PR200804 Targeting Regulatory T Cells to Treat Chronic Migraine and Post-Traumatic Headache

Technical Abstract

This application responds to the FY20 PRMRP Topic Area Chronic Migraine (CM) and Post-Traumatic Headache (PTH) and addresses two Areas of Encouragement: 1) Evaluation of the efficacy of emerging non-opioid pharmaceutical interventions to treat CM and PTH, and 2) Research on the optimal approaches to effective management of pain and co-occurring disorders for CM and PTH so as to decrease polypharmacy.

Background and Rationale: CM and PTH are highly prevalent in US military personnel and veterans due to the stress and mild traumatic brain injury (mTBI) they encounter in the line of duty. The frequent headache is highly debilitating and difficult to treat. Many patients are either not responsive to the current drugs or intolerant to the side effects. Overuse of anti-migraine drugs and opioids leads to medication overuse headache, exacerbating the condition. In addition to recurring headache, mTBI often causes cognitive deficits, emotional difficulties and behavioral disturbances. There is an urgent need to develop novel therapy for CM and PTH with distinct mechanisms of action and are effective for both recurring headache and co-occurring disorders.

Regulatory T (Treg) cells are a specialized subpopulation of $CD3^+CD4^+$ T cells that express high-level of transcription factor Foxp3 in the nucleus and the high-affinity interleukin-2 receptor α (CD25) on the plasma membrane. They possess far-ranging suppressive activity against all types of immune cells, therefore are widely implicated in the maintenance of immune homeostasis. Decrease in Treg cell number and function in the blood have been reported in migraine patients, suggesting a contribution of Treg deficit to migraine pathophysiology.

In a mouse model of CM, repeated administration of nitroglycerin (NTG, a reliable trigger of migraine in patients) significantly increased the total number of $CD3^+$ T cells and macrophages in the trigeminal ganglia (TG) without altering the number of Treg cells. These results suggest a loss of balance between immune activation and Treg-mediated suppression under CM condition, in line with the results of clinical studies. This prompted us to test whether restoring the balance between Treg and other immune cells may reverse migraine chronification. We treated mice with low-dose interleukin-2 (ld-IL2) to preferentially expand and activate endogenous Treg cells. In mice that received repeated NTG administration, ld-IL2 completely reversed CM-related persistent facial skin hyper-sensitivity to noxious stimuli in both male and female mice. The therapeutic effect of ld-IL2 was abolished by Treg depletion and was recapitulated by the adoptive transfer of Treg cells. Importantly, ld-IL2 treatment did not alter basal nociceptive responses, and repeated usage did not induce tolerance, suggesting that targeting Treg constitutes an innovative strategy to treat CM.

Encouraged by these exciting results, we went on to test whether ld-IL2 is effective for PTH. Remarkably, starting ld-IL2 treatment 1 week after mTBI not only accelerated the resolution of acute PTH-related facial mechanical hyper-sensitivity after mTBI, but also prevented the development of chronic PTH-related latent sensitization in both male and female mice. Adoptive transfer of Treg cells 2 weeks after mTBI showed similar anti-PTH effect, supporting Treg as a cellular target to treat both acute and chronic PTH.

At cellular level, we used ratio metric Ca^{2+} imaging to measure the responses of dissociated TG neurons to CGRP (calcitonin gene-related peptide) and PACAP (pituitary adenylate cyclase-activating polypeptide), the two neuropeptides that are strongly implicated in the sensitization of primary afferent neurons during migraine headache. Both repeated NTG administration and mTBI significantly increased the number of TG neurons that respond to CGRP (CGRP-R) and to PACAP (PACAP-R). Ld-IL2 treatment blocked the increase in CGRP-R and PACAP-R TG neurons in both models.

In addition to inducing PTH-related behaviors in mice, mTBI led to impaired recognition memory in novel object recognition (NOR) test, and ld-IL2 treatment prevented the development of mTBI-induced memory deficit. Collectively, these results suggest that ld-IL2, via the expansion/activation of endogenous Treg cells, is a potential treatment for recurring headache in CM and PTH as well as mTBI-induced cognitive deficit.

Hypothesis and Research Objectives: We hypothesize that reduced Treg regulation of immune homeostasis at peripheral contributes to the chronification of headache and cognitive impairment in CM and 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.

Study Design: We will use well-established mouse models of CM and PTH. To ensure the clinical relevance of the mouse study, we will measure multiple endpoints, including both evoked, reflexive behaviors as well as voluntary responses in operant assays in both male and female mice. We will use the numbers of CGRP-R and PACAP-R TG neurons as cellular markers of peripheral sensitization. We will also measure the expression FOS protein, the immediate-early gene product, in neurons in the trigeminocervical complex (TCC, cervical-medullary dorsal horn) to gauge the activation of the trigeminovascular pathway that generates headache.

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

First, we will ask whether mTBI results in a loss of balance between immune activation and Treg-mediated suppression as in the mouse model of CM. We will test whether mTBI reduces Treg cell number and/or the ratio of Treg cells to other immune cells in TG, dura and hippocampus, the brain area essential for memory formation. **Secondly,** we will selectively deplete endogenous Treg cells in DEREG transgenic mice, which express the diphtheria toxin receptor-EGFP fusion protein in Treg cells. We will ask whether Treg depletion alters the development and/or maintenance of CM- and PTH-related behaviors; whether it affects mTBI-induced memory deficit. **Lastly**, we will use Treg depletion to further verify that the effects of ld-IL2 on PTH-related behaviors and mTBI-induced NOR deficit are mediated through Treg cells.

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

Our preliminary study showed that neutralizing antibodies against transforming growth factor beta (TGFb) or interleukin-10 (IL10) completely blocked the effects of ld-IL2 in the CM model. Moreover, incubation of TG culture with exogenous TGFb and IL10 completely reversed NTG-induced increase in CGRP-R and PACAP-R TG neurons. **First,** we will use conditional knockout mice to selectively eliminate TGFb or IL10 signaling in primary afferent neurons. We will investigate whether ld-IL2 reverses CM-related behavioral and neuronal sensitization through both TGFb and/or IL10 signaling pathways in TG neurons. **Secondly,** we will test whether ld-IL2/Treg reverses mTBI-induced behavioral and neuronal changes through a similar mechanism. If this is not the case, we will explore other suppressive mechanisms employed by Treg cells including the conversion of extracellular ATP to adenosine, the PD-1/PD-L1 pathway, and/or the release of extracellular vesicles.

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.

Although ld-IL2 exhibits excellent safety profiles in recent clinical trials, it has not been approved to treat human diseases yet. The daily injection regimen may also be difficult to follow. Here, we will test FDA-approved drugs simvastatin and vitamin D3 in mouse models of CM and PTH. Both drugs are well tolerated in most people and have been shown to increase the number of Treg cells in previous studies. A randomized, double blind clinical study reports that simvastatin plus vitamin D3 alleviates episodic migraine in some patients but the underlying mechanism is not known. **First,** we will confirm that simvastatin and vitamin D3 increases the number of Treg cells in mouse blood, dura and TG. **Secondly**, we will establish NTG- or mTBI-induced behavioral sensitization, and then treat mice with simvastatin and vitamin D3, alone or in combination, to test whether they can reverse CM- and PTH-related behaviors as well as mTBI-induced memory deficit. If yes, we will investigate whether depletion of Treg cells abolishes the benefits of simvastatin and vitamin D3.

Impact: In the **short term,** results from this study will provide additional support for ld-IL2 as treatment for both the recurring headache and the memory deficit in CM and PTH. It will allow us to predict whether ld-IL2, simvastatin and/or vitamin D3 warrant clinical trials for repurposing them to treat CM and PTH. This ensures the expedient translation of the current study outcomes to clinical trials in the foreseeable future.

In the **long term**, the present study will thoroughly validate Treg as a cellular target for CM and PTH. It will elucidate whether and how endogenous Treg cells contribute to the development and/or maintenance of recurring headache and memory deficit in CM and PTH. Understanding the mechanisms underlying the therapeutic effects of ld-IL2 will lead to the identification of novel molecular targets for CM and PTH. Collectively, outcomes from this project will facilitate mechanism-based drug development for CM and PTH, improving the productivity and quality of life of our soldiers and veterans as well as civilian patients.