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TITLE: The Annexin A2 Pathway in Proliferative Vitreoretinopathy: A New Therapeutic Target

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14. ABSTRACT

Proliferative vitreoretinopathy (PVR) occurs in patients with penetrating ocular injury, of which there are more than 200,000 worldwide per year among both military and civilian personnel. It is a major challenge in ophthalmology and retinal surgery. Experts agree that PVR results from proliferation and migration of retinal pigment epithelial (RPE) cells, through a retinal wound and over the vitreal surface of the retina. There, RPE cells secrete collagen and other matrix proteins that form an epiretinal scar-like membrane that exerts tractional forces on the retina, often leading to retinal detachment and loss of vision. At present there are no reliable means of treating or preventing PVR. This program would develop a potential new biologic therapy, based on targeting the annexin A2 cell surface fibrinolytic system and stimulators of the system (macrophage inflammatory protein (MIP) 1-alpha and MIF 1-beta) for early point-of-care prevention of PVR.

15. SUBJECT TERMS
None listed.

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1. Introduction

The primary goal of this research project is to test the hypothesis that blockade of the annexin A2 protease system will prevent the development of dispase-induced PVR in mice by inhibiting the recruitment of circulating innate inflammatory cells into the retina and impeding the delamination of RPE cells and their transition to migratory scar-forming cells within the retina. A secondary goal is to explore the postulate that blockade of the macrophage inflammatory protein system will prevent or curtail the development of PVR by blocking assembly of the A2 protease system on the surface of migrating RPE cells.

2. Keywords

annexin A2, macrophages, macrophage inflammatory protein, proliferative vitreoretinopathy, retinal pigmented epithelial cell

3. Accomplishments

Goals and accomplishments slated for Year 2 are listed according to the Statement of Work for this project.

SPECIFIC AIM 1: DEMONSTRATE THE ABILITY OF ANTI-A2 BLOCKING ANTIBODIES TO PREVENT PROLIFERATIVE VITREORETINOPATHY (PVR) IN TWO STANDARD ANIMAL MODELS

Major Tasks 1-3 were completed in year 1.

Major Task 4: Confirm lead IgG candidate optimal dose
In Year 1, we used two doses of the two lead candidate antibodies (02L21A and 36H22A at 5 and 15 ug/ml). Eyes were harvested at 4 weeks, sections prepared, and tissue stained with hematoxylin and eosin. Double blind scoring revealed strong effects of both doses at 4 weeks. To extend these findings, we have now verified, in preliminary experiments, that the 15 ug/ml dose provides an effect similar to that of 45 ug/ml. Therefore, we have not conducted further extensive studies with the highest dose originally proposed (45 ug/ml). Antibodies 02L21A and 36H22A show efficacy similar to 1A7, the original IgG; these two antibodies display about 50% inhibition of PVR compared to scores obtained with either no antibody or an inactive control (1D4) (**Figure 1**). This task is now 100% complete.

Major Task 5: Determine benefit of multiple doses of the lead IgG candidate: We have been unable to obtain meaningful data in the PVR model upon administration of a second injection of intravitreal antibody. Following the first two injections (dispase, followed by antibody), we find that mouse eyes do not tolerate a third injection. Specifically, the globe softens, making it difficult to perform a third injection without compromising the tissue architecture and traumatizing the retina. Therefore, we have discontinued this approach, and consider the task complete.

Major Task 6: Evaluate lead IgG candidate in second animal model: We have completed the initial phase of this task. All relevant lab members have completed training in large animal experimentation. We have worked through new institutional protocols and logistics concerning the purchase of rabbits from USDA approved vendors. We are in the process of scheduling this experiment, which must be conducted in collaboration with Research Animal Resource Center (RARC) veterinarians. Once rabbits have been purchased, quarantine procedures are completed, and RARC technician time has been scheduled, we will initiate the experimental phase. This task is approximately 10% complete.

SPECIFIC AIM 2: CONFIRM THAT BLOCKADE OF MIP-1α/β CAN PREVENT PVR *IN VIVO*

Major Task 1 was completed in Year 1.

Major Task 2: Identify the optimal anti-MIP dose in mice
All of the preliminary experiments presented above were conducted at an anti-MIP dose of 30 ug/ml (3 ul per eye). We have now compared 3 doses of anti-MIPalpha and anti-MIPbeta in combination (10, 30, and 90 ug/ml) (**Figure 2**). The results indicate dose-dependent inhibition of PVR, with a maximal effect at 90 ug/ml. Inhibition with 90 ug/ml is highly significant based on overall histology and RPE scores (**Figure 3**). This task is now 100% complete.

Major Task 3: Determine the effect of multiple anti-MIP doses: As noted above under **Aim 1, Major Task 5,** we have been unable to obtain meaningful data in the PVR model upon administration of a second injection of intravitreal antibody. Following the first two injections (dispase, followed by antibody), we find that mouse eyes do not tolerate a third injection. Specifically, the globe softens, making it difficult to perform a third injection without compromising the tissue architecture and traumatizing the retina. Therefore, we have discontinued this approach, and consider the task complete.

Major Task 4 Demonstrate that anti-MIPs prevent PVR in rabbits: As noted above (**Aim 1, Major Task 6**), we have completed the initial phase of this task. All relevant lab members have completed training in large animal experimentation. We have worked through new institutional protocols and logistics concerning the purchase of rabbits from USDA approved vendors. We are in the process of scheduling this experiment, which must be conducted in collaboration with Research Animal Resource Center (RARC) veterinarians. Once rabbits have been purchased, quarantine procedures are completed, and RARC technician time has been scheduled, we will initiate the experimental phase. This task is approximately 10% complete.

SPECIFIC AIM 3: DEFINE THE PHARMACOKINETICS AND POTENTIAL TOXICITY OF LEAD CANDIDATES IN MICE

Major Tasks 1-3 are scheduled for completion in Year 3.

Major Task 4: Work on this task has begun. Since writing the grant proposal, regulations concerning the use of rabbits in research at our institution have become significantly more complex. For example, we are no longer allowed to transfer rabbits from the animal facility to the laboratory in which instruments for OCT, ERG, angiographic imaging, and fundus photography are typically conducted. We are working with RARC and the equipment owners to resolve this issue. On a more positive note, Ms. Dena Almeida, Lab Coordinator, has completed training in OCT, angiographic imaging, fluorescein angiography, and fundus photography, and is equipped to perform these studies once we can sort through the logistics. This task is now approximately 15% complete, and full completion is expected in Year 3.

Training Impact: This project has provided Dr. Valentina Dallacasagrande with invaluable experience in techniques involving induction and evaluation of PVR in mice. She has mastered our injection protocol, the scoring rubric, statistical analysis, and OCT and fundoscopic procedures involved in the project. In addition, she presented a project on closely related research at an international conference (the Gordon Conference on Plasminogen Activation and Extracellular Proteolysis in Ventura, California) last year. These new skills and experience contributed to her ability to achieve a permanent position in industry in January of 2023.

In addition, the project has had a positive impact on training of our technical staff. It has greatly enriched their ability to execute highly specialized technical skills, which are needed to complete the project. Out technical staff also now have an enhanced mechanistic understanding of the evolution and potential treatment of PVR.

Dissemination:

We are currently revising a major, original paper on anti-annexin treatment for experimental PVR in mice for *Nature Communications.*

Plans for the next reporting period:
As indicated on the attached SOW, we will, in Year 3, determine the efficacy of anti-A2 in PVR in rabbits (**Aim 1, Task 6**). In addition, we will focus on determining the efficacy of anti-MIP in PVR in rabbits (**Aim 2, Task 4**). We will also complete studies on pharmacokinetics and potential toxicity of lead anti-A2 antibodies in rabbits (**Aim 3, Major Tasks 1-4**).

4. Impact

Impact on current discipline: In the previous funding period, prior to the current grant, we established that development of PVR in the mouse dispase model is critically dependent upon expression of annexin A2 in the host. This work suggested that A2 supports the cell transformation and migration of retinal pigment epithelial cells that underlie the formation of fibrotic membranes on the surface of the retina. These epiretinal membranes can develop into scars that may contract leading to retinal detachment and major vision loss. Given that the current treatment for PVR remains unsatisfactory, we have initiated studies in mice determining whether annexin A2 may be a new potential therapeutic target. Over the past year, we have developed an expanded scoring system to gauge the severity of dispase-induced PVR in mice, using a comprehensive array of assessment tools including overall histology, evaluation of retinal pigment epithelial cell migration, fundoscopy, and optical coherence tomography (OCT). Our data confirm that immuno-inhibition of A2 may prevent or significantly reduce the development of epiretinal membranes in the dispase model of murine PVR. In addition, we find that inhibition of macrophage inflammatory protein (MIP) 1-alpha and MIP1-beta also reduces the degree of PVR in mice. These new data provide further evidence that blockade of these agents may have therapeutic value. They also establish a molecular pathway connects a MIP-annexin A2 axis in the development of PVR. A portion of this work is under revision of *Nature Communications*.

Impact on other disciplines: Once complete, findings from this study could provide new treatment avenues and a better mechanistic understanding of other "scarring" disorders, such as renal, pulmonary, or hepatic fibrosis.

Impact on technology transfer: Nothing yet to report.

Impact on society: Nothing yet to report.

5. Changes/Problems

Changes in approach: Nothing to report.

Problems or delays: As mentioned in last year's progress report, we experienced a delay in [1] receiving critical equipment for evaluation of antibodies in mice (specialized needles for ocular injection) due to Covid-19, and [2] closure of our institution's Visual Function core. We overcame both of these problems by [1] changing the order of the scheduled experiments, as reflected in the revised SOW, and [2] training our own personnel in the procedures previously performed in the core. These include OCT, fluorescein angiography, and fundus photography.

In March of 2023, our Tri-Institutional Therapeutics Discovery Institute completed work on the development of new antibodies for this project, but discontinued their involvement in assessing its developability due to "insufficient understanding of the lead antibody profiles." We are therefore now working to identify potential industrial partners through the Weill Cornell Medicine Enterprise Innovation Office. We have initiated meetings with this office, which is actively searching for new collaborative partners..

Changes significantly impacting expenditures: In January of 2023, Dr. Valentina Dallacasagrande left our research group in order to pursue a position in industry. Funds originally directed toward her compensation are now being utilized to cover the increased effort for Dr. Min Luo, who is now covering tasks originally assigned to Dr. Dallacasagrande.

6. Products

Nothing to report.

7. Participants & Other Collaborating Organizations

Name	Katherine A. Hajjar, MD
Project Role	PI
Researcher Identifier	Orcid: 0000-0003-3977-4356
Nearest person month worked	2.4
Contribution to project	Planning and oversight of all experiments; review of data; trouble shooting as needed; manuscript and progress report preparation
Funding Support	This grant and other institutional funds

Name	Dena Almeida
Project Role	Research Specialist (Hajjar Lab)
Researcher Identifier	N/A
Nearest person month worked	7.4
Contribution to project	Intravitreal and anterior chamber injections; tissue harvest, processing, and archiving; histologic staining and quantitative evaluation; assist with visual function testing
Funding Support	This grant and institutional funds

Name	Min Luo, PhD
Project Role	Research Associate (Hajjar Lab)
Researcher Identifier	N/A
Nearest person month worked	7.4
Contribution to project	Assist with tissue histological analyses as a masked reader of stained mouse tissues; assist with injection procedures, visual function studies, and mouse breeding and genotyping as needed.
Funding Support	This grant and institutional funds

Name	Valentina Dallacasagrande, PhD
Project Role	Research Associate (Hajjar Lab)
Researcher Identifier	N/A
Nearest person month worked	3.9
Contribution to project	Assist with mouse husbandry and breeding, mouse sirgivcal procedures, tissueharvest, and processing.
Funding Support	This grant and institutional funds

Name	Szilard Kiss, MD
Project Role	Collaborator (Ophthalmologist at Weill Cornell Medicine)
Researcher Identifier	N/A
Nearest person month worked	N/A
Contribution to project	Provide advice on evaluation of histologic samples and visual function studies throughout the project; assist with rabbit studies in Year 2.
Funding Support	Institutional funds

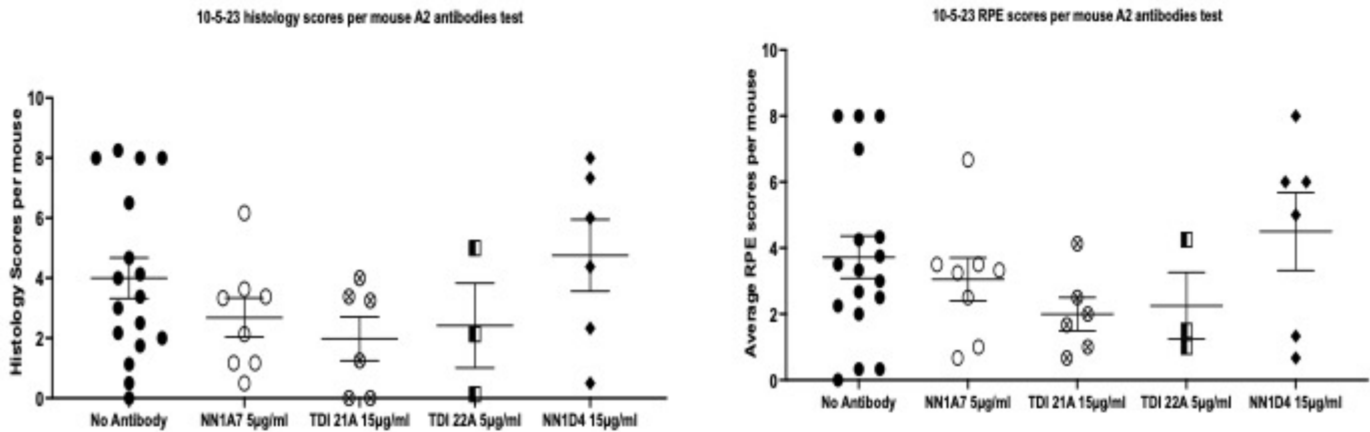
Name	Marcelo Nociari, PhD (replaces Ryan Schreiner, PhD); researcher in Department of Ophthalmology at Weill Cornell Medicine
Project Role	Collaborator
Researcher Identifier	N/A
Nearest person month worked	N/A
Contribution to project	Will provide instrumentation and training for visual function studies, as needed
Funding Support	Institutional funds

8. Special Reporting Requirements

Not applicable.

9. Appendices

Figure 1: Effect of TDI antibodies (TDI 21A and TDI22A) in comparison to previous agent (NN1A7) and negative control (NN1D4) on development of PVR in mice.



(updated 10-5-23)

Each sample represents averaged combined scores from Dena+Val+Lucy+Harley per mouse.
Each mouse picked one representative slide.
Note: Each eye deliver 3μl antibodies, all antibodies were delivered one day after dispase injury.

Figure 2: Dose-dependent effect of anti-MIP antibodies on development of PVR in mice

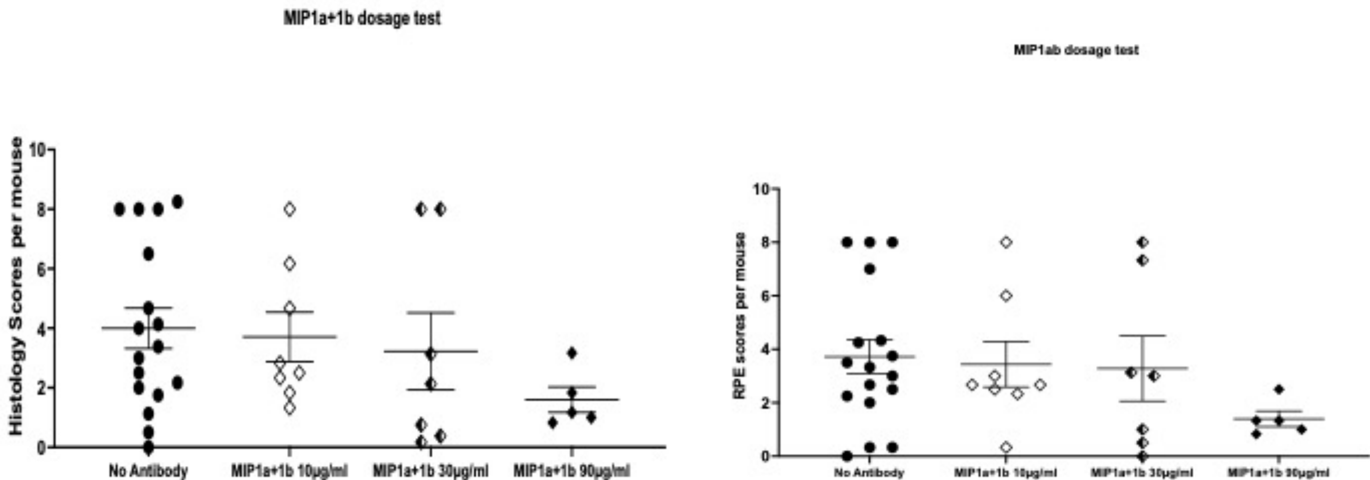


Figure 3: Comparison of optimal dose anti-MIP1alpha and anti-MIP1beta versus no antibody in prevention of PVR in mice.