Oral administration of cannabis with lipids leads to high levels of cannabinoids in the intestinal lymphatic system and prominent immunomodulation

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Supplementary Information

Table S1. Times of maximum concentration in plasma (t_{max}) and one-hour prior to t_{max} (t_{max} – 1h) following oral administration of lipid-free formulation (12 mg/kg) and long-chain triglyceride (LCT)-based formulation (12 mg/kg) of cannabidiol (CBD) and Δ^9-tetrahydrocannabinol (THC) to rats[18].

Cannabinoid	Formulation	t_{max} (h)	t_{max} – 1h
CBD	lipid-free	3	2
	LCT-based	3	2
THC	lipid-free	2	1
	LCT-based	3	2

Table S2. Chromatographic conditions for the detection of cannabidiol (CBD) and Δ^9-tetrahydrocannabinol (THC) in rat plasma, intestinal lymph fluid, mesenteric lymph nodes (MLN), spleen, and human chylomicron (CM) samples[18,53,54].

	Medium	Mobile phase	Stationary phase	Flow rate ($mL.min^{-1}$)	Oven temperature (°C)	IS	Detector/ conditions
CBD	Plasma and lymph	ACN and Water (62:38, v/v)	ACE C18-PFP 150 × 4.6 mm, 3 µm	1	55	DDT	UV/ 220 nm
	MLN	ACN and Water (75:25, v/v)	ACE Excel Super C18 100 × 4.6 mm, 5 µm	0.8	43	DDT	UV/ 230 nm
	Spleen	ACN and Water (75:25, v/v)	ACE Excel Super C18 100 × 4.6 mm, 5 µm	0.8	43	DDT	UV/ 230 nm
	Human-CM	ACN and Water (75:25, v/v)	ACE Excel Super C18 100 × 4.6 mm, 5 µm	0.8	43	DDT	UV/ 210 nm
THC	Plasma and lymph	ACN and Water (62:38, v/v)	ACE C18-PFP 150 × 4.6 mm, 3 µm	1	55	DDT	UV/ 220 nm
	MLN	0.1% (v/v) formic acid in ACN and Water (90:10, v/v)	Waters XBridge C18 75 × 2.1 mm, 2.5 µm	0.3	60	$VitD_3$	MS/MS THC: + 315.2/193.0 $VitD_3$: + 385.3/259.3
	Spleen	0.1% (v/v) formic acid in ACN and Water (90:10, v/v)	Waters XBridge C18 75 × 2.1 mm, 2.5 µm	0.3	60	$VitD_3$	MS/MS THC: + 315.2/193.0 $VitD_3$: + 385.3/259.3
	Human-CM	ACN and Water (90:10, v/v)	ACE Excel Super C18 100 × 4.6 mm, 5 µm	0.6	43	PB	UV/ 220 nm

IS, internal standard; DDT, 4,4-dichlorodiphenyltrichloroethane; PB, probucol.

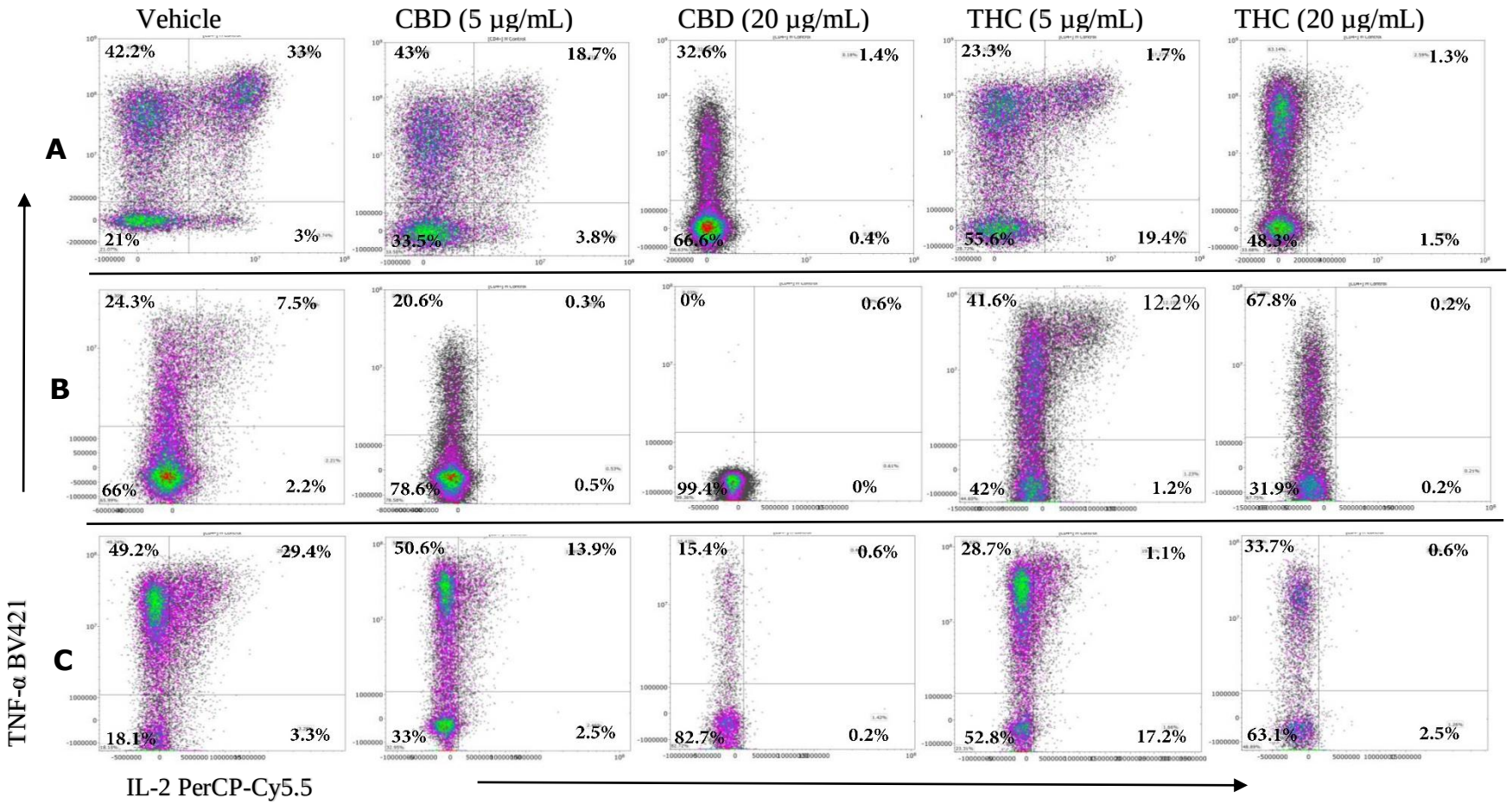

Figure S1. Representative flow cytometry histograms showing the effects of cannabidiol (CBD) and Δ^9-tetrahydrocannabinol (THC) at concentrations of 5 and 20 µg/mL on TNF-α and IL-2 expressing $CD3^+$ T cells isolated from human participants. Cells were stimulated by phorbol myristate acetate and ionomycin (PMA & I) in the presence of brefeldin A. **Panel A:** Effect of CBD and THC on PBMC from a healthy volunteer, **Panel B:** Effect of CBD and THC on PBMC from a multiple sclerosis (MS) patient, **Panel C:** Effect of CBD and THC on PBMC from a patient on chemotherapy to treat non-seminomatous germ cell tumours (NSGCT).

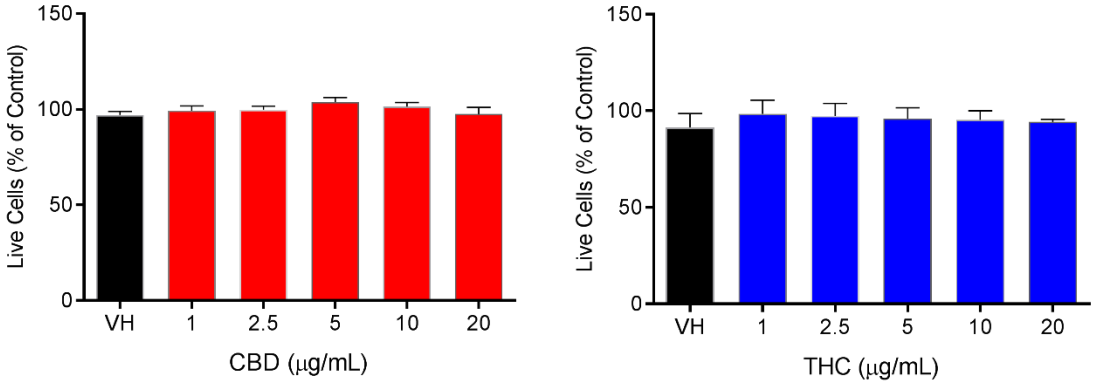

Figure S2. Effects of cannabidiol (CBD) and Δ^9-tetrahydrocannabinol (THC) at concentrations of 1-20 μg/mL on the viability of $CD3^+$ T cells isolated from healthy human participants (n = 5). Statistical analysis was performed using one-way ANOVA with Fisher's LSD test. No statistical differences were observed compared to the vehicle (DMSO)-treated cells (VH).

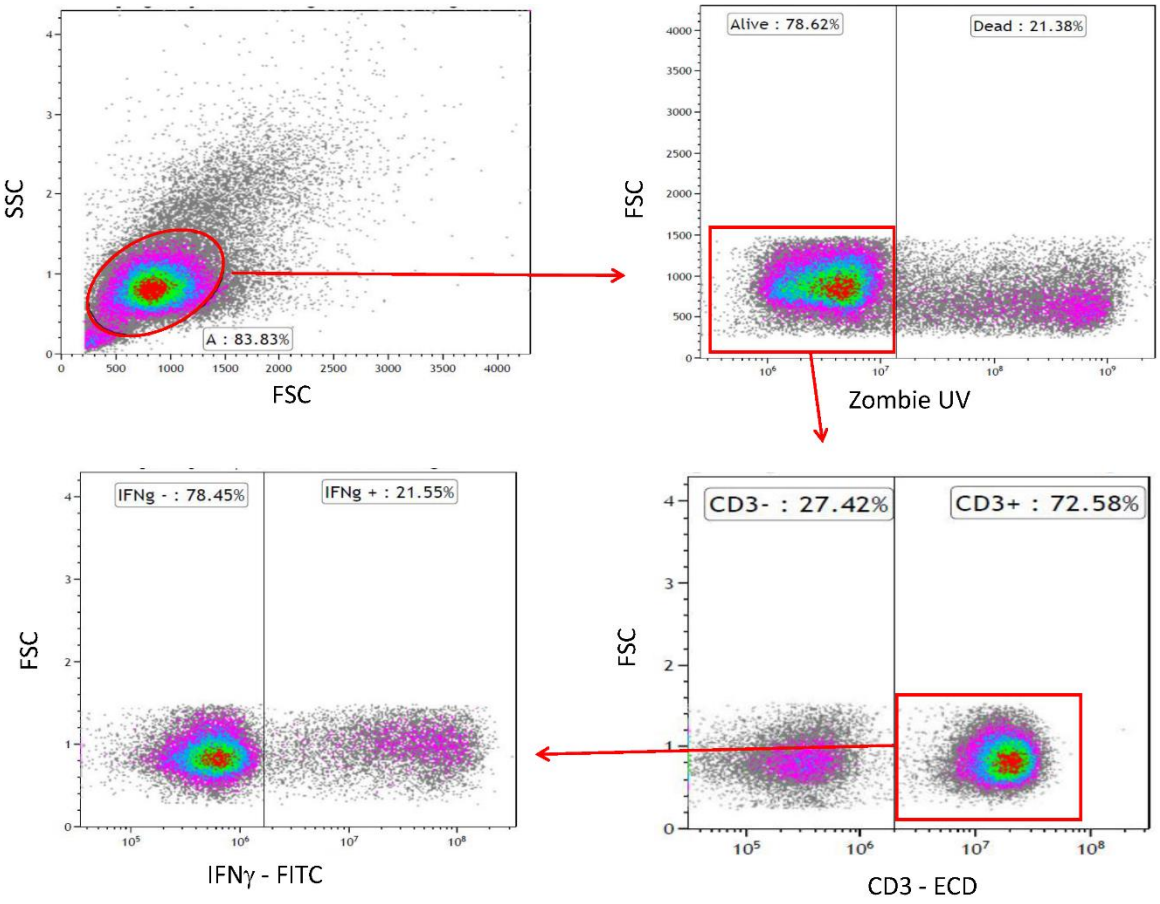

Figure S3. The gating strategy used for the analysis of flow cytometry data for the assessment of intracellular cytokines (IFNγ as an example) as a response to treating immune cells with cannabidiol (CBD) and Δ^9-tetrahydrocannabinol (THC).