Chronic Delta-9-Tetrahydrocannabinol (THC) Treatment Counteracts SIV-induced Modulation of Proinflammatory microRNA Cargo in Basal-ganglia-derived Extracellular- Vesicles

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Abstract

Background:

Early invasion of the central nervous system (CNS) by human immunodeficiency virus (HIV) (1, 2), results in neuroinflammation, potentially through extracellular vesicles (EVs) and their micro RNAs (miRNA) cargoes (3, 4). Although the basal ganglia (BG) is a major target and reservoir of HIV in the CNS (5, 6), whether BG produces EVs and the effect of HIV and/or the phytocannabinoid— delta-9-tetrahydrocannabinol (THC) on BG-EVs and HIV neuropathogenesis remain unknown.

Methods:

We used simian immunodeficiency virus (SIV) model of HIV and THC treatment in rhesus macaques (7) to demonstrate for the first time that BG contains EVs (BG-EVs), and that BG-EVs cargo and function are modulated by SIV and THC. We also used primary astrocytes from the brains of wild type (WT) and CX3CR1^{+/GFP} mice to investigate the significance of BG-EVs in CNS cells.

Results:

Significant changes in BG-EV associated miRNA specific to SIV infection and THC treatment were observed. BG-EVs from SIV infected rhesus macaques (SIV EVs) contain 11 significantly downregulated miRNA. Remarkably, intervention with THC led to significant upregulation of 37 miRNAs in BG-EVs (SIV-THC EVs). Most of these miRNAs regulate pathways related to inflammation/immune regulation, TLR signaling, Neurotrophin TRK receptor signaling, and cell death/response. BG-EVs activated WT and CX3CR1^{+/GFP} astrocytes and reprogrammed astrocyte transcriptome in an EV and CX3CR1 dependent manners.

Conclusions:

Our findings reveal a role for BG-EVs as a vehicle with potential to disseminate HIV and THC induced changes within the CNS.

Background

The advent of antiretroviral therapy (ART) has increased the lifespans of people living with HIV (PLWH) (8). However, high percentage of PLWH remain comorbid with drug abuse, (cocaine and marijuana (9-11)) leading to rapid disease progression (12-17), immune activation, or protection from immune activation in cases of dual drug use (marijuana and cocaine) (9). Although HIV induced neuroinflammation may drive HIV-associated neurocognitive disorder (HAND), the mechanisms by which cannabinoids, such as THC, a component of marijuana modulates HIV-induced neuroinflammation are not completely understood.

Investigating the longitudinal effects of HIV and THC on the brain is difficult to conduct in humans because humans are polydrug users, cigarette smokers, and alcohol consumers. Humans also have variability in patterns and lengths of drug, alcohol, and cigarette usage. These extrinsic factors make studies with human subjects' complex, and data associated with such studies correlational. Animal models, such as SIV-infected rhesus macaque (SIV/RM) model (18, 19) of HIV provides a valuable approach and more controlled environment to study HIV-induced neuroinflammation, the response to long-term treatment with THC, and the effects of HIV/SIV alone or SIV and THC on EV cargo composition and function.

Previously, we showed that the anti-inflammatory effects of long-term low dose THC was associated with its ability to stimulate the release of bioactive blood derived extracellular (BEVs) (18) that induced divergent actin cytoskeletal and signaling cues in SIV infected RM (18). Whether or not THC is capable of reversing neuroinflammatory effects of HIV/SIV is unknown. In addition to the effects of HIV proteins and RNA on persistent inflammation/immune activation, EVs play multifaceted roles in the function/dysfunction of HIV target cells, such as monocytes, microglial, astroglial and non-target cells, including neurons. Broadly defined, EVs released by many cell types (20-32) encompassing exosomes, microvesicles, and apoptotic bodies carry bioinformation (proteins, DNA, diverse RNA profiles) and regulate intercellular/organ communications (24, 29, 31). EVs carry markers of the producer cells and if the producer cells are healthy or diseased, EVs will carry markers corresponding to their state (33, 34). Thus, EVs are important in research (26, 33, 35-37) and drug discovery/therapeutics (38).

The cargo of EVs, including RNA mediate dynamic intercellular crosstalk. EV-associated RNA (exRNA) consists of diverse RNA biotypes that are incorporated into or associated with various circulating carriers, including lipoproteins (39, 40), EVs (41, 42) and extracellular or membraneless condensates (MCs) (42). EV-associated exRNA include but not limited to miRNAs (32). Through association with EVs, miRNAs are protected from degradation by RNases (43).

EVs traverse the blood brain barrier (BBB) and they have been shown to regulate neuroprotective factors (44). Emerging evidence indicate that brain-derived EVs are linked to neurogenesis, neural development, synaptic communication, nerve regeneration, and neuroinflammation (45-48). EVs are used to establish molecular signatures associated with drug abuse comorbid with HIV (18, 44, 49, 50), and as such, may serve as intercellular conveyors of bioactive molecules within the CNS. Indeed, brain-derived EVs have been purified from cultured neurons, oligodendrocytes, microglia, astrocytes, and cerebrospinal fluid (CSF) (51-53). However, there are no available studies describing the properties and functions of EVs from the BG, a series of interconnected subcortical nuclei and a major target/reservoir of HIV in the CNS (5, 6). BG dysfunction is a hallmark of HIV infection and cognitive impairment in PLWH with neuronal death in the BG (54). Moreover, HIV targets the BG leading to loss of dopaminergic neurons (55). A prior study showed an increase in miR-29b in the BG of morphine-dependent SIV-infected RMs compared with controls (4).

In this study, we demonstrate that BG contains bioactive EVs. A significant number of miRNAs are significantly decreased in the BG of SIV-infected RMs. We further demonstrate that low-dose chronic THC treatment counteracts the suppressive effects of SIV on BG derived EV miRNA repertoire, and conversely restores the levels of all of the suppressed miRNAs. Furthermore, BG-EVs internalized by astrocytes alter astrocyte activation and gene expression profiles in an EV and CX3CR1 dependent manner.

Methods

Macaques and viruses: A total of 12 age and weight-matched Mamu-A0*1⁺/B08⁺/B17⁻ specific-pathogen-free (free of SIV, D retrovirus, STLV and Herpes B) male Indian rhesus macaques were randomly assigned to three experimental groups. One group (n=4; Group 1) received twice daily injections of vehicle (VEH/SIV) (1:1:18 of emulphor: alcohol: saline) and second (n=4; Group 2) received twice-daily injections of Δ^9 -THC (THC/SIV) beginning four weeks prior to SIV infection until 6 months post-SIV infection(56). Group 3 (n=4) macaques served as uninfected controls. Chronic administration of VEH (Group 1) or Δ^9 -THC (Group 2) was initiated four weeks before SIV infection at 0.18mg/kg as used in previous studies (7, 56-58). This dose of Δ^9 -THC was found to eliminate responding in a complex operant behavioral task in almost all animals (58). Groups 1 and 2 macaques were infected intravenously with 100 TCID₅₀ dose of the CCR5 tropic SIVmac251 or SIVmac239. Beginning the day of SIV infection, the THC dose was increased for each subject to 0.32mg/kg, over a period of approximately two weeks when responding was no longer affected by 0.18mg/kg on a daily basis (i.e., tolerance developed), and maintained for the duration of the study. The optimization of the THC dosing in RMs accounts for the development of tolerance during the initial period of administration. Because in previously published studies (7, 58) this dose of THC showed protection, the same dose was used in this study. SIV levels in plasma and gingiva were quantified using the TaqMan One-Step Real-time RT-qPCR assay that targeted the LTR gene (56, 57). At necropsy, basal ganglia tissues were collected in RNAlater (Thermo Fisher Scientific) and Z-fix for total RNA extraction and embedding in paraffin blocks.

Δ^9 -THC administration: THC (NIDA/NIH) was prepared as an emulsion using alcohol, emulphor, and saline (1:1:18) as vehicle before use. THC was administered intramuscularly (I/m) and initiated 1 month post SIV infection (MPI) at 0.32mg/kg as reported previously (56, 57) until necropsy (6MPI). This dose of THC showed protection in previous studies in our previously published studies (7, 56-58).

BG-EV purification and characterization: The schematic and workflow for isolation of basal ganglia EVs is shown in **Figure S1**. Briefly, small chunks of RNAlater-stored BG tissues, ranging from 35-118mg, were finely chopped and digested with collagenase III. Samples were clarified and supernatant was purified on a 20 x 0.5cm Sephadex G-50 size exclusion column, using a particle purification liquid chromatography (PPLC) system as previously described (42). Fifty fractions of 200 μ L were collected, and the 3D UV-Vis (230 – 650nm) fractionation profiles were recorded. A no-tissue collagenase control was used as background. After background subtraction and PPLC analysis for particle size and concentration, EV-containing fractions were pooled and stored in small aliquots at -80°C. For further characterization, EVs were diluted in 0.1X PBS (1/1000). Zeta potential (ζ -potential) measurements were acquired using nanoparticle tracking analysis (ZetaView) as described previously (59).

Energy Dispersive X-ray Transmission Electron Microscope with Immunogold-Labeling (TEM-EDX-IL): Equal volumes of BG-EVs from each group were pooled (n=4). 10 μ L were spotted onto TEM grids. Specimens were incubated with anti-CD9 at 4°C overnight. Following washing, samples were incubated with 10nm gold-conjugated anti-mouse IgG for 1 hour, washed, and then followed by a post-stain with uranyl acetate (1%). Specimens were characterized using TEM.

BG-EV RNA isolation: 2mL of PPLC-purified BG-EVs (equivalent of 9.6×10^{11} to 5.7×10^{12} particles or 254 to 984 μ g of EV proteins) were concentrated under reduced pressure at low temperature, and the total RNA was isolated using miRNeasy serum/plasma kit, per manufacturer's protocol. RNA was eluted and the eluate was measured using a NanoDrop 1000.

Small RNA-Seq: Libraries were prepared using 25ng of RNA and 20 cycles of PCR following the manufacturer's recommendations. The libraries were pooled to equal nanomolarity concentrations and then purified and size selected using Pippin Prep. The library pool was profiled using a TapeStation and Qubit before sequencing on the NextSeq 550. Sequencing was performed with single 75bp reads.

Bioinformatics: sRNA-Seq data were processed for filtering, trimming, and QC analyses before generating count matrices. Count matrices were obtained using trimmed reads (minimum length 15) by alignment to the *Macaca mulatta* genome and the *Macaca mulatta* dataset miRBase (miRNA database).

Mouse model: All animal procedures were approved by the Institutional Animal Care and Use Committee (IACUC; Animal welfare assurance No A3011-0), Stony Brook, School of Medicine and conducted in accordance with National Institutes of Health "Guide for the Care and Use of Laboratory Animals" guidelines. Experiments were performed using neonatal (P1-P3) C57BL/6J (WT) and CX3CR1-GFP (60) pups. The brains from these mice were used for preparation of astrocyte primary cultures.

Generation of primary cortical astrocytes: Primary astrocytes were isolated from P1-P3 mouse pups (61, 62). Briefly, T-75 flasks were coated with Poly-D-Lysine for 1 hour at 37°C. Flasks were washed, filled with DMEM 10% FBS and placed at 37°C, 5% CO₂. Brains were dissected, the cerebellum removed, and the cortices placed on ice in 1X HBSS to slow metabolic function. Four cortices were then placed in one mL of papain solution for 15 minutes at 37°C for chemical dissociation, with a brief mechanical dissociation through a p1000 pipette tip. After additional incubation at 37°C for 15 minutes, p1000 pipette dissociation, a p200 pipette was used to further digest the tissue into a homogenous single-cell suspension. Papain activity was neutralized using 20 mL of DMEM 10% FBS media and the solution spun at 3000 RPM for 10 minutes. The pellet was resuspended in 1 mL DMEM 10% FBS, placed into T-75 flask containing 37°C media. On days 3, 6, and 9 of culture, a portion of 10mL of media were removed and replaced with equivalent volume of fresh media. On day 10 of culture, microglia were de-attached and removed. On day 12 of culture, OPCs were de-attached (in a 16-18 hour shake at 300 RPM) and removed. After removal of the OPCs, the remaining cells are astrocytes and were trypsinized and plated in a 10cm dish.

BG-EV internalization: PBS control or BG-EVs were stained with SYTO™ RNaselect™ Green Fluorescent cell stain and purified using Exosome Spin Columns. Labelled BG-EVs were added to cells for kinetic imaging using Lionheart FX automated scope.

Immunofluorescence of activation markers: 10,000 astrocytes were treated with PBS (vehicle) or 100µg/mL of pooled BG-EVs (n=4, 25µg/sample) for 24 hours. Cells were imaged immediately after treatment and at 24 hours post-treatment. Subsequently, cells were fixed, permeabilized, incubated with anti-GFAP, and detected with appropriate fluoroscently-conjugated secondary antibody. Cells were finally stained with DAPI and imaged again using a confocal microscope (Leica Sp8-x) or an automated scope (Lionheart FX).

Real-time quantitative PCR (RT-qPCR): 250,000 astrocytes plated overnight were treated with PBS or with 100 µg/mL of pooled BG-EVs (n=4, 25µg/sample) for 24 hours. RNA was extracted from cells and used for cDNA synthesis. RT-qPCR was performed using a 7500 FAST machine and Power Track SYBR Green master mix.

Cell viability analysis: Percentage of viable cells was inferred by quantitation of cellular ATP, measured using CellTiter-Glo Luminiscent viability assay (63, 64).

Statistical analysis: Differential expression data were generated using Graphpad Prism. The significance cutoff was set to fold change (FC) >1.5 or <-1.5 and a p-value <0.05.

Results

SIV infection and long-term low dose THC treatment do not alter BG-EV physicochemical properties

Clarified supernatant from BG digest is a mixture of collagenase, EVs, and non-EVs. We used a Particle Purification Liquid Chromatography (PPLC) system (42) to i) gain insight into the spectra of BG digest from the three experimental groups (uninfected controls, VEH/SIV and SIV/THC), and ii) identify and collect pure EVs devoid of other factors, such as non-EV membraneless condensates that often times co-purify with EVs (42). Schematic for BG-EV isolation workflow is shown in **Figure S1**. The elution profiles of BG digest from uninfected controls, VEH/SIV, and SIV/THC groups are similar (**Figure 1A**). 3D UV-Vis measurements (fraction/wavelength/intensity) showing 3D-surface plot of EV fractions (**Figure 1B**) and MC UV-peak that blue-shifted to 262 nm (**Figure 1C**) were generated using PPLC analytics. Qualitative turbidity indices in the visible range, defined as and was used for further identification of the different fractions where R₂ index detected in fractions 7-16, indicate the presence of EVs, while fractions 19-28 contain MCs (**Figure 1D**). As indicated in **Figure 1E**, the sizes (133 to 157 nm) of BG uninfected controls, VEH/SIV, and THC/SIV groups are not different. In addition, BG-EV concentration per mg of tissue was similar for all groups (**Figure 1F**), but the ζ-potential of BG-EV membrane showed that BG-EVs bear highly negative surface charge (**Figure 1G**). Furthermore, there are no differences in the total protein level of all BG-EVs (**Figure 1H**).

SIV infection and long-term low dose THC treatment do not alter ultrastructural, elemental, and surface cargo of BG-EVs and BG MCs

TEM-EDX-IL analysis showed that BG-EVs (~100-300 nm) are enriched in CD9 (**Figures 2A-B**). We also analyzed the non-EV MC component of BG. Similar to seminal plasma(42), BG MCs exhibit dense, sharp-edged and membraneless structures with primary particle size of ~10-20 nm that aggregates into large structures up to ~500 nm (**Figure 2C**). Trace elements detected in the EDX spectra of BG MCs confirmed the ribonucleo-proteinaceous nature of BG MCs (**Figure 2D**). The analysis of the ultrastructure and chemical composition of EVs via this method provides rigor and is of the utmost importance for measuring the chemical composition of EVs and other particles.

SIV downregulates proinflammatory miRNA in BG-EVs and THC counteracts the effect of SIV

Table 1. Sequencing Metrics* across each sample.

Sample ID	Raw Reads	Trimmed reads (minimum length 5)	Percentage reads (minimum length 5)	Trimmed reads (minimum length 15)	Percentage reads (minimum length 15)	Reads aligned to mmul genome	Percentage reads aligned to mmul genome	Reads aligned to mmul miRBase	Percentage reads aligned to mmul miRBase
IR97	15,040,626	14,916,991	99.2	14,726,019	97.9	5,801,187	39.39	197,985	1.34
IT18	10,553,653	10,383,011	98.4	10,123,258	95.9	8,449,318	83.46	3,437,862	33.96
GT18	8,502,723	8,407,642	98.9	8,250,430	97.0	7,014,323	85.02	2,224,050	26.96
IV95	12,202,389	11,909,194	97.6	11,402,173	93.4	8,043,460	70.54	180,656	1.58
JD66	13,541,260	13,405,955	99.0	13,232,393	97.7	11,158,775	84.33	593,001	4.48
JR36	9,531,380	9,304,961	97.6	8,964,346	94.1	7,063,324	78.79	353,166	3.94
JI45	11,677,881	11,421,702	97.8	10,918,406	93.5	8,837,853	80.94	3,226,625	29.55
JC85	10,290,459	10,095,165	98.1	9,675,224	94.0	6,639,058	68.62	752,874	7.78
IV90	9,073,363	8,899,837	98.1	8,538,395	94.1	6,826,220	79.95	2,065,222	24.19

* After adapters are trimmed, reads are filtered based on length (5 bp, 15 bp). Filtering reads shorter than 5 bp determines RNA degradation. 15 bp is the minimum length for meaningful alignments.

We analyzed miRNA content of BG-EVs using the Sequencing Metrics shown in **Table 1**. Relative to uninfected control BG-EVs, 11 significantly downregulated miRNAs were identified in BG-EVs of VEH/SIV (**Figure 3A**, blue arrows). Remarkably, 37 miRNAs were significantly upregulated in THC/SIV, and these also included the 11 miRNAs downregulated in VEH/SIV BG (**Figure 3B**, red arrows), along with miR-21 that was upregulated by THC (**Figure 3C**, red arrow). DIANA pathway analysis of predicted miRNA target genes (65) identified various clusters, including inflammation/immune regulation (red arrow heads), TLR signaling (orange arrow heads), Neurotrophin TRK receptor signaling and response to stress (green arrow heads), cell death and viral process (black arrow heads) among the top-pathways (**Figure 3D**). Moreover, BG-EVs from VEH/SIV contain reduced levels of neurotoxic miRNAs (66) (**Figure 3E**), with significant differences in let-7a-5p and let-7c-5p. These data reveal that the neuroprotective/anti-inflammatory factors induced by THC may be mediated by EV-associated miRNAs. The list of downregulated miRs in BG-EVs from VEH-untreated/SIV compared to control RMs is shown in **Table 2**, while **Table 3** presents downregulated miRs in BG-EVs from VEH/SIV compared to controls.

Table 2. List of downregulated miRs in BG-EVs from VEH-untreated/SIV compared to control rhesus macaques

miRNA	Mean Log Read Count (control)	N (control)	Mean Log Read Count (VEH-untreated/SIV)	N (VEH-untreated/SIV)	Log2 Fold change	-log (P-value)
mml-miR-7203-5p	0.1	3	1.465	3	-3.87283	4
mml-miR-105-5p	0.1003	3	1.099	3	-3.4538	2.251812
mml-miR-504-3p	0.1003	3	1.055	3	-3.39485	2.091515
mml-miR-627-5p	0.1	3	0.9929	3	-3.31165	2.236572
mml-miR-374a-3p	0.1003	3	0.954	3	-3.24967	1.752027
mml-miR-6827-5p	0.1	3	0.8882	3	-3.15088	1.866461
mml-miR-1298-3p	0.1	3	0.7518	3	-2.91035	1.434152
mml-miR-582-5p	0.1	3	0.7426	3	-2.89259	1.407823
mml-miR-135a-2-3p	0.4101	3	1.469	3	-1.84079	2.481486
mml-miR-770-5p	0.392	3	1.398	3	-1.83444	2.283997

Primary astrocytes internalize BG-EVs

The internalization of the labeled EVs by astrocytes revealed a green signal (**Figure 4A**) that increased with increasing concentration (**Figure 4B**) and extended time (**Figure 4C**). Treating cells with increasing concentrations of EVs for 18 h showed that EVs from uninfected control and THC/SIV macaques at all concentrations did not alter astrocyte viability. However, 200 µg/mL of BG-EVs from VEV/SIV macaques reduced astrocyte viability but the same concentration of EVs from THC/SIV BG had not impact on viability (**Figure 4D**). This functional data suggest that high amounts of EVs from SIV infected animals may have the potential to compromise astrocyte survival. The reversal of SIV EV-induced decrease in cell viability by THC/SIV EVs suggests that EVs from HIV/SIV infected brain (BG) cells contain factors/molecules that may induce oxidative stress and impair cellular metabolism that are absent or excluded in EVs from BG of THC/SIV macaques.

BG-EVs activate primary astrocytes

Reactive astrocytes are polarized by insults towards neurotoxic A1 (inflammatory/harmful) or neuroprotective A2 (protective) phenotypes, with A1 phenotypes being linked to neurodegenerative diseases and aging(67). In our study, all the EVs irrespective of background (uninfected, VEH/SIV, THC/SIV) upregulated the expression of GFAP, a marker of reactive/activated astrocytes (**Figures 5A-D**). WT and CX3CR1^{+/GFP} astrocytes treated with VEH, SIV, and THC/SIV EVs significantly elevated GFAP (**Figures 5B, 5D**). However, in CX3CR1^{+/GFP} cells, THC/SIV EVs significantly suppressed GFAP levels compared to uninfected control EVs (**Figure 5D**). While BG-EVs elevated GFAP expression in WT and CX3CR1^{+/GFP} cells, CX3CR1^{+/GFP} cells are more activated as shown by significantly higher GFAP levels (**Figure 5E**).

BG-EVs reprogram the transcriptome of primary astrocytes

Primers shown in **Table 4** were used to quantify the mRNA profile of some genes in astrocytes. EVs from all groups increased mRNA expression of CD40 (TNF-receptor superfamily) in WT and CXCR1^{+/GFP} astrocytes (**Figure 6A**). EVs also elevated TNFα mRNA in WT and CX3CR1^{+/GFP} astrocytes (**Figure 6B**). Interestingly, THC/SIV EVs significantly reduced CD40 mRNA in WT but not CX3CR1^{+/GFP} astrocytes (**Figure 6A**) but suppressed TNFα mRNA in both WT and CX3CR1^{+/GFP} astrocytes (**Figure 6B**).

Table 3. List of upregulated miRs in BG-EVs from THC-treated/SIV compared to VEH-untreated/SIV rhesus macaques

miRNA	Mean Log Read Count (VEH-untreated/SIV)	N (VEH-untreated/SIV)	Mean Log Read Count (THC-treated/SIV)	N (THC-treated/SIV)	Log2 Fold change	-log (P-value)
mml-miR-7203-5p	1.533	3	0.1	3	3.938286	4
mml-miR-504-3p	1.283	3	0.1003	3	3.677128	4
mml-miR-105-5p	1.233	3	0.1003	3	3.619779	4
mml-miR-627-5p	1.092	3	0.1	3	3.448901	3.69897
mml-miR-6827-5p	1.033	3	0.1	3	3.368768	3.30103
mml-miR-191-3p	0.9053	3	0.1003	3	3.174074	2.173925
mml-miR-107-5p	0.8257	3	0.1003	3	3.041296	1.835647
mml-miR-323b-5p	0.8105	3	0.1	3	3.018812	2.19382
mml-miR-190b	0.767	3	0.1003	3	2.934905	1.605548
mml-miR-374a-5p	0.731	3	0.1003	3	2.86555	1.47237
mml-miR-374a-3p	0.7101	3	0.1003	3	2.823701	1.396856
mml-miR-16-1-3p	0.7001	3	0.1	3	2.807561	1.735182
mml-miR-542-5p	0.6414	3	0.1	3	2.681224	1.511449
mml-miR-892c-5p	0.6414	3	0.1	3	2.681224	1.511449
mml-miR-448	0.6191	3	0.1	3	2.630172	1.430626
mml-let-7g-3p	0.6065	3	0.1	3	2.600508	1.386158
mml-miR-487b-5p	0.6065	3	0.1	3	2.600508	1.386158
mml-miR-379-3p	0.6021	3	0.1	3	2.590003	1.369572
mml-miR-206	1.491	3	0.2594	3	2.52303	4
mml-miR-30c-1-3p	1.033	3	0.2007	3	2.363728	2.29243
mml-miR-1296-5p	1.003	3	0.2007	3	2.321209	2.161151
mml-miR-4766-5p	0.7518	3	0.159	3	2.241322	1.337242
mml-miR-532-3p	0.7418	3	0.159	3	2.222004	1.302771
mml-miR-7184-3p	0.8925	3	0.2007	3	2.152812	1.701147
mml-miR-	1.689	3	0.392	3	2.107244	4

770-5p						
mml-miR-455-5p	0.8488	3	0.2007	3	2.080384	1.536107
mml-miR-1255a-5p	0.8157	3	0.2007	3	2.022998	1.415669
mml-miR-135a-2-3p	1.58	3	0.4101	3	1.945877	4
mml-miR-490-3p	1.27	3	0.3333	3	1.929935	2.79588
mml-miR-7205-5p	1.702	3	0.4475	3	1.927271	4
mml-miR-128a-5p	0.9108	3	0.2594	3	1.811956	1.548214
mml-miR-21-3p	1.184	3	0.3471	3	1.770246	2.318759
mml-miR-194-5p	1.026	3	0.301	3	1.769195	1.835647
mml-miR-874-5p	1.001	3	0.301	3	1.733607	1.735182
mml-miR-184	1.39	3	0.4184	3	1.73213	2.958607
mml-miR-320c	0.9847	3	0.3181	3	1.630204	1.605548
mml-miR-137-5p	1.492	3	0.4924	3	1.599345	3.09691

Table 4. Mouse primer* sequences used in this study.

Gene	Forward sequence (5'→3')	Reverse sequence (5'→3')
GAPDH	CCCCTTCATTGACCTCAACTACA	CGCTCCTGGAGGATGGTGAT
CD40	TGAGGATAAGAACTTGGAGGTC	CCGGGACTTTAAACCACAGA
TNFα	CACCACCATCAAGGACTC	AGGTCTGAAGGTAGGAAGG
MMP-2	TCAAGGACCGGTTTATTTGG	GCGAAGAACACAGCCTTCTC
MMP-9	CAGCCGACTTTTGTGXXCTT	GCTTCTCTCCCATCATCTGG
CX3CR1	AGGACACAGCCAGACAAG	TCAGGGGAGAAAGCAAG

*Primers were synthesized by Integrated DNA technologies IDT, Iowa, USA

Because HIV regulates MMP expression in astrocytes (68), we assessed the regulation of astrocyte MMP levels in response to BG-EV treatment. The EVs irrespective of background elevated MMP2 mRNA in WT astrocytes but suppressed MMP2 mRNA in CXCR1^{+/GFP} cells (**Figure 6C**). In contrast to MMP2, EVs irrespective of background suppressed MMP9 mRNA in WT astrocytes while upregulating MMP9 mRNA in CXCR1^{+/GFP} astrocytes (**Figure 6D**). Compared to WT, EVs more significantly elevated CD40 (**Figure 6E**), TNFα (**Figure 6F**), MMP2 (**Figure 6G**), and MMP9 (**Figure 6H**) mRNA in CXCR1^{+/GFP} astrocytes. The exceptions were suppression of TNFα by uninfected control EVs (**Figure 6F**) and MMP9 by PBS and THC/SIV EVs in CXCR1^{+/GFP} cells (**Figure 6H**). These data show that EVs regulate astrocyte CD40, MMP2, and MMP9 in a CX3CR1-dependent manner, since WT astrocytes express significantly higher CX3CR1 mRNA compared to CX3CR1^{+/GFP} astrocytes (**Figure 6I**). Although some reports indicate that astrocytes do not express CX3CR1 (69, 70), prior studies showed that astrocytes express CX3CR1 and GFAP (71), both in vitro and in vivo and during brain development or neurological insult (72). Moreover, primary simian astrocytes also express CX3CR1 mRNA (73).

Functional enrichment analysis (using String) revealed the interactome of GFAP, CD40, TNFα, MMP2, and MMP9 (**Figure 6J**). GFAP, CD40, TNFα, MMP2, and MMP9 are enriched in numerous gene ontology (GO) biological processes (**Table 5**) and KEGG pathways (**Table 6**). Of particular interest was the enrichment of Positive regulation of ikappaB phosphorylation, regulation of chronic inflammatory response,

regulation of immunoglobulin secretion, positive regulation of glial cell proliferation, and leukocyte tethering/rolling (**Table 5**). We also found the enrichment of asthma, bladder cancer, allograft rejection, malaria, and viral protein interaction with cytokine and cytokine receptor (**Table 6**) to be interesting. These data are noteworthy because increased TNF α and GFAP expression are found in the brains of patients with certain CNS disorders (74), and exogenous TNF α suppressed GFAP expression in cultured astrocytes (75).

Table 5. Top-10 GO biological process, depicting functional enrichment in the network shown in Figure 8J.

#Term ID	term description	observed gene count	background gene count	strength	false discovery rate
GO:1903721	Positive regulation of i-kappab phosphorylation	2	6	3.09	0.0019
GO:0002676	Regulation of chronic inflammatory response	2	11	2.82	0.0036
GO:0051023	Regulation of immunoglobulin secretion	2	19	2.59	0.0051
GO:0060252	Positive regulation of glial cell proliferation	2	20	2.57	0.0054
GO:0050901	Leukocyte tethering or rolling	2	21	2.54	0.0055
GO:1904707	Positive regulation of vascular associated smooth muscle cell proliferation	3	35	2.5	0.00082
GO:0030574	Collagen catabolic process	2	30	2.39	0.0085
GO:0031102	Neuron projection regeneration	2	32	2.36	0.0094
GO:0001774	Microglial cell activation	2	33	2.35	0.0097
GO:2001240	Negative regulation of extrinsic apoptotic signaling pathway in absence of ligand	2	33	2.35	0.0097

Table 6. Top-10 KEGG pathways, depicting functional enrichment in the network shown in Figure 8J.

#Term ID	term description	observed gene count	background gene count	strength	false discovery rate
mmu05310	Asthma	2	24	2.49	0.0034
mmu05219	Bladder cancer	2	40	2.26	0.0048
mmu05330	Allograft rejection	2	50	2.17	0.0062
mmu05144	Malaria	2	53	2.14	0.0062
mmu04061	Viral protein interaction with cytokine and cytokine receptor	2	87	1.93	0.0136
mmu01522	Endocrine resistance	2	91	1.91	0.0136
mmu04657	IL-17 signaling pathway	2	90	1.91	0.0136
mmu05322	Systemic lupus erythematosus	2	93	1.9	0.0136
mmu05418	Fluid shear stress and atherosclerosis	3	141	1.89	0.0024
mmu04620	Toll-like receptor signaling pathway	2	98	1.88	0.0136

Discussion

HIV invades the brain about two weeks after infection (76, 77), infecting resident CNS cells, including microglia and astrocytes (78, 79). Glial cells maintain brain homeostasis (80) via scavenging for excess toxic neurotransmitters, maintaining BBB integrity, regulation of immune activation/inflammation, and release of neurotrophic factors. Moreover, activation of glial cells contributes to neuropathology induced by mitochondrial toxins (81). HIV and its proteins (Tat and gp120) have been implicated in mediating astrocyte toxicity (82, 83). Although astrocytes make up about 40% of the total CNS cell population, their exact function in HIV-induced neuroinflammation remains unclear.

Our data using the SIV-infected RM model exposed to chronic THC treatment, along with WT and CX3CR1^{+/GFP} astrocytes revealed that BG contain EVs with previously unrecognized functions. There was no significant difference in BG-EV size distributions, concentrations, protein content, and zeta potential (**Figure 1**). Similarly, we detected no difference in the structure of EVs (**Figure 2**) from BG samples of uninfected controls, VEH/SIV, and THC/SIV. However, significant differences were observed in the BG-EV miRNA profile (**Figure 3**). The cargo composition and functions of the BG-EVs were modulated by HIV/SIV infection. BG-EVs from these animals exhibited a proinflammatory profile and induced an activated/proinflammatory state in primary CNS astrocytes. Strikingly, long term low dose THC treatment of SIV infected RMs selectively counteracts the generalized proinflammatory nature of SIV BG-EVs.

It is evident that EVs can be used as biomarkers for specific conditions, as suggested for neuron derived EVs from PLWH bearing biomarkers of cognitive impairment (84). EVs are also prospective carriers of drugs and other exogenous compounds with the potential to regulate neuropathogenesis (85). Using the SIV infected RM model, we detected several miRNAs that regulate inflammation/immune regulation, TLR signaling, Neurotrophin TRK receptor signaling, cell death/response to stress to be significantly downregulated. Neurotrophins, including brain derived neurotrophic factors (BDNF) are a family of closely related proteins identified to control many aspects of survival, development, and functions of neurons. Continued presence of neurotrophins is essential as it controls synaptic function and plasticity, sustain neuronal survival, morphology, and differentiation, in addition to other roles outside the nervous system (86). Furthermore, HIV suppresses BDNF expression and reduces BDNF activity, resulting in neurodegeneration in infected individuals (87, 88). Remarkably, intervention with THC led to significant upregulation of all the SIV-downregulated miRNAs. THC also upregulated 26 miRNAs and some neurotoxic miRNAs, including the let-7 family members (66) that regulate biological processes, such as apoptosis (89, 90), immune system modulation (91, 92), TLR7 activation (93), axon guidance (94), and BBB (95). Additionally, let-7c has been shown to promote polarization of macrophages from M1 to M2 phenotype (92). These neurotoxic miRNAs were either downregulated or were unchanged in the SIV infected group. The various pathways altered by SIV or THC have been implicated in neuroinflammation. These observations indicate that the changes SIV and/or THC imprinted on the brain, manifest in BG-EVs, which may then serve as a conduit for dissemination of miRNAs to CNS cells. It is also possible that BG-EVs may spread their miRNA cargos to distant sites in the periphery via cell-to-cell transfer.

Aside from serving as biomarkers, EVs mediate intercellular communication. In our study, astrocytes tolerated up to 100 µg BG-EVs from all groups. However, increasing BG-EV concentration beyond 100 µg showed that the tolerance of astrocytes was dependent on the origin (uninfected control, VEH/SIV, THC/SIV) and concentration (50, 100, 200 µg) of the EVs. The viability of astrocytes remained unchanged and comparable to PBS treated cells upto 200 µg EV concentration. However, viability of astrocytes significantly declined in the presence of 200 µg of SIV EVs. THC/SIV EVs prevented SIV EV-mediated decline in the viability of astrocytes.

In steady state, astrocytes express GFAP. However, the expression of GFAP is increased during activation (96). In our study, SIV EVs significantly increased the level of astrocyte GFAP (**Figure 5**). The conversion of GFAP^{low} astrocytes into GFAP^{high} astrocytes which occurred via interaction with EVs is indicative of their potential to activate these cells. Activated astrocytes, and other brain resident cells are key contributors to HIV-induced neuroinflammation. These cells release neurotoxic factors and inflammatory mediators such as TNFα (97) that may lead to deleterious consequences, including neurotoxicity. TNFα is mitogenic to astrocytes and increased levels of TNFα are associated with elevated GFAP expression (98). Astrocytes promote chronic inflammation and progressive neurodegeneration via overexpression of TNFα (99). Increased production of TNFα is linked to HIV-induced immunological abnormalities (100-102) and apoptotic effects in astrocytes, a major feature of cellular injury in HAND (103). In the CNS, CD40 is expressed by astrocytes and its interaction with CD40L on other resident CNS cells such as T, monocytic, natural killer, and mast cells mediates intracellular signaling events that promote the production of proinflammatory factors and neurotoxins (104). The reprogramming of astrocytes from CD40/TNFα^{low} to CD40/TNFα^{high} by EVs is suggestive of the proinflammatory nature of BG-EVs. Similarly, the conversion of CD40/TNFα^{high} to CD40/TNFα^{intermediate} by THC/SIV EVs is suggestive of the potential anti-inflammatory nature of THC/SIV EVs. These observations are significant because the hallmarks of HAND include widespread microglial activation, accompanied by reactive astrogliosis and their secretory products, including cytokines and chemokines (105, 106).

In addition to TNFα, other inflammatory mediators were induced by BG-EVs. MMP2 and MMP9 mRNA were variably altered in astrocytes by all EVs in a CX3CR1-dependent manner. In some cases, THC/SIV EVs counteracted SIV EV-induced effects on MMP2 and MMP9 mRNA. These observations are intriguing because under pathological conditions, dysregulated expression of MMPs induces inflammation and promotes progression of neurodegenerative diseases (107). MMP2 and MMP9 are ECM-degrading enzymes involved in inflammation and tissue remodeling. Through their induction of soluble TNFα and proteolytic activity on the ECM, MMPs may promote brain injury. It is likely that astrocyte activation by EVs may trigger the expression of inflammatory mediators (TNFα, MMP2, and MMP9) observed in this study. Furthermore, MMPs are thought to be involved in the pathogenesis of HAND and other neurodegenerative disorders via degradation of ECM and compromising the BBB (108-110). Additionally, MMP2 and MMP9 are present in the CSF, plasma, or brain tissue of HIV

patients (109). The suppressive effect of THC/SIV EVs on inflammatory mediators show the potential of such THC EVs to ameliorate the effect of pathological EVs.

With regards to the role of CX3CR1 in HIV infection, the ligand of CX3CR1, CX3CL1, also known as Fractalkine (FKN) is increased in the CSF of HIV-infected individuals who exhibit neurocognitive impairment (111, 112). However, exogenous FKN has been shown to protect cultured neurons from neurotoxicity induced by Tat or Tat + morphine induced dendritic losses (113, 114). It is worth mentioning that CX3CR1 isoforms produced by alternative splicing may function as fusion co-receptors for HIV envelope protein (115), although the significance of CX3CR1 among other HIV co-receptors for HIV entry is still not clear. Nonetheless, HIV-infected individuals homozygous for CX3CR1-I249M280 (that affects two amino acids – isoleucine-249 and methionine-280) exhibit a more rapid progression to AIDS (116), perhaps due to reduced FKN binding. Our findings, together with the literature evidence, suggest a possible involvement of CX3CR1 mediated response to BG-EV reprogramming of astrocyte gene expression and function.

The ability of THC, a component of cannabis used as recreational agent and appetite stimulant by PLWH (117-121) to reprogram BG-EVs and affect their functions is significant. Chronic cannabis use may slow disease progression, prolong survival, reduce viral load, and attenuate infection-induced inflammation/immune activation in SIV infected RMs (7, 57, 58, 122-126) and ART-treated PLWH (127, 128). The effect of THC is systemic—affecting many organs. As a result, THC and other cannabinoids are recommended for the treatment of digestive disorders (129-134) and FDA approved their use for clinical management of wasting, appetite stimulation, and alleviation of ART toxicity in PLWH (117, 118, 120, 121, 135).

Conclusion

In summary, the findings of this study suggest that HIV/SIV infection reprograms the BG leading to the release of pathogenic EVs that may potentially promote CNS inflammation and toxicity. However, cannabinoid mediated modulation of EV cargo composition as shown in this study maybe a mechanism for the regulation of HIV/SIV-induced changes. This is significant because exploration of the potential of THC EVs in a preclinical animal model seems logical to investigate whether the clinical advantages of THC EVs will result in beneficial outcome. This findings of this study also paves the way for investigation into the effects of the combined administration of THC:CBD [1:1 or 1:3 ratio] on neuroinflammation and their effects on BG-EV composition and function. The implication of our findings goes beyond HIV induced inflammation. Glia cells (microglia and astrocytes) are involved in the pathogenesis of pain (136). Activated/reactive astrocytes play a role in neuropathic pain (137, 138), inflammatory pain (139, 140), as well as bone cancer pain (141). Activated astrocytes are also involved in Parkinson's disease, spinal cord injury (142, 143), and traumatic brain injury (144). In line with their role in the pathogenesis of pain, studies are warranted to assess the effect of CNS EVs in mediating the development and maintenance of pain.

Abbreviations

BG – Basal ganglia

EV – Extracellular vesicles

CBD – Cannabidiol

CNS – Central nervous system

THC – delta-9-tetrahydrocannabinol

MC – membraneless condensates

VEH – Vehicle

Declarations

Consent for publication: All authors read and approved the publication of this manuscript.

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Author contributions: CMO and MM conceptualized the study. HK, MM-W, MM, MAT conducted the experiments. HK, MM-W, MMM, MAT, SET, MM, and CMO conducted data analyses. HK, MM-W, MMM, MAT, SET, MM, and CMO wrote the original draft of the paper, provided text, and reviewed and edited the manuscript.

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References

1. Gray F, Scaravilli F, Everall I, Chretien F, An S, Boche D, et al. (1996): Neuropathology of early HIV-1 infection. *Brain pathology (Zurich, Switzerland)*. 6:1-15.
2. An SF, Giometto B, Scaravilli F (1996): HIV-1 DNA in brains in AIDS and pre-AIDS: correlation with the stage of disease. *Ann Neurol*. 40:611-617.
3. Sharma H, Chinnappan M, Agarwal S, Dalvi P, Gunewardena S, O'Brien-Ladner A, et al. (2018): Macrophage-derived extracellular vesicles mediate smooth muscle hyperplasia: role of altered miRNA cargo in response to HIV infection and substance abuse. *Faseb j*. 32:5174-5185.
4. Hu G, Yao H, Chaudhuri AD, Duan M, Yelamanchili SV, Wen H, et al. (2012): Exosome-mediated shuttling of microRNA-29 regulates HIV Tat and morphine-mediated neuronal dysfunction. *Cell death & disease*. 3:e381.
5. Chaganti J, Marripudi K, Staub LP, Rae CD, Gates TM, Moffat KJ, et al. (2019): Imaging correlates of the blood-brain barrier disruption in HIV-associated neurocognitive disorder and therapeutic implications. *Aids*. 33:1843-1852.
6. Mintzopoulos D, Ratai EM, He J, Gonzalez RG, Kaufman MJ (2019): Simian immunodeficiency virus transiently increases brain temperature in rhesus monkeys: detection with magnetic resonance spectroscopy thermometry. *Magnetic resonance in medicine*. 81:2896-2904.
7. Molina PE, Winsauer P, Zhang P, Walker E, Birke L, Amedee A, et al. (2011): Cannabinoid administration attenuates the progression of simian immunodeficiency virus. *AIDS Res Hum Retroviruses*. 27:585-592.
8. UNAIDS (2020): Global HIV & AIDS statistics – Fact sheet.
9. Castro FOF, Silva JM, Dorneles GP, Barros JBS, Ribeiro CB, Noronha I, et al. (2019): Distinct inflammatory profiles in HIV-infected individuals under antiretroviral therapy using cannabis, cocaine or cannabis plus cocaine. *Aids*. 33:1831-1842.
10. Galaj E, Bi GH, Yang HJ, Xi ZX (2020): Cannabidiol attenuates the rewarding effects of cocaine in rats by CB2, 5-HT(1A) and TRPV1 receptor mechanisms. *Neuropharmacology*. 167:107740.
11. Mimiaga MJ, Reisner SL, Grasso C, Crane HM, Safren SA, Kitahata MM, et al. (2013): Substance use among HIV-infected patients engaged in primary care in the United States: findings from the Centers for AIDS Research Network of Integrated Clinical Systems cohort. *American journal of public health*. 103:1457-1467.
12. Mohammadi A, Darabi M, Nasry M, Saabet-Jahromi MJ, Malek-Pour-Afshar R, Sheibani H (2009): Effect of opium addiction on lipid profile and atherosclerosis formation in hypercholesterolemic rabbits. *Experimental and toxicologic pathology : official journal of the Gesellschaft fur Toxikologische Pathologie*. 61:145-149.
13. Roohafza H, Taleai M, Sadeghi M, Haghani P, Shokouh P, Sarrafzadegan N (2013): Opium decreases the age at myocardial infarction and sudden cardiac death: a long- and short-term outcome evaluation. *Archives of Iranian medicine*. 16:154-160.
14. Nabati S, Asadikaram G, Arababadi MK, Shahabinejad G, Rezaeian M, Mahmoodi M, et al. (2013): The plasma levels of the cytokines in opium-addicts and the effects of opium on the cytokines secretion by their lymphocytes. *Immunology letters*. 152:42-46.
15. Saha B, Momen-Heravi F, Kodys K, Szabo G (2016): MicroRNA Cargo of Extracellular Vesicles from Alcohol-exposed Monocytes Signals Naive Monocytes to Differentiate into M2 Macrophages. *J Biol Chem*. 291:149-159.
16. Momen-Heravi F, Saha B, Kodys K, Catalano D, Satishchandran A, Szabo G (2015): Increased number of circulating exosomes and their microRNA cargos are potential novel biomarkers in alcoholic hepatitis. *Journal of translational medicine*. 13:261.
17. Momen-Heravi F, Bala S, Kodys K, Szabo G (2015): Exosomes derived from alcohol-treated hepatocytes horizontally transfer liver specific miRNA-122 and sensitize monocytes to LPS. *Scientific reports*. 5:9991.
18. Lyu Y, Kopcho S, Mohan M, Okeoma CM (2020): Long-Term Low-Dose Delta-9-Tetrahydrocannabinol (THC) Administration to Simian Immunodeficiency Virus (SIV) Infected Rhesus Macaques Stimulates the Release of Bioactive Blood Extracellular Vesicles (EVs) that Induce Divergent Structural Adaptations and Signaling Cues. *Cells*. 9.
19. Mohan M, Kaushal D, Aye PP, Alvarez X, Veazey RS, Lackner AA (2013): Focused examination of the intestinal epithelium reveals transcriptional signatures consistent with disturbances in enterocyte maturation and differentiation during the course of SIV infection.

20. Lotvall J, Valadi H (2007): Cell to cell signalling via exosomes through esRNA. *Cell adhesion & migration*. 1:156-158.
21. Admyre C, Grunewald J, Thyberg J, Gripenback S, Tornling G, Eklund A, et al. (2003): Exosomes with major histocompatibility complex class II and co-stimulatory molecules are present in human BAL fluid. *Eur Respir J*. 22:578-583.
22. Admyre C, Johansson SM, Qazi KR, Filen JJ, Lahesmaa R, Norman M, et al. (2007): Exosomes with immune modulatory features are present in human breast milk. *J Immunol*. 179:1969-1978.
23. Baum MK, Rafie C, Lai S, Sales S, Page B, Campa A (2009): Crack-cocaine use accelerates HIV disease progression in a cohort of HIV-positive drug users. *Journal of acquired immune deficiency syndromes*. 50:93-99.
24. Bobrie A, Colombo M, Raposo G, Thery C (2011): Exosome secretion: molecular mechanisms and roles in immune responses. *Traffic*. 12:1659-1668.
25. Caby MP, Lankar D, Vincendeau-Scherrer C, Raposo G, Bonnerot C (2005): Exosomal-like vesicles are present in human blood plasma. *International immunology*. 17:879-887.
26. Madison MN, Roller RJ, Okeoma CM (2014): Human semen contains exosomes with potent anti-HIV-1 activity. *Retrovirology*. 11:102.
27. Palanisamy V, Sharma S, Deshpande A, Zhou H, Gimzewski J, Wong DT (2010): Nanostructural and transcriptomic analyses of human saliva derived exosomes. *PLoS One*. 5:e8577.
28. Pisitkun T, Shen RF, Knepper MA (2004): Identification and proteomic profiling of exosomes in human urine. *Proc Natl Acad Sci U S A*. 101:13368-13373.
29. Simons M, Raposo G (2009): Exosomes—vesicular carriers for intercellular communication. *Current opinion in cell biology*. 21:575-581.
30. Smith JA, Daniel R (2016): Human vaginal fluid contains exosomes that have an inhibitory effect on an early step of the HIV-1 life cycle. *Aids*. 30:2611-2616.
31. Thery C, Ostrowski M, Segura E (2009): Membrane vesicles as conveyors of immune responses. *Nature reviews Immunology*. 9:581-593.
32. Vojtech L, Woo S, Hughes S, Levy C, Ballweber L, Sauteraud RP, et al. (2014): Exosomes in human semen carry a distinctive repertoire of small non-coding RNAs with potential regulatory functions. *Nucleic Acids Res*. 42:7290-7304.
33. Madison MN, Okeoma CM (2015): Exosomes: Implications in HIV-1 Pathogenesis. *Viruses*. 7:4093-4118.
34. Welch JL, Madison MN, Margolick JB, Galvin S, Gupta P, Martinez-Maza O, et al. (2017): Effect of prolonged freezing of semen on exosome recovery and biologic activity. *Scientific reports*. 7:45034.
35. Madison MN, Jones PH, Okeoma CM (2015): Exosomes in human semen restrict HIV-1 transmission by vaginal cells and block intravaginal replication of LP-BM5 murine AIDS virus complex. *Virology*. 482:189-201.
36. Welch JL, Kaddour H, Schlievert PM, Stapleton JT, Okeoma CM (2018): Semen exosomes promote transcriptional silencing of HIV-1 by disrupting NF- κ B/Sp1/Tat circuitry. *J Virol*.
37. Becker A, Thakur BK, Weiss JM, Kim HS, Peinado H, Lyden D (2016): Extracellular Vesicles in Cancer: Cell-to-Cell Mediators of Metastasis. *Cancer Cell*. 30:836-848.
38. Hu G, Yang L, Cai Y, Niu F, Mezzacappa F, Callen S, et al. (2016): Emerging roles of extracellular vesicles in neurodegenerative disorders: focus on HIV-associated neurological complications. *Cell death & disease*. 7:e2481.
39. Turchinovich A, Weiz L, Langheinz A, Burwinkel B (2011): Characterization of extracellular circulating microRNA. *Nucleic Acids Res*. 39:7223-7233.
40. Vickers KC, Palmisano BT, Shoucri BM, Shamburek RD, Remaley AT (2011): MicroRNAs are transported in plasma and delivered to recipient cells by high-density lipoproteins. *Nature cell biology*. 13:423-433.
41. Skog J, Würlinger T, van Rijn S, Meijer DH, Gainche L, Sena-Esteves M, et al. (2008): Glioblastoma microvesicles transport RNA and proteins that promote tumour growth and provide diagnostic biomarkers. *Nature cell biology*. 10:1470-1476.
42. Kaddour H, Lyu Y, Shouman N, Mohan M, Okeoma CM (2020): Development of Novel High-Resolution Size-Guided Turbidimetry-Enabled Particle Purification Liquid Chromatography (PPLC): Extracellular Vesicles and Membraneless Condensates in Focus. *International journal of molecular sciences*. 21.
43. Lässer C, Alikhani VS, Ekström K, Eldh M, Paredes PT, Bossios A, et al. (2011): Human saliva, plasma and breast milk exosomes contain RNA: uptake by macrophages. *Journal of translational medicine*. 9:9.
44. Hu G, Yao H, Chaudhuri AD, Duan M, Yelamanchili SV, Wen H, et al. (2012): Exosome-mediated shuttling of microRNA-29 regulates HIV Tat and morphine-mediated neuronal dysfunction. *Cell death & disease*. 3:e381.
45. Duarte A, Cisternas P, Caviades A, Batiz LF, Lafourcade C, Wyneken U, et al. (2017): Astrocytes at the Hub of the Stress Response: Potential Modulation of Neurogenesis by miRNAs in Astrocyte-Derived Exosomes. *Stem cells international*. 2017:1719050.

46. Budnik V, Ruiz-Cañada C, Wendler F (2016): Extracellular vesicles round off communication in the nervous system. *Nature reviews Neuroscience*. 17:160-172.
47. Balusu S, Van Wonterghem E, De Rycke R, Raemdonck K, Stremersch S, Gevaert K, et al. (2016): Identification of a novel mechanism of blood-brain communication during peripheral inflammation via choroid plexus-derived extracellular vesicles. *EMBO Mol Med*. 8:1162-1183.
48. Park S, Ahn ES, Kim Y (2015): Neuroblastoma SH-SY5Y cell-derived exosomes stimulate dendrite-like outgrowths and modify the differentiation of A375 melanoma cells. *Cell biology international*. 39:379-387.
49. Kaddour H, Lyu Y, Welch JL, Paromov V, Mandape SN, Sakhare SS, et al. (2020): Proteomics Profiling of Autologous Blood and Semen Exosomes from HIV-infected and Uninfected Individuals Reveals Compositional and Functional Variabilities. *Molecular & cellular proteomics : MCP*. 19:78-100.
50. Lyu Y, Kaddour H, Kopcho S, Panzner TD, Shouman N, Kim EY, et al. (2019): Human Immunodeficiency Virus (HIV) Infection and Use of Illicit Substances Promote Secretion of Semen Exosomes that Enhance Monocyte Adhesion and Induce Actin Reorganization and Chemotactic Migration. *Cells*. 8.
51. Pegtel DM, Peferoen L, Amor S (2014): Extracellular vesicles as modulators of cell-to-cell communication in the healthy and diseased brain. *Philos Trans R Soc Lond B Biol Sci*. 369.
52. Fröhlich D, Kuo WP, Frühbeis C, Sun JJ, Zehendner CM, Luhmann HJ, et al. (2014): Multifaceted effects of oligodendroglial exosomes on neurons: impact on neuronal firing rate, signal transduction and gene regulation. *Philos Trans R Soc Lond B Biol Sci*. 369.
53. Antonucci F, Turola E, Riganti L, Caleo M, Gabrielli M, Perrotta C, et al. (2012): Microvesicles released from microglia stimulate synaptic activity via enhanced sphingolipid metabolism. *The EMBO Journal*. 31:1231-1240.
54. Persidsky Y, Buttini M, Limoges J, Bock P, Gendelman HE (1997): An analysis of HIV-1-associated inflammatory products in brain tissue of humans and SCID mice with HIV-1 encephalitis. *J Neurovirol*. 3:401-416.
55. Nath A, Anderson C, Jones M, Maragos W, Booze R, Mactutus C, et al. (2000): Neurotoxicity and dysfunction of dopaminergic systems associated with AIDS dementia. *Journal of psychopharmacology (Oxford, England)*. 14:222-227.
56. Kumar V, Torben W, Mansfield J, Alvarez X, Vande Stouwe C, Li J, et al. (2019): Cannabinoid Attenuation of Intestinal Inflammation in Chronic SIV-Infected Rhesus Macaques Involves T Cell Modulation and Differential Expression of Micro-RNAs and Pro-inflammatory Genes. *Frontiers in immunology*. 10:914.
57. Chandra LC, Kumar V, Torben W, Vande Stouwe C, Winsauer P, Amedee A, et al. (2015): Chronic administration of Delta9-tetrahydrocannabinol induces intestinal anti-inflammatory microRNA expression during acute simian immunodeficiency virus infection of rhesus macaques. *J Virol*. 89:1168-1181.
58. Winsauer PJ, Molina PE, Amedee AM, Filipeanu CM, McGoey RR, Troxclair DA, et al. (2011): Tolerance to chronic delta-9-tetrahydrocannabinol (Delta(9)-THC) in rhesus macaques infected with simian immunodeficiency virus. *Experimental and clinical psychopharmacology*. 19:154-172.
59. Kaddour H, Panzner TD, Welch JL, Shouman N, Mohan M, Stapleton JT, et al. (2020): Electrostatic Surface Properties of Blood and Semen Extracellular Vesicles: Implications of Sialylation and HIV-Induced Changes on EV Internalization. *Viruses*. 12.
60. Jung S, Aliberti J, Graemmel P, Sunshine MJ, Kreutzberg GW, Sher A, et al. (2000): Analysis of fractalkine receptor CX3CR1 function by targeted deletion and green fluorescent protein reporter gene insertion. *Molecular and cellular biology*. 20:4106-4114.
61. Bronstein R, Torres L, Nissen JC, Tsirka SE (2013): Culturing microglia from the neonatal and adult central nervous system. *Journal of visualized experiments: JoVE*.
62. O'Meara RW, Ryan SD, Colognato H, Kothary R (2011): Derivation of enriched oligodendrocyte cultures and oligodendrocyte/neuron myelinating co-cultures from post-natal murine tissues. *JoVE (Journal of Visualized Experiments)*. e3324.
63. Lyu Y, Kopcho S, Alvarez FA, Okeoma BC, Okeoma CM (2020): Development of a Cationic Amphiphilic Helical Peptidomimetic (B18L) As A Novel Anti-Cancer Drug Lead. *Cancers*. 12.
64. Lyu Y, Mahauad-Fernandez WD, Okeoma CM (2020): Development and Characterization of the Shortest Anti-Adhesion Peptide Analogue of B49Mod1. *Molecules*. 25.
65. Vlachos IS, Zagganas K, Paraskevopoulou MD, Georgakilas G, Karagkouni D, Vergoulis T, et al. (2015): DIANA-miRPath v3. 0: deciphering microRNA function with experimental support. *Nucleic acids research*. 43:W460-W466.
66. Derkow K, Rössling R, Schipke C, Krüger C, Bauer J, Fähring M, et al. (2018): Distinct expression of the neurotoxic microRNA family let-7 in the cerebrospinal fluid of patients with Alzheimer's disease. *PLoS One*. 13:e0200602.
67. Liddelow SA, Barres BA (2017): Reactive Astrocytes: Production, Function, and Therapeutic Potential. *Immunity*. 46:957-967.

68. Lvque T, Le Pavec G, Boutet A, Tardieu M, Dormont D, Gras G (2004): Differential regulation of gelatinase A and B and TIMP-1 and -2 by TNFlpha and HIV virions in astrocytes. *Microbes Infect.* 6:157-163.
69. Yeo SI, Kim JE, Ryu HJ, Seo CH, Lee BC, Choi IG, et al. (2011): The roles of fractalkine/CX3CR1 system in neuronal death following pilocarpine-induced status epilepticus. *Journal of neuroimmunology.* 234:93-102.
70. Hatori K, Nagai A, Heisel R, Ryu JK, Kim SU (2002): Fractalkine and fractalkine receptors in human neurons and glial cells. *J Neurosci Res.* 69:418-426.
71. Chen G, Zhou Z, Sha W, Wang L, Yan F, Yang X, et al. (2020): A novel CX3CR1 inhibitor AZD8797 facilitates early recovery of rat acute spinal cord injury by inhibiting inflammation and apoptosis. *International journal of molecular medicine.* 45:1373-1384.
72. Meucci O, Fatatis A, Simen AA, Miller RJ (2000): Expression of CX3CR1 chemokine receptors on neurons and their role in neuronal survival. *Proc Natl Acad Sci U S A.* 97:8075-8080.
73. Croitoru-Lamourey J, Guillemin GJ, Boussin FD, Mognetti B, Gigout LI, Chret A, et al. (2003): Expression of chemokines and their receptors in human and simian astrocytes: evidence for a central role of TNF alpha and IFN gamma in CXCR4 and CCR5 modulation. *Glia.* 41:354-370.
74. Rostworowski M, Balasingam V, Chabot S, Owens T, Yong VW (1997): Astrogliosis in the neonatal and adult murine brain post-trauma: elevation of inflammatory cytokines and the lack of requirement for endogenous interferon-gamma. *The Journal of neuroscience : the official journal of the Society for Neuroscience.* 17:3664-3674.
75. Oh YJ, Markelonis GJ, Oh TH (1993): Effects of interleukin-1 beta and tumor necrosis factor-alpha on the expression of glial fibrillary acidic protein and transferrin in cultured astrocytes. *Glia.* 8:77-86.
76. Chakrabarti L, Hurtrel M, Maire MA, Vazeux R, Dormont D, Montagnier L, et al. (1991): Early viral replication in the brain of SIV-infected rhesus monkeys. *Am J Pathol.* 139:1273-1280.
77. Valcour V, Chalermchai T, Sailasuta N, Marovich M, Lerdlum S, Suttichom D, et al. (2012): Central nervous system viral invasion and inflammation during acute HIV infection. *J Infect Dis.* 206:275-282.
78. Narasipura SD, Henderson LJ, Fu SW, Chen L, Kashanchi F, Al-Harhi L (2012): Role of -catenin and TCF/LEF family members in transcriptional activity of HIV in astrocytes. *J Virol.* 86:1911-1921.
79. Lutgen V, Narasipura SD, Barbian HJ, Richards M, Wallace J, Razmpour R, et al. (2020): HIV infects astrocytes in vivo and egresses from the brain to the periphery. *PLoS Pathog.* 16:e1008381.
80. Liu J, Xu E, Tu G, Liu H, Luo J, Xiong H (2017): Methamphetamine potentiates HIV-1gp120-induced microglial neurotoxic activity by enhancing microglial outward K(+) current. *Molecular and cellular neurosciences.* 82:167-175.
81. Kirkley KS, Popichak KA, Hammond SL, Davies C, Hunt L, Tjalkens RB (2019): Genetic suppression of IKK2/NF-B in astrocytes inhibits neuroinflammation and reduces neuronal loss in the MPTP-Probenecid model of Parkinson's disease. *Neurobiology of disease.* 127:193-209.
82. Bansal AK, Mactutus CF, Nath A, Maragos W, Hauser KF, Booze RM (2000): Neurotoxicity of HIV-1 proteins gp120 and Tat in the rat striatum. *Brain research.* 879:42-49.
83. Iskander S, Walsh KA, Hammond RR (2004): Human CNS cultures exposed to HIV-1 gp120 reproduce dendritic injuries of HIV-1-associated dementia. *J Neuroinflammation.* 1:7.
84. Sun B, Dalvi P, Abadjian L, Tang N, Pulliam L (2017): Blood neuron-derived exosomes as biomarkers of cognitive impairment in HIV. *Aids.* 31:F9-f17.
85. Haney MJ, Klyachko NL, Zhao Y, Gupta R, Plotnikova EG, He Z, et al. (2015): Exosomes as drug delivery vehicles for Parkinson's disease therapy. *Journal of controlled release : official journal of the Controlled Release Society.* 207:18-30.
86. Barde YA, Edgar D, Thoenen H (1982): Purification of a new neurotrophic factor from mammalian brain. *The EMBO Journal.* 1:549-553.
87. Bachis A, Major EO, Mocchetti I (2003): Brain-derived neurotrophic factor inhibits human immunodeficiency virus-1/gp120-mediated cerebellar granule cell death by preventing gp120 internalization. *The Journal of neuroscience : the official journal of the Society for Neuroscience.* 23:5715-5722.
88. Nosheny RL, Bachis A, Acquas E, Mocchetti I (2004): Human immunodeficiency virus type 1 glycoprotein gp120 reduces the levels of brain-derived neurotrophic factor in vivo: potential implication for neuronal cell death. *Eur J Neurosci.* 20:2857-2864.
89. Li L, Liu H, Song H, Qin Y, Wang Y, Xu M, et al. (2017): Let-7d microRNA Attenuates 6-OHDA-Induced Injury by Targeting Caspase-3 in MN9D Cells. *J Mol Neurosci.* 63:403-411.
90. Wang S, Tang Y, Cui H, Zhao X, Luo X, Pan W, et al. (2011): Let-7/miR-98 regulate Fas and Fas-mediated apoptosis. *Genes Immun.* 12:149-154.

91. Jiang S, Yan W, Wang SE, Baltimore D (2018): Let-7 Suppresses B Cell Activation through Restricting the Availability of Necessary Nutrients. *Cell Metab.* 27:393-403.e394.
92. Banerjee S, Xie N, Cui H, Tan Z, Yang S, Icyuz M, et al. (2013): MicroRNA let-7c regulates macrophage polarization. *J Immunol.* 190:6542-6549.
93. Lehmann SM, Krüger C, Park B, Derkow K, Rosenberger K, Baumgart J, et al. (2012): An unconventional role for miRNA: let-7 activates Toll-like receptor 7 and causes neurodegeneration. *Nature neuroscience.* 15:827-835.
94. Wang X, Chen Q, Yi S, Liu Q, Zhang R, Wang P, et al. (2019): The microRNAs let-7 and miR-9 down-regulate the axon-guidance genes Ntn1 and Dcc during peripheral nerve regeneration. *J Biol Chem.* 294:3489-3500.
95. Rom S, Dykstra H, Zuluaga-Ramirez V, Reichenbach NL, Persidsky Y (2015): miR-98 and let-7g* protect the blood-brain barrier under neuroinflammatory conditions. *J Cereb Blood Flow Metab.* 35:1957-1965.
96. Pekny M, Pekna M (2004): Astrocyte intermediate filaments in CNS pathologies and regeneration. *J Pathol.* 204:428-437.
97. Brabers NA, Nottet HS (2006): Role of the pro-inflammatory cytokines TNF-alpha and IL-1beta in HIV-associated dementia. *European journal of clinical investigation.* 36:447-458.
98. Zhang L, Zhao W, Li B, Alkon DL, Barker JL, Chang YH, et al. (2000): TNF-alpha induced over-expression of GFAP is associated with MAPKs. *Neuroreport.* 11:409-412.
99. Wilms H, Sievers J, Rickert U, Rostami-Yazdi M, Mrowietz U, Lucius R (2010): Dimethylfumarate inhibits microglial and astrocytic inflammation by suppressing the synthesis of nitric oxide, IL-1beta, TNF-alpha and IL-6 in an in-vitro model of brain inflammation. *J Neuroinflammation.* 7:30.
100. Marfaing-Koka A, Aubin JT, Grangeot-Keros L, Portier A, Benattar C, Merrien D, et al. (1996): In vivo role of IL-6 on the viral load and on immunological abnormalities of HIV-infected patients. *J Acquir Immune Defic Syndr Hum Retrovirol.* 11:59-68.
101. Breen EC, Rezai AR, Nakajima K, Beall GN, Mitsuyasu RT, Hirano T, et al. (1990): Infection with HIV is associated with elevated IL-6 levels and production. *J Immunol.* 144:480-484.
102. Li G, Makar T, Gerzanich V, Kalakonda S, Ivanova S, Pereira EFR, et al. (2020): HIV-1 Vpr-Induced Proinflammatory Response and Apoptosis Are Mediated through the Sur1-Trpm4 Channel in Astrocytes. *mBio.* 11.
103. Persidsky Y, Limoges J, McComb R, Bock P, Baldwin T, Tyor W, et al. (1996): Human immunodeficiency virus encephalitis in SCID mice. *Am J Pathol.* 149:1027-1053.
104. Chen K, Huang J, Gong W, Zhang L, Yu P, Wang JM (2006): CD40/CD40L dyad in the inflammatory and immune responses in the central nervous system. *Cellular & molecular immunology.* 3:163-169.
105. Adle-Biassette H, Chrétien F, Wingertsman L, Héry C, Ereau T, Scaravilli F, et al. (1999): Neuronal apoptosis does not correlate with dementia in HIV infection but is related to microglial activation and axonal damage. *Neuropathology and applied neurobiology.* 25:123-133.
106. Giometto B, An SF, Groves M, Scaravilli T, Geddes JF, Miller R, et al. (1997): Accumulation of beta-amyloid precursor protein in HIV encephalitis: relationship with neuropsychological abnormalities. *Ann Neurol.* 42:34-40.
107. Brkic M, Balusu S, Libert C, Vandenbroucke RE (2015): Friends or Foes: Matrix Metalloproteinases and Their Multifaceted Roles in Neurodegenerative Diseases. *Mediators Inflamm.* 2015:620581.
108. Liuzzi GM, Mastroianni CM, Santacroce MP, Fanelli M, D'Agostino C, Vullo V, et al. (2000): Increased activity of matrix metalloproteinases in the cerebrospinal fluid of patients with HIV-associated neurological diseases. *J Neurovirol.* 6:156-163.
109. Ghorpade A, Persidskaia R, Suryadevara R, Che M, Liu XJ, Persidsky Y, et al. (2001): Mononuclear phagocyte differentiation, activation, and viral infection regulate matrix metalloproteinase expression: implications for human immunodeficiency virus type 1-associated dementia. *J Virol.* 75:6572-6583.
110. Toschi E, Barillari G, Sgadari C, Bacigalupo I, Cereseto A, Carlei D, et al. (2001): Activation of matrix-metalloproteinase-2 and membrane-type-1-matrix-metalloproteinase in endothelial cells and induction of vascular permeability in vivo by human immunodeficiency virus-1 Tat protein and basic fibroblast growth factor. *Mol Biol Cell.* 12:2934-2946.
111. Sporer B, Kastenbauer S, Koedel U, Arendt G, Pfister HW (2003): Increased intrathecal release of soluble fractalkine in HIV-infected patients. *AIDS Res Hum Retroviruses.* 19:111-116.
112. Erichsen D, Lopez AL, Peng H, Niemann D, Williams C, Bauer M, et al. (2003): Neuronal injury regulates fractalkine: relevance for HIV-1 associated dementia. *Journal of neuroimmunology.* 138:144-155.
113. Suzuki M, El-Hage N, Zou S, Hahn YK, Sorrell ME, Sturgill JL, et al. (2011): Fractalkine/CX3CL1 protects striatal neurons from synergistic morphine and HIV-1 Tat-induced dendritic losses and death. *Molecular neurodegeneration.* 6:78.

114. Tong N, Perry SW, Zhang Q, James HJ, Guo H, Brooks A, et al. (2000): Neuronal fractalkine expression in HIV-1 encephalitis: roles for macrophage recruitment and neuroprotection in the central nervous system. *J Immunol.* 164:1333-1339.
115. Garin A, Tarantino N, Faure S, Daoudi M, Lécureuil C, Bourdais A, et al. (2003): Two novel fully functional isoforms of CX3CR1 are potent HIV coreceptors. *J Immunol.* 171:5305-5312.
116. Faure S, Meyer L, Costagliola D, Vaneensberghe C, Genin E, Autran B, et al. (2000): Rapid progression to AIDS in HIV+ individuals with a structural variant of the chemokine receptor CX3CR1. *Science.* 287:2274-2277.
117. Haney M, Gunderson EW, Rabkin J, Hart CL, Vosburg SK, Comer SD, et al. (2007): Dronabinol and marijuana in HIV-positive marijuana smokers. Caloric intake, mood, and sleep. *Journal of acquired immune deficiency syndromes.* 45:545-554.
118. Riggs PK, Vaida F, Rossi SS, Sorkin LS, Gouaux B, Grant I, et al. (2012): A pilot study of the effects of cannabis on appetite hormones in HIV-infected adult men. *Brain research.* 1431:46-52.
119. Kader R, Govender R, Seedat S, Koch JR, Parry C (2015): Understanding the Impact of Hazardous and Harmful Use of Alcohol and/or Other Drugs on ARV Adherence and Disease Progression. *PLoS One.* 10:e0125088.
120. Margolis AM, Heverling H, Pham PA, Stolbach A (2014): A review of the toxicity of HIV medications. *Journal of medical toxicology : official journal of the American College of Medical Toxicology.* 10:26-39.
121. Vidot DC, Lerner B, Gonzalez R (2017): Cannabis Use, Medication Management and Adherence Among Persons Living with HIV. *AIDS and behavior.* 21:2005-2013.
122. Kumar V, Torben W, Kenway CS, Schiro FR, Mohan M (2016): Longitudinal Examination of the Intestinal Lamina Propria Cellular Compartment of Simian Immunodeficiency Virus-Infected Rhesus Macaques Provides Broader and Deeper Insights into the Link between Aberrant MicroRNA Expression and Persistent Immune Activation. *J Virol.* 90:5003-5019.
123. Molina PE, Amedee A, LeCapitaine NJ, Zabaleta J, Mohan M, Winsauer P, et al. (2011): Cannabinoid neuroimmune modulation of SIV disease. *J Neuroimmune Pharmacol.* 6:516-527.
124. Molina PE, Amedee AM, LeCapitaine NJ, Zabaleta J, Mohan M, Winsauer PJ, et al. (2014): Modulation of gut-specific mechanisms by chronic delta(9)-tetrahydrocannabinol administration in male rhesus macaques infected with simian immunodeficiency virus: a systems biology analysis. *AIDS Res Hum Retroviruses.* 30:567-578.
125. Simon L, Song K, Vande Stouwe C, Hollenbach A, Amedee A, Mohan M, et al. (2016): Delta9-Tetrahydrocannabinol (Delta9-THC) Promotes Neuroimmune-Modulatory MicroRNA Profile in Striatum of Simian Immunodeficiency Virus (SIV)-Infected Macaques. *J Neuroimmune Pharmacol.* 11:192-213.
126. Kumar V WT, Joshua Mansfield, Vande Stowe Curtis, Siddappa Byrareddy, Jian Li, Bapi Pahar, Molina PE, Mohan M. (2019): Cannabinoid attenuation of intestinal inflammation in chronic SIV-infected rhesus macaques involves differential modulation of T cell activation, microRNAs and pro-inflammatory gene expression. *Frontiers in immunology.*
127. Milloy MJ, Marshall B, Kerr T, Richardson L, Hogg R, Guillemi S, et al. (2015): High-intensity cannabis use associated with lower plasma human immunodeficiency virus-1 RNA viral load among recently infected people who use injection drugs. *Drug and alcohol review.* 34:135-140.
128. Rizzo MD, Crawford RB, Henriquez JE, Aldhamen YA, Gulick P, Amalfitano A, et al. (2018): HIV-infected cannabis users have lower circulating CD16+ monocytes and IFN-gamma-inducible protein 10 levels compared with nonusing HIV patients. *Aids.* 32:419-429.
129. Hasenoehrl C, Storr M, Schicho R (2017): Cannabinoids for treating inflammatory bowel diseases: where are we and where do we go? *Expert review of gastroenterology & hepatology.* 11:329-337.
130. Hasenoehrl C, Taschler U, Storr M, Schicho R (2016): The gastrointestinal tract - a central organ of cannabinoid signaling in health and disease. *Neurogastroenterol Motil.* 28:1765-1780.
131. Goyal H, Singla U, Gupta U, May E (2017): Role of cannabis in digestive disorders. *Eur J Gastroenterol Hepatol.* 29:135-143.
132. Gerich ME, Isfort RW, Brimhall B, Siegel CA (2015): Medical marijuana for digestive disorders: high time to prescribe? *Am J Gastroenterol.* 110:208-214.
133. Naftali T, Mechulam R, Lev LB, Konikoff FM (2014): Cannabis for inflammatory bowel disease. *Dig Dis.* 32:468-474.
134. Schicho R, Storr M (2014): Cannabis finds its way into treatment of Crohn's disease. *Pharmacology.* 93:1-3.
135. Hawkins T (2010): Understanding and managing the adverse effects of antiretroviral therapy. *Antiviral Res.* 85:201-209.
136. Eto K, Kim SK, Takeda I, Nabekura J (2018): The roles of cortical astrocytes in chronic pain and other brain pathologies. *Neuroscience research.* 126:3-8.
137. Vega-Avelaira D, Moss A, Fitzgerald M (2007): Age-related changes in the spinal cord microglial and astrocytic response profile to nerve injury. *Brain Behav Immun.* 21:617-623.

138. Stuesse SL, Crisp T, McBurney DL, Schechter JB, Lovell JA, Cruce WL (2001): Neuropathic pain in aged rats: behavioral responses and astrocytic activation. *Experimental brain research*. 137:219-227.
139. Raghavendra V, Tanga FY, DeLeo JA (2004): Complete Freund's adjuvant-induced peripheral inflammation evokes glial activation and proinflammatory cytokine expression in the CNS. *Eur J Neurosci*. 20:467-473.
140. Sweitzer SM, Colburn RW, Rutkowski M, DeLeo JA (1999): Acute peripheral inflammation induces moderate glial activation and spinal IL-1 β expression that correlates with pain behavior in the rat. *Brain research*. 829:209-221.
141. Lu C, Liu Y, Sun B, Sun Y, Hou B, Zhang Y, et al. (2015): Intrathecal Injection of JWH-015 Attenuates Bone Cancer Pain Via Time-Dependent Modification of Pro-inflammatory Cytokines Expression and Astrocytes Activity in Spinal Cord. *Inflammation*. 38:1880-1890.
142. Yun SP, Kam TI, Panicker N, Kim S, Oh Y, Park JS, et al. (2018): Block of A1 astrocyte conversion by microglia is neuroprotective in models of Parkinson's disease. *Nat Med*. 24:931-938.
143. Qian D, Li L, Rong Y, Liu W, Wang Q, Zhou Z, et al. (2019): Blocking Notch signal pathway suppresses the activation of neurotoxic A1 astrocytes after spinal cord injury. *Cell cycle (Georgetown, Tex)*. 18:3010-3029.
144. Myer DJ, Gurkoff GG, Lee SM, Hovda DA, Sofroniew MV (2006): Essential protective roles of reactive astrocytes in traumatic brain injury. *Brain*. 129:2761-2772.

Figures

Figure 1

Physicochemical characterization of BG-EVs: **A)** Absorbance at 280 nm of PPLC-isolated collagenase-digested BG eluates from VEH, SIV and THC/SIV groups (n=4/group). **B)** 3D-surface plot featuring the EV-turbidimetric signature. **C)** Contour representation highlighting the non-membranous molecular condensates (MCs) whose UV-peak blue-shifted to 262 nm. **D)** R2 index detected in fractions 7-16, confirming the presence of EVs in these fractions, whereas fractions 19-28 contained the MCs. **E,F)** PPLC-derived particle size (E) and concentration (F). **G)** Zeta (ζ)-potential of BG-EVs as measured by NTA (ZetaView). **H)** Quantification of EV-associated total protein as measured by the Bradford assay (top) and assessment of the protein weight per EV particle (Bottom). N=4 per group. Error bars represent standard error of the mean. No statistical differences were noted.

Figure 2

TEM-EDX elemental analysis of BG structures: **A)** Representative TEM images of CD9-immunolabeled EVs from BG tissues, from the three RM groups. The black spots on the structures correspond to 6-nm-gold-nanoparticles-labeled goat anti-mouse secondary antibody. **B)** A total of 25 TEM images were analyzed for size and counted for the immunogold label per vesicle and the means were reported per group. Error bar represent standard error of the mean. The number above the bars represent the number of vesicles analyzed per group. **C)** Representative images of MC from BG tissues. Seen are dense, sharp-edged and membraneless structures of primary particle size of ~10-20 nm that aggregate into large structures up to ~500 nm. **D)** Corresponding EDX spectra of the MC structures revealing presence of SPONCH (Sulphur, S; Phosphorus, P; Oxygen, O; Nitrogen, N; Carbon, C; and H, Hydrogen) elements, as a signature of biomolecules. Despite the strong signal of copper, Cu and chromium, Cr which constitute the main material of the specimen holder, and the fact that H is beyond the EDX detection limit and C may also be a specimen artifact, persistent traces of SPON, sulfur, phosphorus, oxygen, and nitrogen, were detected, supporting a ribonucleo-proteinaceous nature of the MC structures.

Figure 3

Changes in miRNA expression in BG-EVs from chronically SIV-infected rhesus macaques administered vehicle (VEH) or THC: **A-C)** Volcano plots showing the relationship between fold-change and statistical significance of differentially expressed miRNAs in **A)** VEH/SIV rhesus macaques relative to control, **B)** THC/SIV relative to VEH/SIV rhesus macaques, and **C)** THC/SIV relative to control rhesus macaques. The blue and red vertical lines correspond to 1.5-fold down and up, respectively, and the horizontal black lines represent $p < 0.05$. The miRNAs of interest are denoted with arrows. **D)** Heatmap of significant pathways predicted by DIANA-miRPath (v.3) of the human counterparts of the 11 SIV-downregulated miRs, showing inflammation and immune regulation pathway cluster (red arrows), a TLR signaling pathway cluster (orange arrows), in addition to Neurotrophin TRK receptor signaling pathway and response to stress (green arrows), as well as cell death and

viral process (black arrows), among others. **E)** Raw counts of selected neurotoxic miRNAs, downregulated in the VEH/SIV group. Statistical differences were assessed by a 2-way Anova test with Tukey's correction (n=3). *, p<0.05.

Figure 4

BG-EVs are taken up by astrocytes: We incubated 10,000 astrocytes from *CX3CR1^{+/GFP}* mice with increasing concentrations (0, 12.5, 25, 50, 100 and 200 μg/mL) of SytoSELECT labeled BG-EVs in the presence of NucBlue (a live cell stain, 30 μL/mL) and seeded in a glass-bottom 96 well plate 24-hour prior to experiment. Kinetics imaging (every 3h) was recorded over the course of 18 h using automated Lionheart FX software(18) (Biotek) after which total GFP (ex/em) and Nucblue (ex/em) intensity were recorded using a plate reader (Synergy H1 Biotek). Astrocytes were assessed for viability using Cell Titer Glow assay (Promega). **A)** Representative 10x images at 18 h time-point after addition of BG-EVs. **B)** Total gfp and nucblue intensity as measured by the plate reader. **E)** Viability of astrocytes treated with BG-EVs. Ordinary One-way ANOVA multiple comparison test (Tukey's test) was used to assess statistical differences. *, p<0.05; **, p<0.01; ***, p<0.001; ****, p<0.0001; ns, non-significant. Error bars represent Standard Deviation.

Figure 5

BG-EVs elevate Glial Fibrillary Acidic Protein (GFAP) levels: 10,000 primary mouse astrocytes per well were seeded in a glass-bottom 96 well plate 24-hour prior to experiment. Cells were treated with vehicle PBS or with 100 µg/mL pooled BG-EVs (n=4, 25 µg/pool from each group) for 24 hours, in triplicate wells and 9 field of views. Cells were then fixed, immuno-stained for GFAP, and imaged using an automated scope (Lionheart FX, Biotek). **A,C)** Representative 10x images for **A)** WT and **C)** CX3CR1+GFP+ astrocytes. Scale bar = 50 µm **B,D)** Mean fluorescence intensity (MFI) of GFAP calculated using single-cell analysis for **B)** WT and **D)** CX3CR1+GFP+ astrocytes. Ordinary One-way ANOVA multiple comparison test (Dunnett's test) was used to assess statistical differences in B and D. *, p<0.05; ***, p<0.001; ****, p<0.0001. Error bars represent S.E.M. of 600-1000 cells per treatment. **E)** Graph depicting genotype-dependent differential response to the treatments between WT and CX3CR1+GFP+ astrocytes. Unpaired T-test with Welch's correction was used to assess statistical differences. **, p<0.01; ***, p<0.001; ****, p<0.0001. Error bars represent S.E.M. of 600-1000 cells per treatment.

Figure 6

BG-EVs mediate distinct changes in the transcriptome of astrocytes: 200,000 primary mouse astrocytes per well were seeded in a 12 well-plate 24-hour prior to experiment. Cells were treated with vehicle PBS or with 100 µg/mL pooled BG-EVs (n=4, 25 µg/pool from each group) and incubated for 24 hours. Cellular RNA was extracted, and gene expression was assessed by RT-qPCR. **A-D)** Differential analysis of **A)** CD40, **B)** TNFα, **C)** MMP2, and **D)** MMP9. Top graph is WT and bottom graph is CX3CR1+GFP+ (TG) astrocytes. **E-H)** Graphs depicting the differential response to the treatments between WT and CX3CR1+GFP+ (TG) astrocytes. Ordinary One-way ANOVA multiple comparison test (Dunnett's test) was used to assess statistical differences in A-D. Unpaired T-test with Welch's correction was used to assess statistical differences between SIV and THC/SIV groups in panels A-H. Error bars represent S.E.M. *, p<0.05; **, p<0.01; ***, p<0.001; ****, p<0.0001; ns, not significant. **I)** The levels of untreated astrocyte CX3CR1 mRNA expression relative to normalization against GAPDH. The RT-qPCR products representative of mRNA levels were normalized to GAPDH signal and shown on the bar, while the amplicons separated on agarose gel is the inset. **J)** Protein-Protein Interaction (PPI) network of the altered genes in astrocytes.

Supplementary Files

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