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TITLE: Age-Associated Microglia/Macrophage Response Inhibits Remyelination

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14. ABSTRACT

Purpose: The purpose of this research is to evaluate whether reactive oxygen species are an age-associated inhibitor of remyelination. We will evaluate ROS deposition during remyelination, evaluate the sources of ROS, and use ROS lowering medications to evaluate how impacts remyelination

Scope: We will use animal models of remyelination, microglial fate mapping, ROS measuring techniques and single cell RNA sequencing of ROS enriched cells to evaluate the source of ROS production. We will use medications to lower ROS and evaluate how ROS impacts remyelination

Major findings: Currently we have conducted microglial fate mapping and found that age is associated with a delayed accumulation of microglia without altering monocyte-derived macrophage entry.

15. SUBJECT TERMS
None listed.

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1. Introduction

Background: Multiple sclerosis (MS) is associated with ongoing neurodegeneration driven by the loss of neurons and axons. Remyelination protects axons from degeneration, slowing the permanent disability related to axonal loss. For these reasons, boosting remyelination for people with MS is an active area for clinical trials as remyelination therapies could benefit most people with MS. However, remyelination naturally declines with age, potentially limiting how long any remyelination therapy may help people with MS. We currently understand that much of the age-related remyelination decline relates to the impaired macrophages and microglia immune response. In this proposal, we will investigate a new potential, age-related, remyelination inhibitor: the production of reactive oxygen species (ROS) from microglia and macrophages. **Hypothesis:** We find more ROS production by microglia/macrophage and lipid peroxidation in middle-aged mice. ROS is a known toxin that may either kill or stall oligodendrocyte lineage cells during remyelination. **We, therefore, hypothesize that a population of aged, but not young, microglia /macrophages exaggerate ROS production that inhibits remyelination.**

Aim1: Study design: We will differentiate microglia and macrophage using transgenic models and dissect ROS production in young and middle-aged mice following focal demyelination produced by intraspinal injection of LPC (lysolecithin). We will assess essential ROS producing enzymes, NADPH oxidase and examine the extent of lipid peroxidation in young and middle-aged mice.

Aim 2. Study design: To probe ROS production after demyelination in middle-aged and young mice, we will isolate microglia and macrophage, stratify them based on the production of ROS and conduct single-cell RNA sequencing. This approach, called Tox-seq, will identify the age and ROS-associated microglial and macrophage phenotypes that likely contribute to age-dependent remyelination decline. We will use ligand-receptor interaction to define potential candidates that impair oligodendrocyte differentiation or promote microglia/macrophage ROS production. We will screen candidates in culture.

Aim 3. Study design: We propose that ROS may be a remyelination obstacle enriched during aging. We find more lipid peroxidation and NADPH oxidase in middle-aged mice, and ROS is known to impair oligodendrogenesis. We will test a NOX inhibitor in middle-aged mice to determine if lowering ROS production improves remyelination in these mice.

From this work, we will also lay the groundwork for the expanded hypothesis that an age-associated population of microglia or macrophage actively inhibits remyelination. From the data collected here, we will define such phenotypes and identify new targets that we will examine in future years.

2. Keywords

Multiple Sclerosis, Remyelination, Myelin, Microglia, Macrophages, Reactive Oxygen species

3. Accomplishments

What were the major goals of the project?

In the first 12 months our goals were to:
1. Accomplish subtask 1: amend local AUP protocols
2. Accomplish subtask 2: Submit documents for DoD Animal Care and Use Review Office (ACURO)
3. Accomplish subtask 3: complete animal breeding
4. Nearly complete subtask 4: conduct LPC surgeries on those animals breed in subtask 3
5. Complete Subtask 6: conduct single cell RNA sequencing

What was accomplished under these goals?

For the most point, we have made great progress accomplishing these tasks. I recruited a person onto this project and they are starting to make progress. We have accomplished subtask 1 and 2, and have ACURO approval to conduct our research. We have also accomplished subtask 3 and 4 to complete the breeding and conduct LPC surgeries on our mice to understand how remyelination changes in young and middle-aged mice. To this affect, we have completed an analysis of these mice to differentiate microglia and monocyte-derived macrophages. We have found that monocyte-derived macrophages did not differ between young and middle-aged mice. Instead, microglia recruitment to the site of damage is delayed (Fig 1)

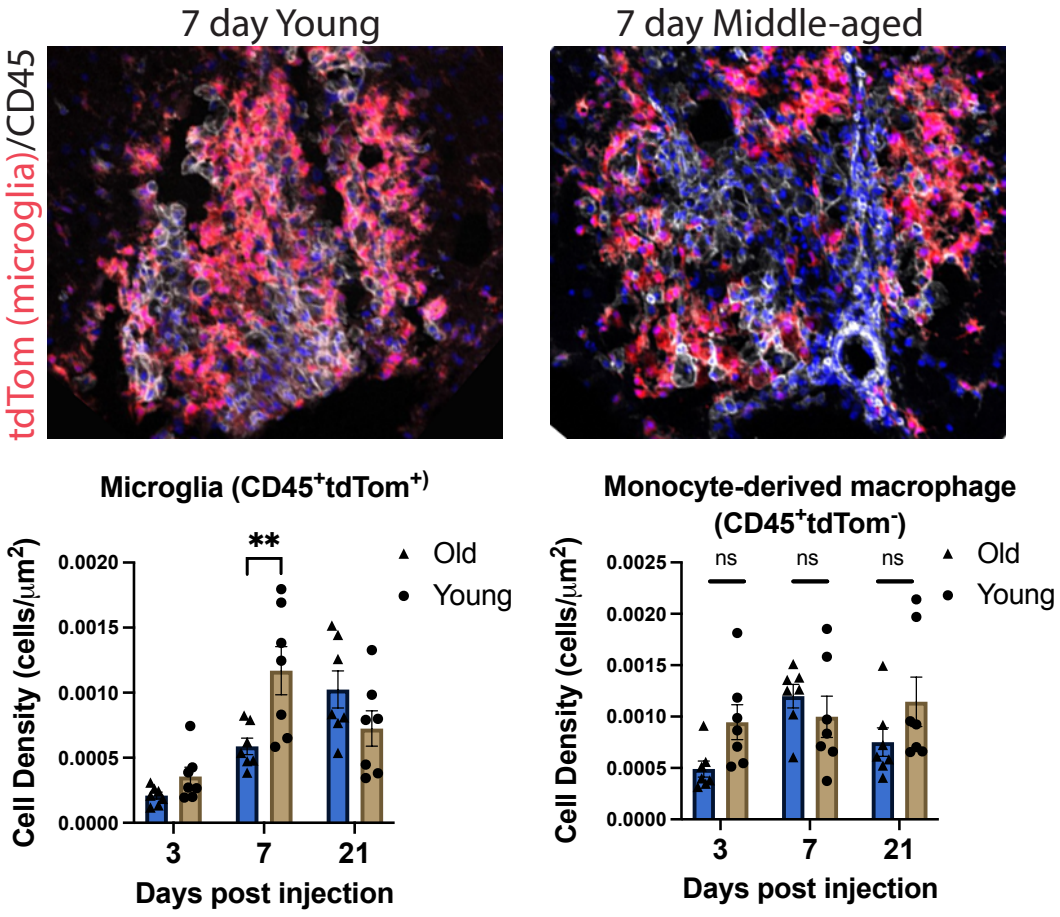

Figure 1: Microglia were fate mapped by giving Cx3Cr1^{creER};Rosa^{tdTom} tamoxifen at 2 weeks of age. Virtually all microglia, but less than 1% of monocytes retain tdTomato (tdTom). We injected Cx3Cr1^{creER};Rosa^{tdTom} with LPC to monitor microglia (CD45+tdTom+) and monocyte-derived macrophages (CD45+tdTom-) during remyelination in young (8-12wk old) and middle aged (8-12month old) mice. We find microglia accumulation is delayed in older mice, but ultimately reach equivalent levels

We did have one stated goal that was not met, complete subtask 6. We were delayed for several reasons (see below) and will work to complete this task in the upcoming year.

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

One activity that we accomplished this year is training and development for a new person in my research group. This person (Sharmistha Panda) started research in Jan 2022 and accomplished learning the animal ethics, mouse handling, mouse IP injections, aseptic technique, and will soon learn the animal surgery. They have also supported writing a book chapter related to microglia and remyelination. In addition to this work, they have acquired new technical skills such as immunohistochemistry, confocal microscopy, image analysis, and statistical analysis and presentation.

How were the results disseminated to communities of interest?

Results from this project were yet to be disseminated, but we plan to present this research for the first time in Dec at a provincial MS meeting.

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

With much of the research training accomplished we are expecting to make good progress to catch-up (completing subtask 6) and complete the other subtasks. If we are falling too far behind I may need to accelerate progress by hiring another part time technician to further support the research.

4. Impact

What was the impact on the development of the principal discipline(s) of the project?

Fundamental to the research at hand is understanding why remyelination slows with age. It is an important question because current medications being tested in early clinical trials are finding improved remyelination (based on biomarkers) in younger, but not older, people with MS. Given MS is a disease of decades duration, age is a critical concern to develop new remyelination approaches. A large body of literature finds that microglia function is impaired in aging animals during remyelination. Often the microglial response is described as reduced. We used new tools and find that microglia, but not monocyte derived macrophages, have delayed—not reduced—accumulation (Fig1). These data suggest that strategies to boost remyelination during age should accelerate the early microglial response. In terms of MS, microglia should be an important target to accelerate remyelination, especially how age impacts microglia function.

What was the impact on other disciplines?
For other neurodegenerative diseases the slowed microglia response during remyelination in older animals may provide a clue as to why age is an important factor to drive neurodegeneration. If microglia are important in protecting tissue, and their response becomes delayed with age, the brain tissue may reach a tipping point where limited microglia protection augments neurodegeneration. Potentially, accelerating how microglia respond to neurodegenerative signals could better protect the brain in neurodegenerative diseases like Alzheimer's disease

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
We have currently not changed our approach, but may need to change make changes (outlined in next paragraph).

Actual or anticipated problems or delays and actions or plans to resolve them
We have faced several problems this year. The first important problem is that we were very delayed with regards to the start of the key personnel on this project. Specifically, Sharmistha Panda was set to arrive Sept 2021, but due to covid-related visa delays she did not arrive until just before Christmas and only really began in the lab Jan 2022. Thus we lost 4 months of her productivity.
We are facing other problems. Currently all of the lipid peroxidation antibodies that we used to start this project have all been discontinued by vendors. We are testing new antibodies and hoping that some can work. As an alternative we are exploring assays that may give better sensitivity. These plate-reader based assayscan measure different lipid peroxidation bio-products in homogenized tissue. This would not add substantial funds to our project
Another plan we are considering changing is to conduct scRNAseq (tox seq) on all glial cells during remyelination. The current plan is to conduct tox seq on just the microglia/macrophages. Expanding Tox Seq to all glial cells would provide more data for the same costs. This approach would provide us a more holistic view of those ROS positive cells during remyelination in aged mice. Our thinking is that new studies highlight other cellular sources of ROS and we can capture other ROS positive cells using a slight modification to our tox seq protocol. Specifically, we would sort viable cells (all of them) instead of microglia/macrophages (CD11b+ cells) prior to scRNAseq. This adjustment would not change the costs of this project

Changes that had a significant impact on expenditures
Nothing to report

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
Nothing to report

Significant changes in use or care of vertebrate animals.
Nothing to report

Significant changes in use of biohazards and/or select agents
Nothing to report

6. Products

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:	**Sharmistha Panda**
Project Role	**Graduate Student**
Researcher identifier	**n/a**
Nearest person worked months	**8 months**
Contribution to project	**Is leading the assessment of ROS in tissue, the single cell RNA seq, and will conduct drug testing**
Funding Support	**100% from this CDMRP funding**

Name:	**Kelly Lee**
Project Role	**Research technician**
Researcher identifier	**n/a**
Nearest person worked months	**4months**
Contribution to project	**Support flow cytometry, animal surgeries, animal breeding, animal ethics, and research training for this project**
Funding Support	**40% from this from this CDMRP funding and 60% from a CIHR grant held in my name**

Name:	**Andre Fare**
Project Role	**Summer student**
Researcher identifier	**n/a**
Nearest person worked months	**3months**
Contribution to project	**Worked on RNAscope and histology for the project**
Funding Support	**100% from this from this CDMRP funding**

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

Nothing to report

9. Appendices

Nothing to report