and cdc42 appeared to protect the development of lupus-associated glomerular nephritis.

5. CONCLUSIONS:
We conclude that RhoB is a viable therapeutic target to reduce autoantibody production without compromising B cell function in autoantibody-mediated autoimmune diseases.

6. PUBLICATIONS, ABSTRACTS, AND PRESENTATIONS: The data has been included in a manuscript in preparation "The small GTPase RhoB mediates the development of autoimmune arthritis and be therapeutically targeted to attenuate disease".

The data was included in a pre-application (PR140281, "RhoB as a novel therapeutic target for the treatment of Lupus") to the DOD-Lupus FY14 PRMRP Investigator-Initiated Research Award Program. Disappointingly, the pre-application proposal was not accepted for full submission.

7. INVENTIONS, PATENTS AND LICENSES: None.

8. REPORTABLE OUTCOMES: We have identified RhoB as a therapeutic target for the treatment of autoantibody-mediated autoimmune diseases.

9. OTHER ACHIEVEMENTS: None.

10. REFERENCES:

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11. APPENDICES: None.