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14. ABSTRACT: 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) system we established that results in neuroinflammation. We think the system is likely to recapitulate facets of GBS in adults. In this model, neuroinflammation is triggered by infection with Zika virus (ZIKV). A hallmark of neuroinflammation in these animals is consistent acute and chronic neural upregulation of the chemokine CXCL12. We hypothesize 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. Regulation by CXCL12 is mediated through interaction with its primary cell-surface receptor, CXCR4. Stimulation of CXCR4 by CXCL12 results in activation of multiple signal transduction pathways leading to the migration of CXCR4+ cells. Multiple mechanisms regulate this central CXCL12-CXCR4 axis.

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REPORT

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) system we established that results in neuroinflammation. We think the system is likely to recapitulate facets of GBS in adults. In this model, neuroinflammation is triggered by infection with Zika virus (ZIKV). A hallmark of neuroinflammation in these animals is consistent acute and chronic neural upregulation of the chemokine CXCL12. We hypothesize 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. Regulation by CXCL12 is mediated through interaction with its primary cell-surface receptor, CXCR4. Stimulation of CXCR4 by CXCL12 results in activation of multiple signal transduction pathways leading to the migration of $CXCR4^{+}$ cells. Multiple mechanisms regulate this central CXCL12-CXCR4 axis.

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 inhibition of the CXCL12-CXCR4 axis will mitigate the symptoms of GBS. We will test our hypothesis using an FDA-approved inhibitor of CXCL12, AMD3100 (Plerixafor). AMD3100 inhibits CXCL12 by binding to CXCR4 at high affinity precluding binding of CXCL12 to CXCR4. AMD3100 was originally developed as an anti-HIV drug. Although AMD3100 is not currently used for treatment of HIV-infected patients due to its inability to block macrophage-tropic HIV strains, the drug is clinically efficacious for some types of cancers, including non-Hodgkin's lymphoma, and is under evaluation for additional types of cancer that may be reliant on stimulation of the CXCL12-CXCR4 axis. In addition, AMD3100 is useful for human transplant patients as it mobilizes hematopoietic stem cells. 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?**

Due to prioritization of NHPs for NIH work on COVID-19 our project has been unfortunately delayed. As described in last year's report, we collaborated with Dr. Joseph Mankowski's lab at Johns Hopkins University Medical School to set up noninvasive *in vivo* confocal microscopy (IVCM) to examine the status of the peripheral nervous system (PNS) for the project was likely to be extremely useful for our project. During the past year we have also carried out cell culture experiments to evaluate the response of neuronal cells to ZIKV infection and treatment with AMD3100. It is important to note that addition of these capabilities does not change the objective of the project. IVCM will enhance data interpretation and provide insight into the potential therapeutic effect of AMD3100 on peripheral neuropathy. Similarly, evaluation of neuronal gene expression in response to virus infection and AMD3100 treatment should facilitate evaluation of the host genomic response in our upcoming in vivo experiments. We will still carry out the suite of approaches and experiments originally outlined in the proposal.

As a prelude to NHP studies and to gain insight into the neuronal gene response to ZIKV infection we infected human neuroblastoma cells (SH-SY5Y) with ZIKV (strain Rio-U1) at an MOI of 0.1. Parallel cultures of cells were treated and maintained with 1 μM of the CXCR4 inhibitor AMD3100 for 10 minutes prior to infection. Quantification of viral replication kinetics based on plaque assays indicated robust virus production (Fig. 1). Moreover, production of infectious virus was similar in the presence and

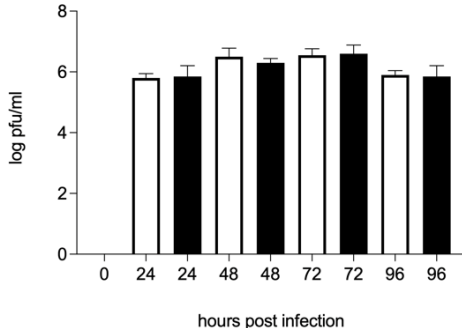

Figure 1. ZIKV replication in SH-SY5Y cells. White – virus titer in the absence of AMD3100. Black – titer in the presence of AMD3100.

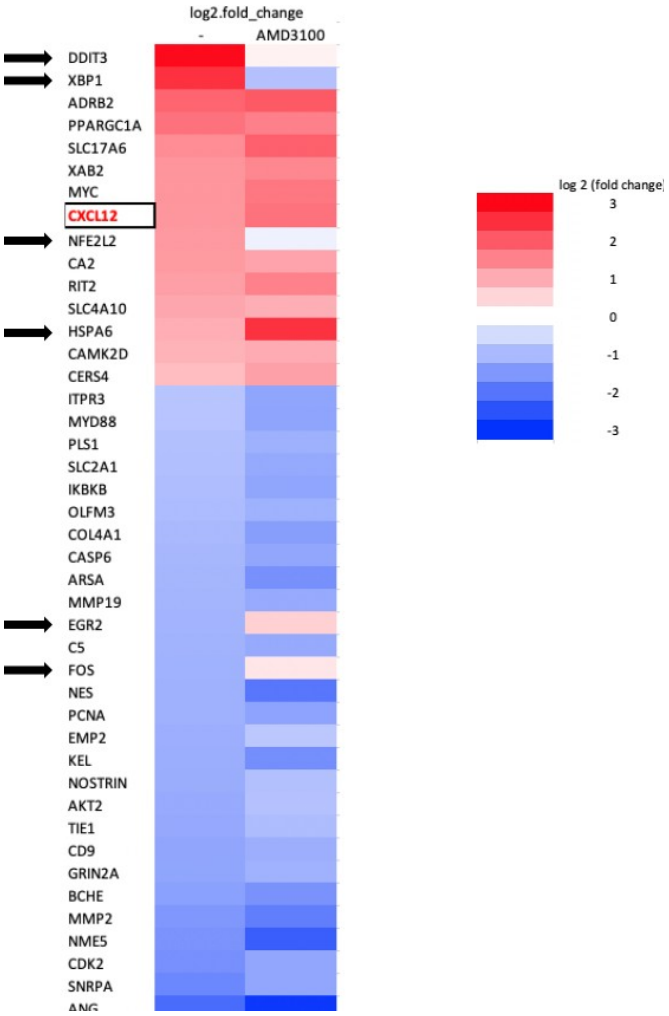

Figure 2. Heatmap showing changes in the expression of host genes following infection of SH-SY5Y cells with ZIKV. The leftward heat map shows differences in expression during ZIKV infection in the absence of AMD3100 and the rightward map in the presence of AMD3100. Only genes exhibiting a 2-fold or greater increase or reduction in expression are shown. CXCL12 is highlighted in red. Arrows depict several genes that display significantly different expression levels in virus-infected cells in the presence of AMD3100.

absence of AMD3100. While virus production was observed over the course of 4 days, we noticed that there was significant cytotoxicity by two days after infection. Since we wanted to define the effect of ZIKV and AMD3100 on neuronal gene expression in the absence of overt cytotoxicity we evaluated gene expression from cells 24 hours after infection.

RNA from infected cells was quantified using nanostring technology using the nCounter® Neuropathology Panel. Nanostring involves the hybridization and capture of target mRNA by virtue of a biotin tag on the initial hybridization probe. Captured mRNAs are then quantified using a second barcoded fluorescent probe. The neuropathology panel contains probes specific to approximately 700 cellular mRNAs previously observed to be changed in their expression in a diverse spectrum of neurological diseases. Genes displaying a two-fold or greater change in expression and highlighted in Fig. 2. ZIKV virus infection resulted in the up- or down-regulation of multiple genes in the set. Interestingly, treatment of cells with AMD3100 significantly changed the expression of a subset of host genes affected by ZIKV infection. Of note, CXCL12, which is central to our project, was upregulated as expected. Further, this chemokine was still highly expressed in the presence of AMD3100. This is consistent with differential expression of some cellular genes due to the inhibition of CXCR4 signal transduction.

- **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 and evaluation of host gene expression 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 additional approaches to augment the project.

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

Until recently, NHP resources at the TNPRC were dedicated to response to the COVID-19 pandemic and a few additional NIH-priority projects. This delayed start of the current project.

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

Since start of the project was delayed, we have not yet dedicated budget for NHPs.

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

Nothing to report

   - **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:	
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