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TITLE: Zika Virus Persistence in Immune-Privileged Organs

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13. SUPPLEMENTARY NOTES

14. ABSTRACT
This project involves the study of Zika virus (ZIKV) persistence within immuneprivileged organs including the testes and eyes. We set out to understand the role of adaptive immunity (e.g., B cells and T cells) as well as interferon regulatory factors (IRF3, IRF5, and IRF7) in controlling ZIKV persistence within immune-privileged organs. During this research period, we discovered that absence of humoral and cellular immune responses results in increased persistence of ZIKV in these organs. Additionally, our initial experiments indicate that interferon regulatory factors, and especially IRF7, also prevents ZIKV persistence in the eye and testes.

15. SUBJECT TERMS
Zika virus, flavivirus, immune-privileged organs, viral persistence

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1. **INTRODUCTION:** The purpose of this study is to examine the role of host immune factors, both adaptive and innate, in controlling persistence of Zika virus (ZIKV) in immune privileged sanctuary sites. In particular, we are examining the role of adaptive immunity and interferon regulatory factors in persistence of ZIKV within the eye and testes.
2. **KEYWORDS:** Zika virus, flavivirus, immune-privileged organs, interferon regulatory factors, adaptive immunity
3. **ACCOMPLISHMENTS:**
    - **What were the major goals of the project?**
        - Major goals of this project, as per the revised and approved statement of work, are to examine persistence of ZIKV within immune privileged organs (e.g., eye, testes, brain) as well as the impact of interferon regulatory factors (IRFs) using IRF3, IRF5, and IRF7 knockout mice. Additionally, we are examining contributions of adaptive immunity (B cells, T cells) using μMT, TCRβ, and Rag1 knockout mice. Timeline for the project is 12 months from the start of project for Irf3/5/7 and 18 months from the state of project for studies of WT, μMT, Tcrβ and Rag1 knockout mice. Major task 1 was revised in a new statement of work approved in early 2018. We have made significant progress on both major tasks, and the overall project is currently ~50% complete.
    - **What was accomplished under these goals?**
        - Major activities: Our major activities have been to examine persistence of ZIKV within the eye and testes in a range of mouse genotypes that lack either interferon regulatory factors or components of the adaptive immune system.
        - Specific objectives: Our specific objectives currently are to measure viral burden at multiple time points and perform histological analysis (e.g., RNA ISH and H&E staining) for these different tissues.
        - Significant results/key outcomes: We discovered that IRF7 and adaptive immunity control viral burden within 1 week after infection. Prior to this, we had done extensive work on TAM receptors under our initial statement of work, which was subsequently revised. We found that TAM receptors had no impact on ZIKV persistence in immune privileged sites.

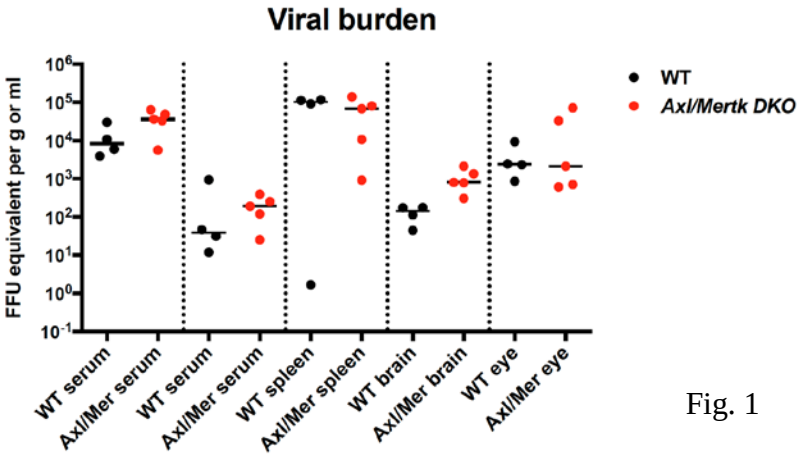

Fig. 1

- Our original statement of work was revised and approved in early 2018 after we discovered that TAM receptors (Axl and Mertk) are not involved in controlling viral infection within immune privileged sites (**Fig. 1**). (Our original proposal focused largely on TAM receptors, but we discovered that they do not contribute to infection in animal models.)

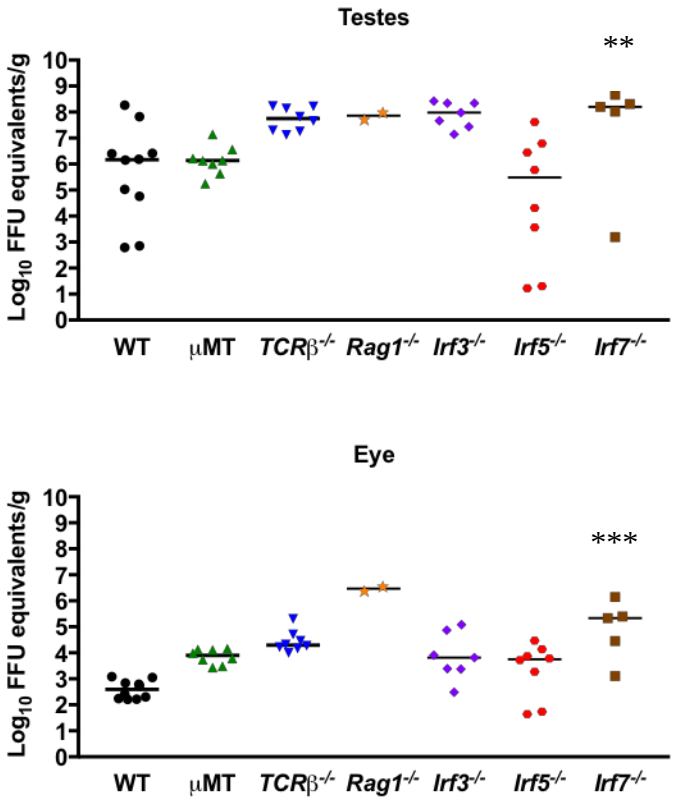

Fig. 2

- In accordance with the revised and approved statement of work from early 2018, we discovered that both adaptive immunity, mediated by antibodies and T cells (μMT, Tcrβ, Rag1), as well as IRF7 play important roles in controlling viral infection of the eyes and the testes 7 days after inoculation with ZIKV (**Fig. 2**).

Data represent two independent experiments, and additional sample processing is underway for a third experiment. Additionally, we are processing tissues and examining viral burden and tissue damage at later time points so that we have a better understanding of how these genes impact persistence of ZIKV in these organs. Histological analysis is currently underway as described in the paproved statement of work..

- **What opportunities for training and professional development has the project provided?**
  - Nothing to report.
- **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 plan to extend our studies by looking at later time points, to increase the number of samples analyzed at early time points, and to complete the histological analysis as described in our statement of work. We do not anticipate major barriers to completion of the proposed studies.

4. **IMPACT:**
   - **What was the impact on the development of the principal discipline(s) of the project?**
     - Our findings are likely to make a major impact on the base of knowledge by demonstrating the genetic and immunological factors that contribute to clearance of ZIKV from immune privileged organs, including the testes and the eye. This is uncharted territory for the field.
   - **What was the impact on other disciplines?**
     - Our work is likely to affect other disciplines, since very little is known about interferon regulatory factors and control of other viral infections that can infect immune-privileged sites. Our work may be relevant for other viruses. Thus, subsequent studies that are outside the scope of our proposed work may lead to novel therapies to treat other viral infections.
   - **What was the impact on technology transfer?**
     - Nothing to report.
   - **What was the impact on society beyond science and technology?**
     - These studies are likely to impact society by demonstrating how viruses like Zika virus are eliminated from tissues, and especially immune-privileged tissues like the eye and reproductive organs, where viruses are known to persist. Viral persistence may contribute to organ damage. therefore, understanding the mechanisms underlying viral persistence may generate avenues for new therapies that enhance removal of virus and virus-infected cells.
5. **CHANGES/PROBLEMS:**
   - **Changes in approach and reasons for change**
     - Our approved statement of work was revised in early 2018, and the new statement of work received approval prior to initiating experiments related to the changes. The main change to the original statement of work was the removal of studies relating to TAM receptors, and the replacement of these

studies with an examination of contributions of IRFs in ZIKV persistence in immune privileged sites. This change was motivated by preliminary data suggesting that the TAM receptor work was not a promising avenue (**Fig. 1**). Approval of the IRF work (new Major Task 1) was obtained prior to initiating work in the revised Major Task 1. We have already begun generating data in accordance with the revised Major Task 1 (**Fig. 2**). However, the change in direction has slightly delayed our progress to certain extent. Therefore, we will be requesting a no-cost extension because of the new direction and previously approved change to our statement of work.

- **Actual or anticipated problems or delays and actions or plans to resolve them**
  - We have not encountered any major problems. The only delay is associated with a revised statement of work in early 2018 when we discovered that TAM receptors have no impact on ZIKV infection. Subsequently, we received approval of a new statement of work and modified animal protocol, and after this, we initiated work on a new Major Task 1 on interferon regulatory factors and persistence of ZIKV in immune privileged organs.
- **Changes that had a significant impact on expenditures**
  - There were no delays in hiring staff. There are no major changes are anticipated to impact the total expenditures, either positively or negatively--only the pace of the expenditures. The only issue is with the revised statement of work in 2018, we are likely to require a no-cost extension to complete the approved statement of work. This is described in more detail in the beginning of the CHANGES/PROBLEMS section.
- **Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents** See below. All changes already approved.
- **Significant changes in use or care of human subjects.** Not applicable / nothing to report.
  - **Significant changes in use or care of vertebrate animals.** Our vertebrate animal protocol was revised in accordance to our revised statement of work, which was approved in early 2018. The modified animal protocol was approved by our IACUC, reported to the agency, and approved by ACURO
- **Significant changes in use of biohazards and/or select agents.** No changes.

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:	*Jonathan Miner*
Project Role:	*PI*
Researcher Identifier (e.g. ORCID ID):	*0000-0001-9551-2962*
Nearest person month worked:	*1.20 calendar months*
Contribution to Project:	*Dr. Miner has supervised these studies and assisted with experiments.*
Funding Support:	*N/A*
Name:	*Amber Smith*
Project Role:	*Laboratory technician*
Researcher Identifier (e.g. ORCID ID):	*N/A*
Nearest person month worked:	*1.70 calendar months*
Contribution to Project:	*Ms. Smith has performed the ZIKV persistence studies.*
Funding Support:	*N/A*
Name:	*Cathrine Miner*
Project Role:	*Laboratory technician*
Researcher Identifier (e.g. ORCID ID):	*N/A*
Nearest person month worked:	*5.80 calendar months*
Contribution to Project:	*Ms. Miner has managed the animal colony for the proposed studies.*
Funding Support:	*N/A*

   - **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**
    - **COLLABORATIVE AWARDS:** Not applicable.
    - **QUAD CHARTS:** Not applicable.
9. **APPENDICES:** Not applicable.