term pain are still poorly understood. METHODS/STUDY POPULATION: Given the shifting dynamics of inflammation, it is important to understand the spatial-longitudinal changes and their effects on TBI-related pain. Utilizing a recently developed transgenic caspase-1 luciferase reporter mouse, we characterized the bioluminescence signal evident in both in vivo and ex vivo tissues following repetitive closed head mTBIs. This allowed us to reveal the spatiotemporal dynamics of caspase-1 activation in individual animals over time. Furthermore, we utilize various proteomic and behavioral assays to evaluate the role of caspase-1 mediated inflammation in the development and progression of injury-associated chronic pain. Lastly, by blocking inflammasome caspase-1 activation with a specific inhibitor, we assess its clinical potential as the next therapeutic approach to pain. RESULTS/ANTICIPATED RESULTS: We established that there were significant increases in bioluminescent signals upon protease cleavage in the brain, thorax, abdomen, and paws in vivo, which lasted for at least one week after each injury. Enhanced inflammation was also observed in ex vivo brain slice preparations following injury events that lasted for at least 3 days. Concurrent with the in vivo detection of the bioluminescent signal were persistent decreases in mouse hind paw withdrawal thresholds that lasted for more than two months postinjury. Using MCC950, a potent small molecule inhibitor of NLRP3 inflammasome-caspase 1 activity, we observed reductions in both caspase-1 bioluminescent signals in vivo and caspase-1 p45 expression by immunoblotting and an increase in hind paw withdrawal thresholds. DISCUSSION/SIGNIFICANCE: Overall, these findings suggest that neuroinflammation in the brain following repeated mTBIs is coincidental with a chronic nociplastic pain state, and repeated mTBI-associated events can be ameliorated by a highly specific small molecule inhibitor of NLRP3 inflammasome activation.

463

Childhood Emotional Neglect on Nucleus Accumbens Connectivity in Adult Survivors of Trauma

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OBJECTIVES/GOALS: Neuroimaging research has found that childhood maltreatment is related to reduced activation of the nucleus accumbens. The long-lasting impact of this relationship is not as well understood. This study aims to explore the association between childhood emotional neglect and reward-related functional connectivity in an adult trauma sample. METHODS/STUDY POPULATION: Participants (N=169, M age=, 32.2; SD=10.3; women=94) experienced a traumatic injury and were recruited from a Level I Trauma Center. Two-weeks post injury, participants completed the Childhood Trauma Questionnaire (emotional neglect M=10.6; SD=5.2), a self-reported, retrospective account of childhood maltreatment, and underwent a resting-state functional magnetic resonance imaging (fMRI) scan. Whole-brain resting-state left and right nucleus accumbens connectivity analyses were completed using the CONN Toolbox. RESULTS/ANTICIPATED RESULTS: Whole-brain left nucleus accumbens connectivity analyses revealed one significant region (angular gyrus (AG); p DISCUSSION/SIGNIFICANCE: Results suggest that childhood emotional neglect is related to nucleus accumbens connectivity and a brain region associated with memory, attention, and theory of mind in adult survivors of trauma. Early life emotional neglect may be contributing to heightened baseline reward sensitivity–particularly for social rewards (implicated by the AG).

464

Chronic HIV infection influences the immune response during acute COVID-19 and long COVID

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OBJECTIVES/GOALS: Despite highly effective antiretroviral therapy, people living with HIV (PLWH) experience chronic immune activation and inflammation which may influence the progression of infections such as SARS-CoV-2. Here, we explore the immune response and clinical outcomes in HIV(+) and HIV(-) individuals experiencing acute COVID-19 and long COVID (LC). METHODS/STUDY POPULATION: We performed flow cytometric analyses on peripheral blood mononuclear cells from the following: 1) HIV(-) individuals experiencing acute COVID-19, 2) PLWH experiencing acute COVID-19, and 3) pre-COVID-19 pandemic PLWH. Additionally, we will perform similar analyses for the following: 1) PLWH experiencing LC, 2) PLWH previously infected with SARS-CoV-2 who recovered, 3) pre-COVID-19 pandemic PLWH, and 4) HIV(-) individuals experiencing LC. Flow cytometry panels include surface markers for immune cell populations, activation and exhaustion surface markers (with and without SARS-CoV-2-specific antigen stimulation), and intracellular cytokine staining. We will also analyze how chronic HIV infection and other clinical and demographic factors (e.g., age, CD4 %) impact persistent symptomatic burden. RESULTS/ANTICIPATED RESULTS: Acute COVID-19 results–Overall, PLWH had higher baseline expression of activation markers OX40 and CD137 on CD4+ and CD8+ T cells, along with increased levels of TNFa producing CD8+ T cells. Interestingly, PLWH had increased expression of exhaustion markers PD1 and TIGIT but decreased expression of TIM3 on CD4+ and CD8+ T cells. Additionally, PLWH had decreased levels of IL-2 and IFNg producing CD4+ T cells which suggests functional exhaustion. Long COVID-19 expected results–we hypothesize that the activation and inflammation seen in chronic HIV infection will lead to more immune dysregulation and subsequently worsened symptomatic burden. Additionally, we hypothesize that PLWH may have different frequencies of certain LC manifestations, such as increased rates of neurocognitive impairment. DISCUSSION/SIGNIFICANCE: Our findings suggest that chronic HIV infection influences acute immune response during SARS-CoV-2 infection, and that PLWH have variable expression of exhaustion markers which warrants further study. Additionally, our findings in the LC cohort will aid in characterizing clinical manifestations and immunologic mechanisms of LC in PLWH.

466

Development of a novel tocotrienol analogue, tocoflexol, as a radiomitigator

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OBJECTIVES/GOALS: We have designed an analogue of the Vitamin E tocotrienols called tocoflexol, which improves their pharmacokinetic limitations to make it an effective radiation medical countermeasure. Our goal is to demonstrate that tocoflexol is an

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