Submitter Name: Jennifer Blackburn Submitter Email: Jennifer.blackburn@yale.edu
PI Name: Ke Xu PI Email: ke.xu@yale.edu

Multiplex Cytokine Profiling and Single Nuclei RNAseq Reveal Anti-Inflammatory Changes Following Intravenous Δ-9 Tetrahydrocannabinol Administration Among Healthy Participants

Jennifer Blackburn[1, 2], Xinyu Zhang[1, 2], Ral Vandenhoudt[1, 2], Brooklyn A Bradley[1, 2], Youshu Cheng[1, 2, 3], Mohini Ranganathan[1, 2], Deepak C. D'Souza[1, 2], Ke Xu[1, 2]

1. Department of Psychiatry, Yale School of Medicine, New Haven, CT
2. Connecticut VA Healthcare System, West Haven, CT
3. Department of Biostatistics, Yale School of Public Health, New Haven, CT

Medical and recreational cannabis use is increasing, however, the underlying mechanisms of cannabis' effect on immune cells remains controversial. Here, we aimed to understand how Δ-9 tetrahydrocannabinol (THC), the principal active cannabinoid in cannabis, alters inflammation in healthy participants.

Participants (n=20) with no history of major medical or psychiatric disorders were recruited to participate in this study. THC (0.32mg/kg) was administered intravenously over 20 minutes and blood samples were collected before, 70 minutes after (T1), and 5 hours after (T2) the infusion. Peripheral blood mononuclear cells (PBMCs) and plasma were immediately separated and cryopreserved and stored at -80ºC, respectively. Plasma cytokine levels were measured using a 6-plex immunoassay panel for Ella™ (Bio-Techne) and a 46-plex panel for Luminex® (Bio-Techne). Transcriptome changes in PBMCs were detected on the 10X Single Cell Multiome platform.

Nine pro-inflammatory cytokines were decreased following THC administration (TNF, CXCL10/IP-10, CXCL1/GROα, CCL2/MCP-1, CCL3/MIP-1α, CCL4/MIP-1β, CCL20/MIP-3α, CCL5/RANTES, and FLT3LG; ps<0.05), while IL-6, which has pro- and anti-inflammatory functions, was increased. Initial Single cell RNA-seq analysis identified the immunoregulators *FKBP5* and *THBS1* as top genes regulated by THC in monocytes and Nature Killer cells. *THBS1* was decreased at both timepoints whereas *FKBP5* initially increased at T1 and decreased at T2. Our data show that a single dose of THC administration in healthy participants inhibited production of multiple pro-inflammatory cytokines and caused dynamic gene expression changes in specific cell types. Future work will investigate if the same alterations are made by THC in altered immune systems in people living with HIV.