

Delta-9-Tetrahydrocannabinol mediated attenuation of gastrointestinal dysfunction in chronic SIV-infected rhesus macaques involves differential modulation of epithelial DNA methylation and T cell activation

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Background. Medicinal and recreational cannabis use is widespread in HIV positive and inflammatory bowel disease patients. While we previously reported the ability of Delta-9-Tetrahydrocannabinol (Δ^9 -THC) to regulate gene/microRNA expression in the GI tract, it is unknown whether THC exerted these effects via epigenetic modulation of gene/microRNA expression. **Methods.** A modified reduced restricted bisulfite sequencing protocol was utilized to assess promoter DNA methylation in colonic epithelial cells (CE) of SIV-infected rhesus macaques (RMs) administered either vehicle (VEH/SIV; n=2) or Δ^9 -THC (THC/SIV; n=3). **Results.** At 6 months post SIV infection, relative to THC-SIV RMs, several CE genes in VEH-SIV RMs showed enhanced promoter hypermethylation. These included Wnt inhibitory factor-1, basic helix-loop-helix family, member a9, ADAM metallopeptidase with thrombospondin type 1 Motif, 20, Mesoderm specific transcript, TERT (Telomerase reverse transcriptase), POU class 3 homeobox 4, leucine zipper downregulated in cancer 1, potassium channels (KCNF1, KCNG3, KCNC1) and Death associated protein kinase 1. Further, immune response (MB21D1, MAMU-B18), apoptosis (phospholipid scramblase-3), epithelial growth (Stratifin, KLC2) and barrier function (CIGALT1) genes and microRNAs (miR-130a/27b/24) showed hypomethylation in CE of VEH-SIV RMs. These changes in CE were accompanied by marked reduction of T-cell activation (Ki67) in the intestinal lamina propria. At peak viral replication (day 14 post SIV), THC-SIV RMs (separate cohort of n=4) showed significantly lower percentages of Ki67⁺CD4⁺ and CD8⁺ T-cells ($p<0.05$) in intestinal lamina propria compared to VEH-SIV RMs (n=4) suggesting substantially reduced activation induced proliferation. Furthermore, THC-SIV RMs had significantly high total CD4⁺ and low total CD8⁺ T cell percentages ($p<0.05$) in peripheral blood. **Conclusions.** Our findings strongly support a role for differential modulation of promoter DNA methylation in THC-mediated suppression of HIV/SIV induced intestinal epithelial dysfunction. Further, these results demonstrate strong translational potential of cannabinoids for the management of intestinal inflammation in not only HIV/SIV but also other chronic inflammatory diseases.