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**TITLE:** Mechanism and Potential Treatment of Guillain Barré Syndrome and Related Neuropathy

**PRINCIPAL INVESTIGATOR:** Antonito Panganiban, Ph.D.

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**14. ABSTRACT** Guillain-Barre' Syndrome (GBS) encompasses a group of polyneuropathies of high medical importance that affect mainly the peripheral nervous system (PNS). In the current model of GBS an adaptive immune response to microbial infection is cross-reactive and destructive to the glycosphingolipids in the protective myelin sheath surrounding axons in the PNS. Damage to myelin results in a several types of clinical manifestation. Recovery usually takes place over the course of several months but a significant number of patients have persistent longer term or permanent neural damage. In our nonhuman primate model of GBS spectrum disease triggered by virusinfection we find multiple hallmarks of neural inflammatory disease, damage to the blood-brain barrier, andupregulation of a key cytokine likely important in the induction of neural damage. I

**15. SUBJECT TERMS**
None listed.

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**REPORT**

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1. **INTRODUCTION:**

Guillain-Barre' Syndrome (GBS) encompasses a group of polyneuropathies of high medical importance that affect the peripheral nervous system (PNS) and can extend to the central nervous system (CNS). This study uses a nonhuman primate (NHP) we established that results in neuroinflammation, which likely recapitulates facets of GBS in adult animals. In this model neuroinflammation is triggered by infection with ZIKV. A hallmark of neuroinflammation in these animals is consistent acute and chronic neural upregulation of the chemokine CXCL12. It is likely that CXCL12 plays dual roles in neuroinflammation. Since CXCL12 regulates lymphocyte migration into the neural parenchyma, it is likely that this chemokine contributes to early inflammation. GBS involves demyelination and CXCL12 is a key chemokine that facilitates myelin repair. Hence, it is likely that CXCL12 also functions positively in myelin repair in the chronic phase. **The main purpose of this Discovery Project is to test an innovative approach for treatment of GBS.** In particular, we will test the hypothesis that targeting the activity of the cytokine CXCL12 will mitigate the symptoms of GBS. We will test our hypothesis by using an FDA-approved highly specific inhibitor of CXCL12. We will determine whether the inhibitor blocks PNS/CNS inflammation, BBB disruption, and protects the myelin sheath. Since they are genetically and physiologically similar to humans, NHPs are often good models for modeling and blocking human disease. Further, drugs developed in NHP models translate clinically to humans more predictably than those developed in other animal models. Consequently, if our hypothesis is correct it should be possible to rapidly move from our NHP model to clinical use.

2. **KEYWORDS:**

Neuropathy, neuropathogenesis, Gullain-Barre' Syndrome (GBS), nonhuman primate, blood-brain barrier (BBB), Zika virus (ZIKV), peripheral nervous system (PNS), central nervous system (CNS)

3. **ACCOMPLISHMENTS:**

- **What were the major goals of the project?**

As indicated in the Introduction, the major goal of the project (Major Task 1) was to determine whether the CXCL12 inhibitor, AMD3100 is able to inhibit or alter facets of neuropathogenesis induced by ZIKV infection in adult macaques. Specific hallmarks to be evaluated included identification and quantification of neural inflammation, disruption of the BBB, and degradation of the myelin sheath. Complementary goals were to determine whether AMD3100 affects virus replication or the host response to virus infection (Major Task 2).

- **What was accomplished under these goals?**

The start of this project last spring coincided with the COVID-19 pandemic. The Tulane National Primate Research Center (TNPRC), where the work of the current project will be carried out, was charged with developing tractable NHP models of SARS-CoV-2 induced pathogenesis to be used for evaluation of COVID-19-related antiviral therapeutics and vaccine assessment. As part of this effort NHPs were reserved primarily for this effort. As a consequence, the current project was unfortunately delayed. However, we will be able to carry out and the project this spring and complete our analysis this summer.

We will be using multiple approaches to assess and quantify PNS/CNS inflammation, BBB disruption, and myelin sheath integrity. It also came to our attention that an additional approach, *in vivo* confocal microscopy (IVCM), for examining status of the PNS was likely to be extremely useful for our project. Since start of our project was delayed, we set up IVCM for the project with the assistance of personnel from Dr. Joseph Mankowski's lab at Johns Hopkins University Medical School. It is important to note that addition of this capability does not change the objective of the project. Rather IVCM should enhance data interpretation and provide insight into the potential therapeutic effect of AMD3100 on peripheral neuropathy. We will still carry out the suite of approaches and experiments outlined in the proposal, including IVCM as a powerful approach to quantify neuropathy.

IVCM as a noninvasive technique for evaluating corneal nerves in NHPs and humans as a way to detect and measure PNS damage by neurotropic viruses and more general neuropathy. In conjunction with an artificial intelligence approach (DeepNerve), it is possible to quantify morphologic changes in NHP corneal nerves as a function of virus infection. The following is a summary of data from IVCM/Deep Nerve analysis to quantify peripheral neuropathy:

Preliminary data presented in the Discovery Project with the NHP ZIKV model of neuropathogenesis indicated that the virus likely affects both the CNS and PNS. Macaque models are used in a variety of diseases characterized by corneal sensory nerve fiber loss. We used automated analysis of nerve fibers leveraging technologies in computer vision and machine learning to process macaque IVCM images. This includes the development and implementation of an innovative deep convolutional neural network (CNN) for IVCM. The deep learning paradigm is to learn both the feature extraction (filters) and classifier using CNNs and supervised learning. Through this process the CNNs are capable of building rich, layered (deep) representations of the data that are then classified through additional layers of representation and learned associations.

We acquired IVCM images from anesthetized macaques using a Heidelberg HRTIII outfitted with the Rostock corneal module. A single scan acquired images at different depths at up to 30 frames a second. Using this information, it was possible to assess and measure three parameters of nerve structure, corneal nerve fiber length (CNFL) corneal nerve fiber density (CNFD) and corneal nerve fiber tortuosity (CNFT). We compared the performance of several neural network/deep learning architectures evaluate IVCM images relative to established manual inspection

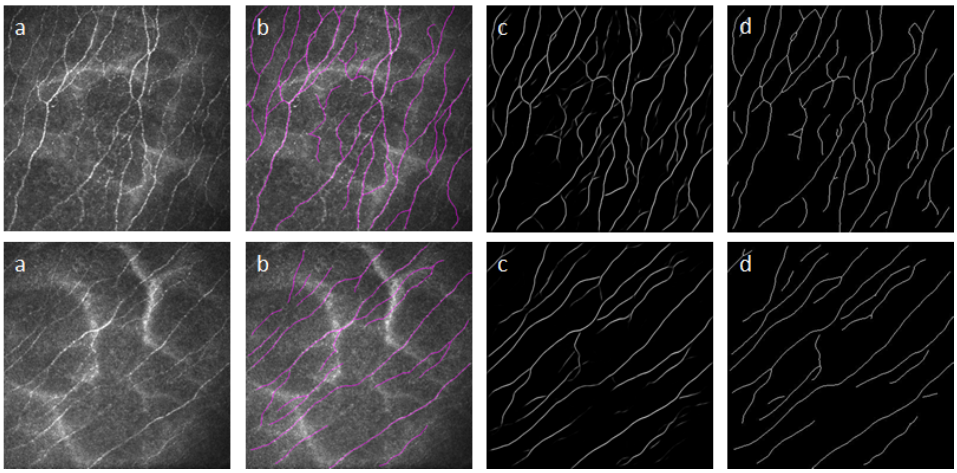


**Figure 1 -** Example macaque data used during the inter-observer evaluation study. The images in the first column (a) are input images; the second column shows their manual tracings (b); the third column images are the probability images from the output of the neural network (c); and the final column gives those images thresholded and skeletonized (d).

protocols by expert readers. The overall processing pipeline is illustrated by the example shown in **Fig. 1.**

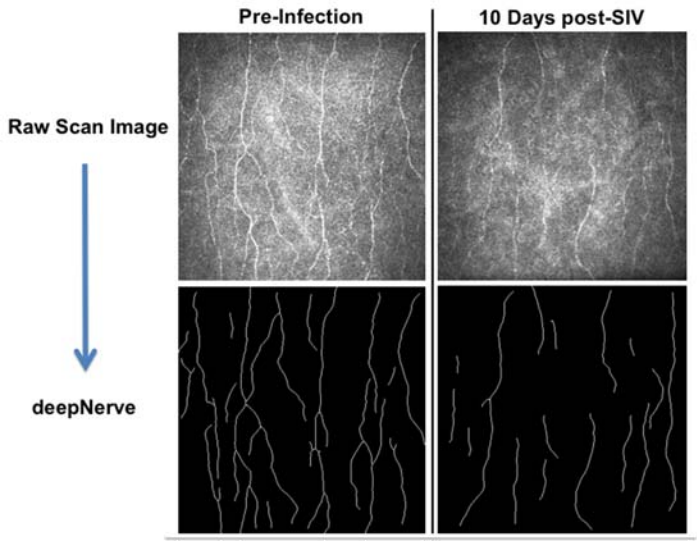


**Figure 2 –** IVCM/Deep Nerve analysis of macaque corneal nerves before and after SIV infection.

While we were not yet able to initiate our ZIKV project it was possible to set up IVCM analysis using available SIV-infected animals. Neurotropic viruses, including HIV and SIV, can cause peripheral neuropathy through multiple potential mechanisms. Immunostaining of excised corneas for the nerve fiber marker beta-III tubulin, which detects nerves in the corneal sub-basal plexus, SIV-infected macaques progressing to AIDS display markedly diminished corneal nerve fiber density. **Fig. 2** illustrates IVCM/Deep Nerve evaluation and quantification of neuropathy in a macaque acutely infected with SIV. Evaluation of 12 animals using this method revealed significant changes in CNFL and CNFD but not CNFT following SIV infection (**Fig. 3**). These data display the power of this automated approach for quantifying peripheral neuropathy using noninvasive inspection of corneal nerves. We will use this approach in our pilot project to inform the likely effect of ZIKV infection on ocular peripheral nerves and determine whether administration of AMD3100 is able to block or diminish neuropathy.

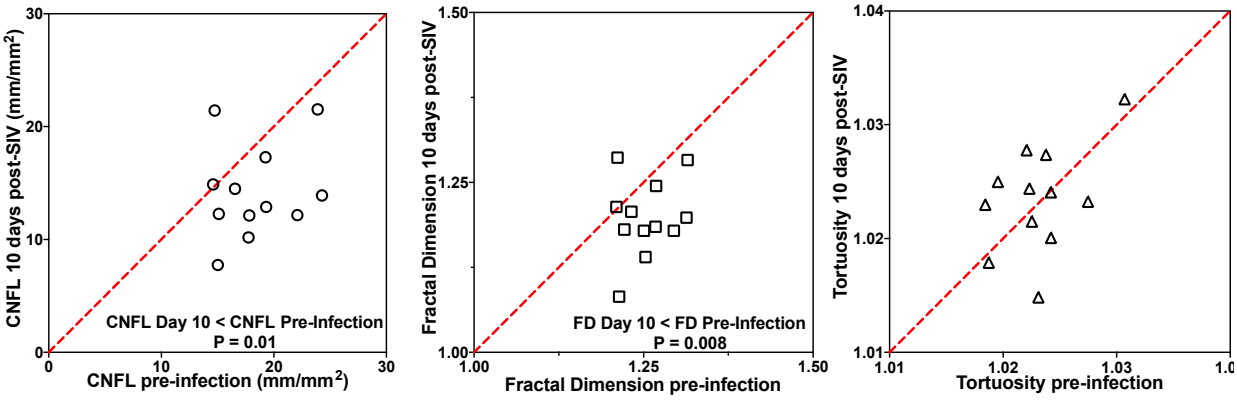


**Figure 3 –** IVCM/Deep Nerve analysis of macaque corneal nerve integrity prior to and after infection with SIV. Twelve macaques were infected with SIV and corneal nerve parameters, CNFL, CNFD, and CNFT quantified using Deep Nerve analysis of images derived from IVCM. Each graph compares CNFL, CNFD, or CNFT in the paired corneal nerve images from individual macaques prior to infection and 10 days post SIV infection. This analysis revealed significant decrease in CNFL and CNFD following infection but no significant change in CNFT following virus infection.

- **What opportunities for training and professional development has the project provided?**

The project was not intended to provide training and professional development opportunities. However, training of the Vet staff in use of the IVCM was significant in that this is a new technique for the TNPRC that will be used during the project.

- **How were the results disseminated to communities of interest?**

Nothing to report

- **What do you plan to do during the next reporting period to accomplish the goals?**

We will implement and complete the entire project as outlined in the original proposal and SOW. In addition, we will use the IVCM to enhance the project.

4. **IMPACT:**
   - **What was the impact on the development of the principal discipline(s) of the project?**

Nothing to report

   - **What was the impact on other disciplines?**

Nothing to report

   - **What was the impact on technology transfer?**

Nothing to report

   - **What was the impact on society beyond science and technology?**

Nothing to report

5. **CHANGES/PROBLEMS:**
   - **Changes in approach and reasons for change**

No changes in approach are planned as we will carry out all techniques and approaches outlined in the original proposal. However, we will include an additional approach to augment the project.

   - **Actual or anticipated problems or delays and actions or plans to resolve them**

For most of 2020 NHP resources at the TNPRC were dedicated to response to the COVID-19 pandemic. This delayed start of the current project.

   - **Changes that had a significant impact on expenditures**

Since start of the project was delayed, there was a corresponding delay in hiring a research technician to assist with the project.

   - **Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents**

Inclusion of IVCM will be added to the vertebrate animal protocol.

   - **Significant changes in use or care of human subjects**

Not applicable

- **Significant changes in use or care of vertebrate animals.**

This project will involve use of vertebrate animals. There are no significant changes to the planned vertebrate animal protocol. It is important to note that the project will not be initiated until all internal (IACUC) and DoD (ACURO) vertebrate animal protocols are approved.

- **Significant changes in use of biohazards and/or select agents**

Not applicable

6. **PRODUCTS:**
   - **Publications, conference papers, and presentations**
     - **Journal publications.**

       Nothing to report

     - **Books or other non-periodical, one-time publications.**

       Nothing to report

     - **Other publications, conference papers, and presentations.**

       Nothing to report

   - **Website(s) or other Internet site(s)**

     Nothing to report

   - **Technologies or techniques**

     Nothing to report

   - **Inventions, patent applications, and/or licenses**

     Nothing to report

   - **Other Products**

     Nothing to report

7. **PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS**
   - **What individuals have worked on the project?**

| | |
|---|---|
| Name: | Antonito Panganiban |
| Project Role: | P. I. |
| Researcher Identifier (e.g. ORCID ID): | 0000-0001-9647-5817 |
| Nearest person month worked: | 1.2 cal. month |
| Contribution to Project: | Dr. Panganiban serves as Principal Investigator of this project. He is responsible for experimental design and coordination with the |

| | |
|---|---|
| | Veterinary staff, and core personnel who will carry out many of the assays to determine the effect of AMD3100 on neuropathy |
| Funding Support: | |

- **Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?**
  Nothing to report

- **What other organizations were involved as partners?**
  Nothing to report

8. **SPECIAL REPORTING REQUIREMENTS**
   Not applicable

9. **APPENDICES:**
   None