decreased PM rigidity compared to acute or normoxic T cell activation, specifically in T cells with upregulated PD1, LAG-3, and Tim-3. Dysfunctional T cells also exhibit changes in specific lipid species. This includes increased free cholesterol and decreased esterified cholesterol among other species. DISCUSSION/SIGNIFICANCE OF IMPACT: Adoptive cell therapy is limited in solid tumors due to a hypoxic, suppressive TME that drives T cell exhaustion. Our data suggest exhausted T cells have fluid plasma membranes that impair synapse formation. Future work will validate this in patient-derived xenograft/TCR-T models that will pave the way for clinical translation.

35

Investigating CD14 as a novel therapeutic target in antiphospholipid syndrome†

Thalia Newman[1] and Jason Knight[2]
[1]Michigan Institute for Clinical & Health Research and [2]University of Michigan Department of Internal Medicine, Division of Rheumatology

OBJECTIVES/GOALS: Autoantibodies targeting beta-2-glycoprotein I (anti-β2GPI) are found in many patients with antiphospholipid syndrome (APS), where they trigger the release of pathogenic neutrophil extracellular traps (NETs). Here, we investigated the relevance of the cell signaling protein CD14 in this process to assess its potential as a therapeutic target. METHODS/STUDY POPULATION: Peripheral blood was collected from healthy donors or primary APS patients (all meeting the most recent ACR/EULAR Criteria). Isolated neutrophils were subjected to bulk RNAseq analysis. Neutrophil expression of CD14 and activation markers was validated by immunoblotting and flow cytometry. For mechanistic studies, affinity-purified anti-β2GPI IgG was added to isolated primary human neutrophils or whole blood in the presence or absence of the CD14 blocking antibody atibuclimab. CD14 and activation marker expression were assessed by flow cytometry, and NETs were visualized by immunofluorescence microscopy and quantified via SYTOX green assay. Ongoing work will involve CD14 blockade in mice subjected to the electrolytic inferior vena cava model of thrombosis, accompanied by APS or control IgG injection. RESULTS/ANTICIPATED RESULTS: Neutrophils from APS patients overexpressed CD14 when compared to healthy controls, and patient neutrophil surface CD14 showed a strong positive association with the activation markers CD11b, CD18, and CD66b. Further, healthy neutrophils activated with patient-derived anti-β2GPI increased surface expression of CD14. Given the association between CD14 and APS-associated neutrophil activity, we asked whether CD14 blockade with atibuclimab could mitigate the effects of anti-β2GPI on neutrophils. Indeed, atibuclimab treatment attenuated anti-β2GPI-induced expression of CD11b, CD18, and CD66b, and NET formation. In vivo, we anticipate that CD14 blockade will reduce APS IgG-enhanced thrombi size and plasma levels of NET biomarkers and inflammatory cytokines. DISCUSSION/SIGNIFICANCE OF IMPACT: Neutrophil CD14 appears to be required for neutrophil activation and NET formation triggered by anti-β2GPI in patients with APS. This work identified neutrophil surface CD14 and its associated downstream pathways as novel therapeutic targets with the potential to restrain not only thrombosis but also the upstream inflammation that underlies APS.

36

Classifying at-home movement states using cortical-basal ganglia activity in Parkinson's disease†*

Rithvik Ramesh, Hamid Fekri Azgomi, Kenneth Louie, Jannine Balakid, Jacob Marks and Doris Wang
University Of California, San Francisco

OBJECTIVES/GOALS: This project aims to improve gait dysfunction treatment in Parkinson's disease (PD) by developing adaptive deep brain stimulation (aDBS) that automatically modulates stimulation in response to specific movement states. We use chronic at-home cortical-pallidal recordings to classify walking vs. non-walking and turning vs. straight walking. METHODS/STUDY POPULATION: Local field potentials from globus pallidus (GP) and electrocorticography from premotor (PM) and primary motor (M1) cortices were recorded in four people with PD (2M/2F) implanted unilaterally (n=2) or bilaterally (n=2) with bidirectional neurostimulators (Summit RC+S, Medtronic, Inc). Concurrent at-home movement was captured with wearable ankle sensors (Rover, Sensoplex Inc). Neural-kinematic signals were segmented into 10-second walking/non-walking and 1-second turning/straight walking epochs. Logistic regression and linear discriminant analysis models classified movement states using power within various frequency bands. Random forests identified walking biomarkers compatible with the Summit RC+S device; real-time decoding performance was evaluated using in silico simulations. RESULTS/ANTICIPATED RESULTS: Over 80 hours of at-home neural-kinematic data were analyzed across 6 hemispheres. M1 alpha (8-13Hz) and beta (13-30Hz) power was lower during walking compared to non-walking in all hemispheres. M1 and PM theta (4-8Hz), alpha, and beta power were lower during turning compared to straight walking in all but one hemisphere. Features from GP were most important for walking/non-walking classification, while cortical features predominated for turning/straight walking. Despite these shared features, the most important frequency ranges for classification varied widely across individuals. Within-subject walking/non-walking classifiers achieved strong performance (AUC:0.77-0.96), with simulations of real-time on-board decoding also demonstrating above-chance decoding in all cases (AUC:0.63-0.85). DISCUSSION/SIGNIFICANCE OF IMPACT: These results support the hypothesis that cortical-basal ganglia oscillations are modulated by specific movement states. Furthermore, this work demonstrates one pipeline that can identify patient-specific movement biomarkers from long-term naturalistic neural-kinematic recordings, advancing the viability of aDBS for gait dysfunction in PD.

https://doi.org/10.1017/cts.2026.10267 Published online by Cambridge University Press