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TITLE: Targeting Regulatory T Cells to Treat Chronic Migraine and Post-Traumatic Headache

PRINCIPAL INVESTIGATOR: Yu-Qing Cao

CONTRACTING ORGANIZATION: Washington University, St. Louis, MO

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Yu-Qing Cao

Email: caoy@anest.wustl.edu

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Washington University Pain Center
Department of Anesthesiology
Washington University in St. Louis 660 S
Euclid, St. Louis 63110

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14. ABSTRACT

In this study we propose test the hypothesis that that reduced regulation of immune homeostasis by regulatory T (Treg) cells at peripheral tissues contributes to the chronification of headache and cognitive impairment in chronic migraine (CM) and post-traumatic headache (PTH). The research objective is to validate Treg as a cellular target for novel, peripherally active therapy for CM and PTH, with mechanisms distinct from the existing treatment options.

We made significant progress during the last funding period. **First,** we are on track of establishing breeding colonies for the transgenic mice to be used in Specific Aims 1 and 2. **Secondly**, we have established the methodology of profiling time-dependent changes of immune cells by mTBI in the peripheral blood to be used in Specific Aims 1A and 3A. **Lastly**, we have investigated the molecular mechanisms underlying the therapeutic effects of low-dose interleukin-2 (ld-IL2) and Treg cells on CM-related behavioral and neuronal sensitization. Our results not only indicate that ld-IL2 and Treg cells engage both peripheral IL-10 and TGFβ signaling pathways to reverse CM-related sensitizations, but also suggest that the IL-10 and TGFβ1 signaling pathways in trigeminal ganglion neurons are potential targets for CM therapy. Similar strategy will be used in Specific Aim 2B to elucidate the mechanisms through which ld-IL2 reverses mTBI-induced behavioral and neuronal changes.

15. SUBJECT TERMS

Chronic migraine, post-traumatic headache, mild-traumatic brain injury, cognitive defect, regulatory T cell, trigeminal ganglion

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1. Introduction

In this study we propose test the hypothesis that that reduced regulation of immune homeostasis by regulatory T (Treg) cells at peripheral tissues contributes to the chronification of headache and cognitive impairment in chronic migraine (CM) and post-traumatic headache (PTH). The research objective is to validate Treg as a cellular target for novel, peripherally active therapy for CM and PTH, with mechanisms distinct from the existing treatment options. The scope of the research contains 3 Specific Aims: **1)** To investigate whether endogenous Treg cells regulate the development and maintenance of recurring headache and cognitive impairment in CM and PTH; **2)** To elucidate the mechanisms through which low-dose interleukin-2 (ld-IL2) treatment and Treg cell transfer reverse CM and PTH; and **3)** To determine whether FDA-approved drugs simvastatin and vitamin D3 are effective for CM, PTH and mild traumatic brain injury (mTBI)-induced cognitive impairment through targeting endogenous Treg cells.

2. Keywords

Chronic migraine (CM), post-traumatic headache (PTH), mild-traumatic brain injury mTBI), cognitive impairment, regulatory T (Treg) cell, low-dose interleukin-2 (ld-IL2), interleukin-10 (IL-10), transforming growth factor beta 1 (TGFβ1), trigeminal ganglion (TG) neurons, peripheral sensitization, calcitonin gene-related peptide (CGRP), pituitary adenylate cyclase-activating polypeptide (PACAP)

3. Accomplishments

What were the major goals of the project?

Obtain IACUC and ACURO approvals on animal study **completed**

Specific Aim 1) To investigate whether endogenous Treg cells regulate the development and maintenance of recurring headache and cognitive impairment in CM and PTH.

Purchase C57BL/6J and DEREG mouse breeders from The Jackson Laboratory and establish the colonies to breed mice for experiments. **70% completed**

Aim 1-A Experiment 1. Using flow cytometry to characterize mTBI-induced changes of circulating Tregs and other immune cells. **30% completed**

Specific Aim 2) To elucidate the mechanisms through which ld-IL2/Treg cells reverse CM and PTH.

Purchase TβRIIf/f, IL10Rαf/f and AvCreERT2 mouse breeders from The Jackson Laboratory, establish breeding colonies and generate AvCreERT2-TβRIIf/f and AvCreERT2-IL10Rαf/f mice for experiments. **40% completed**

Aim 2-B) Experiments 1-4. To test if neutralizing antibodies against TGFβ or IL10 abolishes the effects of ld-IL2 on mTBI-induced behavioral and neuronal changes. **30% completed**

Specific Aim 3) To determine whether FDA-approved drugs simvastatin and vitamin D3 are effective for CM, PTH and mTBI-induced cognitive impairment through targeting endogenous Treg cells.

Experiment 1. Using flow cytometry to examine whether simvastatin and/or vitamin D3 increases circulating Treg cells and whether they alter the number of other immune cells. **20% completed**

Experiment 2. Using immunohistochemistry to examine simvastatin- and/or vitamin D3-induced changes of Tregs and other immune cells in dura, TG and hippocampus. **20% completed**

What was accomplished under these goals?

1) We are by and large on track of establishing breeding colonies for C57BL/6J and DEREG mice (Aim 1) as well as AvCreERT2-TβRIIf/f mice (Aim 2) for experiments in the following years. The generating of AvCreERT2-IL10Rαf/f mice (Aim 2) will be delayed (see section 5 for details).

2) Dr. Hotchkiss and Dr. Unsinger have established the methodology to profile time-dependent changes of immune cells by mTBI in the peripheral blood (for Aims 1A and 3A). In the first experiment, we will quantify total $CD3^+$ T cells as well as T cell subpopulations ($CD4^+$ T helper cells, $CD8^+$ cytotoxic T cells and $Foxp3^+CD25^+CD4^+$ Treg cells) in the peripheral blood. In the second experiment, we will use FITC-conjugated antibodies against mouse CD3, B220, Nk1.1, ly6G and TER119 to exclude T cells, B cells, NK cells, neutrophils and erythroid cells. This allows us to reliably identify the small population of $CD115^+$ and $CD11b^+$ monocytes in the peripheral blood.

3) As part of Specific Aim 2, we have investigated the molecular mechanisms underlying the effects of ld-IL2 and Treg cells on CM-related behavioral and neuronal sensitization. Ld-IL2 treatment increased the production of cytokines IL-10 and TGFβ1 in T cells, especially Treg cells, suggesting that they may mediate the therapeutic effect of ld-IL2. Neutralizing antibodies against either IL-10 or TGFβ completely blocked the effects of ld-IL2 on the facial mechanical hypersensitivity as well as the sensitization of TG neurons resulting from repeated nitroglycerin (NTG, a reliable trigger of migraine in patients) administration in mice, indicating that LD-IL-2 and Treg cells engage both peripheral IL-10 and TGFβ signaling pathways to reverse CM-related sensitizations. In an in vitro assay, incubation of TG culture with exogenous IL-10 or TGFβ1 fully reversed NTG-induced sensitization of TG neurons, suggesting that the IL-10 and TGFβ1 signaling in TG neurons contribute to ld-IL2′ s therapeutic effects. Collectively, these results not only elucidate the molecular mechanisms through which ld-IL2 and Treg cells reverse CM-related sensitizations, but also suggest that the IL-10 and TGFβ1 signaling pathways in TG neurons are potential targets for CM therapy. These results were published in a recent paper in Neurobiology of Pain (attached as appendix).

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 have updated the SOW in response to the delay of Aims 2A and 3A as well as the early initiation of Aims 1A and 2B. Please see the revised SOW for detailed description.

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

Actual or anticipated problems or delays and actions or plans to resolve them:

Due to COVID-related travel restrictions, Dr. Simoes joined the lab later than anticipated (June 1st, 2022). Consequently, both experiments 1 and 2 in Specific Aim 3A will be completed in year 2.

For Specific Aim 2, since IL10Rαf/f mice requires cryo recovery, the arrival of breeders from the vendor was delayed by 5 months. Consequently, the generation of AvCreERT2-IL10Rαf/f mice and the experiments in Aim 2A using these mice will be delayed.

We have taken actions to compensate for the delay of Aim 3A. **First**, we have initiated Specific Aim 1A, which has been planned for months 15-20. Dr. Unsinger and Dr. Hotchkiss has established the methodology for profiling immune cells in wild-type and DEREG mice (for Aims 1A and 3A). **Secondly,** we have initiated Specific Aim 2B, which has been planned for months 29-36.

We have attached a revised SOW to reflect these changes.

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

Significant changes in use or care of human subjects

Nothing to report

Significant changes in use of biohazards and/or select agents

Nothing to report

6. Products

Publications, conference papers, and presentations

Journal publications

One paper published in Neurobiology of Pain during this period:
Guo, Z., Zhang, J., Liu, X., Unsinger, J., Hotchkiss, R. S., and Cao, Y. Q. (2022). Low-dose interleukin-2 reverses chronic migraine-related sensitizations through peripheral interleukin-10 and transforming growth factor beta-1 signaling. Neurobiology of Pain 12, 100096. PMC9207571

Books or other non-periodical, one-time publications

Nothing to report

Other publications, conference papers and presentations

2022 MHSRS meeting abstract:
MHSRS-22-07045 for Pain Management for the Future Fight
Katherine Czerpaniak, Zhaohua Guo and Yu-Qing Cao
Low-dose interleukin-2 reverses post-traumatic headache-related behaviors and mild traumatic brain injury-induced cognitive impairment in a mouse model.

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?

Yu-Qing Cao, Ph.D, Principal Investigator (3 calendar months): Dr. Cao is responsible/for the overall administration and direction of the project.

Leandro Flores do Nascimento, Ph.D, Postdoc (12 Calendar months): Dr. Nascimento has worked on establishing mouse breeding colonies for Specific Aim 1 (C57BL/6J and DEREG mice) and Specific Aim 2(TβRIIf/f, IL10Rαf/f and AvCreERT2 mice).

Lily Feng, B.S., Research Technician II (3 calendar months): Ms. Feng has assisted Dr. Nascimento to establish mouse breeding colonies for Specific Aims 1 and 2.

Roli Simoes, Ph.D., Postdoctoral researcher (2 calendar months): Dr. Simoes has completed the preparation work and is ready to initiate experiments in Specific Aim 3A.

Richard S Hotchkiss, M.D., Collaborator (0.60 calendar months): Dr. Hotchkiss has provided guidance and supervision on using flow cytometry and ELISA to profile immune cells in Specific Aims 1A, 2B and 3A.

Jacqueline Unsinger, Ph.D., Senior Scientist (4.8 calendar months): Dr. Unsinger has established the methodology for Specific Aim 1A and 3A. She has co-authored the Neurobiology of Pain paper (Aim 2B) with Dr. Guo.

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

One journal article and one meeting abstract attached