Delta-9-Tetrahydrocannabinol mediated attenuation of intestinal inflammation in chronic SIV-infected rhesus macaques involves differential modulation of anti-microbial defensins, pro-inflammatory gene and microRNA expression amidst high viral loads

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Background. Increased use of marijuana for both recreational and medical purposes has been reported in both HIV-infected and inflammatory bowel disease patients. We previously showed that chronic administration of Δ^9-tetrahydrocannabinol (Δ^9-THC), the most potent cannabinoid, inhibited viral replication, intestinal inflammation and slowed disease progression in SIV-infected rhesus macaque. In addition, cannabinoids including Δ^9-THC attenuated intestinal inflammation in mouse colitis models and SIV-infected rhesus macaques. Recently, we demonstrated that induction of anti-inflammatory microRNAs (miRNA) in the intestines of acutely SIV-infected rhesus macaques represented one of the mechanisms underlying the anti-inflammatory effects of THC. Nevertheless, it remains unclear whether similar anti-inflammatory microRNA and mRNA induction/modulation occurred during chronic SIV infection. **Methods.** We simultaneously profiled miRNA (TaqMan microRNA OpenArray) and mRNA expression (Microarray) at 6 months post-infection (MPI) in colon of uninfected macaques receiving either vehicle (VEH only; n=6) or Δ^9-THC (THC only; n=3) and SIV-infected macaques administered either vehicle (VEH/SIV; n=13) or THC (THC/SIV; n=8). **Results.** At 6MPI, relative to the VEH only group, 52 (23-up and 29-downregulated) miRNAs showed differential expression in VEH/SIV compared to 32 (11-up and 21-downregulated) in the THC/SIV group. Among the upregulated miRNAs, were several miRNAs, such as miR-106a, miR-22, miR-18a, miR-18b, miR-212, miR-200a, miR-222, miR-29b previously shown to be upregulated in chronic intestinal inflammatory conditions. In addition, the LPS-responsive miR-146b-5p and SIV replication induced miR-190b showed markedly elevated expression only in the VEH-SIV group. Compared to the VEH/SIV group, THC selectively upregulated the expression of 13 miRNAs, out of which miR-422a, and -656-3p were found to directly target MMP8, a proinflammatory matrix metalloproteinase produced by both neutrophils and intestinal epithelium. Interestingly, MMP8 showed significantly reduced expression in the colon of THC/SIV compared to VEH/SIV macaques. We are currently using reporter and overexpression assays to confirm direct targeting of MMP8 by both miRNAs. Most notably, the proinflammatory miR-21, miR-141 and miR-431 previously shown to be markedly upregulated in colons of inflammatory bowel disease patients showed significant downregulation in colons of THC-SIV RMs. Further, all six alpha defensins (1 through 6; 1-4 produced by neutrophils and 5-6 produced by intestinal epithelium) showed significantly reduced expression in THC/SIV compared to the VEH/SIV group, indirectly suggesting attenuated intestinal inflammatory responses. Furthermore, mRNAs encoding tight junction proteins (occludin, claudin-3), anti-inflammatory mucins (MUC13), keratin-8 (stress protection), epithelial proliferation

(PROM1), anti-HIV chemokine CCL5 showed increased expression in colons of THC-SIV macaques. Additional miRNA characterization and immune response studies are in progress. **Conclusions.** Our findings strongly support a role for differential gene and microRNA induction in THC-mediated suppression of HIV/SIV induced intestinal inflammation. 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 of the intestine. Finally, whether THC induces similar miRNA modulation in other tissues requires further investigation.