Kourtney D. Weaver
L2
LSU Health Sciences Center, New Orleans, LA

Flavia M. Souza-Smith
LSUHSC, Department of Physiology

Role of Lymph from Alcohol-Treated Animals on Naïve Perilymphatic Adipose Tissue Immunomodulation

Alcohol impairs innate and adaptive immune responses, harming host defense against infections. Chronic alcohol administration has also been shown to impact early manifestations of metabolic dysfunction such as insulin resistance in rodent models. In our previous studies, we found that alcohol induces mesenteric lymphatic leakage and perilymphatic adipose tissue (PLAT) immunometabolic dysregulation. Whether the lymph from alcohol-treated animals is directly causing PLAT immunometabolic dysregulation is still to be determined. We hypothesize that lymph contents from alcohol-treated animals will immunomodulate naïve PLAT explants.

To test this hypothesis, male Fischer 344 rats received Lieber-DeCarli liquid diet containing 36% of calories from alcohol for 10 weeks. Control groups received isocaloric liquid died and were pair-fed. Lymph from alcohol and control animals were collected and co-cultured with PLAT explants from 5 age-matched animals for 48 hours. PLAT explants were also cultured with media containing no lymph to serve as a negative control. The transcriptome was analyzed by isolating RNA from PLAT and supernatant samples and measuring PLAT gene expression of CD3 and CD4 T cells, CD26 and CD38 (CD4 T cells activation), FOXP3 (Tregs), and IL-6, IL-1b, IL-10 cytokines via qPCR. Gene expression was normalized to RPS-13. In addition, secretome analysis was performed using ELISA to detect adiponectin in supernatant samples. ELISA was also used to measure protein concentrations of IL-6, IL-1B, and adiponectin in PLAT protein extract. Protein concentrations detected by ELISA were normalized total protein concentration using BCA protein assay and by PLAT weight.

Levels of gene and protein expression were compared between PLAT samples cultured with lymph from control-animals and lymph from alcohol-treated animals. We observed a trend of an alcohol-induced increase of CD4 gene expression in PLAT samples. FOXP3 and IL-10 gene expression were higher, but not significant, in PLAT samples cultured with lymph from control animals compared to alcohol-treated animals. There was no observed difference in expression of the remaining genes between groups. There was an increased protein expression of IL-6 in PLAT cultured with lymph from alcohol-treated animals compared to control. Protein expression of adiponectin was also increased in PLAT cultured with lymph from alcohol-treated animals. We observed no difference in IL-1B protein concentration between groups. Neither gene expression nor protein were detectable in supernatant samples. Future studies with this model include repeating these experiments with a second cohort of animals to increase sample size. Proteomic analysis of lymph contents from control and alcohol-treated animals will also be conducted. Overall, these studies were the first step in understanding the consequences of chronic alcohol on lymph and its effects on PLAT immune cell milieu.