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Activation of Cannabinoid Type Two Receptors (CB₂) Diminish Inflammatory Responses in Macrophages and Brain Endothelium

Yuri Persidsky,

Department of Pathology and Laboratory Medicine, Temple University School of Medicine, Philadelphia, PA 19140, USA

Center for Substance Abuse Research, Temple University School of Medicine, Philadelphia, PA 19140, USA

Shongshan Fan,

Department of Pathology and Laboratory Medicine, Temple University School of Medicine, Philadelphia, PA 19140, USA

Center for Substance Abuse Research, Temple University School of Medicine, Philadelphia, PA 19140, USA

Holly Dykstra,

Department of Pathology and Laboratory Medicine, Temple University School of Medicine, Philadelphia, PA 19140, USA

Center for Substance Abuse Research, Temple University School of Medicine, Philadelphia, PA 19140, USA

Nancy L. Reichenbach,

Department of Pathology and Laboratory Medicine, Temple University School of Medicine, Philadelphia, PA 19140, USA

Center for Substance Abuse Research, Temple University School of Medicine, Philadelphia, PA 19140, USA

Slava Rom, and

Department of Pathology and Laboratory Medicine, Temple University School of Medicine, Philadelphia, PA 19140, USA

Center for Substance Abuse Research, Temple University School of Medicine, Philadelphia, PA 19140, USA

Servio H. Ramirez

Department of Pathology and Laboratory Medicine, Temple University School of Medicine, Philadelphia, PA 19140, USA

yuri.persidsky@tuhs.temple.edu.

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Center for Substance Abuse Research, Temple University School of Medicine, Philadelphia, PA 19140, USA

Shriners Hospitals Pediatric Research Center, Temple University School of Medicine, Philadelphia, PA 19140, USA

Abstract

Chronic neuroinflammatory disorders (such as HIV associated neurodegeneration) require treatment that decreases production of inflammatory factors by activated microglia and macrophages and protection of blood brain barrier (BBB) injury secondary to activation of brain endothelium. Cannabinoid type 2 receptor (CB₂) is highly expressed on macrophages and brain microvascular endothelial cells (BMVEC) and is upregulated in inflammation and HIV infection. It has been shown that CB₂ activation dampened inflammatory responses in macrophages and BMVEC. In this study, we assessed by PCR array the expression of a wide range of genes increased in macrophages and BMVEC in inflammation. TNF α treatment upregulated 33 genes in primary human BMVEC, and two highly selective CB₂ agonists diminished expression of 31 and 32 genes. These results were confirmed by functional assays (BBB protection after inflammatory insult and decreased migration of monocytes across BMVEC monolayers after CB₂ stimulation). Similarly, CB₂ stimulation in primary human macrophages led to the suppression of 35 genes out of the 50 genes upregulated by LPS. Such changes in gene expression paralleled diminished secretion of proinflammatory factors. These results indicate the potential utility of CB₂ agonists for the treatment of neuroinflammation.

Keywords

Cannabinoid type 2 receptor; Neuroinflammation; Brain endothelial cell; Macrophage; Blood brain barrier

Despite immune recovery in individuals undergoing combination antiretroviral therapy (cART), the frequency of HIV-associated neurocognitive disorders (HAND) remains high for reasons that are not well understood (Cross et al. 2013). Interruption of cART results in virus resurgence within days in macrophages, considered to be a primary viral reservoir. Current HIV neuropathogenesis models indicate that HIV-associated neurodegeneration is driven by chronic inflammatory responses outside of the brain due to blood brain barrier (BBB) injury as well as a low level of HIV replication in central nervous system (CNS) reservoir cells (macrophages, microglia) and their chronic activation (Marcotte et al. 2013). Development and testing of novel treatment approaches, such as highly selective cannabinoid receptor 2 (CB₂) agonists that target inflammation and virus replication in macrophages and protect the BBB, should be beneficial for amelioration of HIV infection and HAND (Buch 2013; Purohit et al. 2014).

Emerging evidence suggests that modulating CB₂ in immune and other cell types holds unique therapeutic potential in a variety of inflammatory and other diseases. Selective CB₂ agonists possess potent anti-inflammatory, immunomodulatory and neuroprotective properties, and they do not activate the central cannabinoid 1 (CB₁) receptors responsible for the psychotropic effects of marijuana. Notably, the main psychoactive ingredient of

marijuana, (9)-tetrahydrocannabinol (THC), a mixed CB₁/CB₂ agonist, has recently been demonstrated to slow disease progression in a primate model of HIV infection, simian immunodeficiency virus (SIV) (Molina et al. 2011a, b, 2014). Although the anti-inflammatory effects of CB₂ are becoming better understood, the anti-retroviral effects of selective CB₂ agonists in the setting of HIV-1 infection in vivo have not yet been studied. Due to the non-selective nature of THC and the myriad of active compounds in marijuana, CB₂ agonists could offer far greater advantages for treatment of HIV-related pathologies.

The plausibility that CB₂ could be a key modulator of HIV CNS infection and inflammatory responses can be considered on the basis of augmented CB₂ expression in brain microglia/macrophages from patients with HIV encephalitis (Persidsky et al. 2011). Findings that are extended in vitro where commercially available CB₂ agonists have been reported to dampen HIV replication in human macrophages (Ramirez et al. 2013). Furthermore, CB₂ agonists decreased leukocyte adhesion to brain vascular endothelium, suppressed monocyte migration across the BBB and prevented enhanced BBB permeability in mice with systemic inflammation (Ramirez et al. 2012; Rom et al. 2013), all of these effects point to applicability of CB₂ agonist for the treatment of HIV infection in the periphery and in the CNS.

In this study, we performed a screening study in both primary brain endothelial cells and macrophages to identify genes (associated with inflammation) that could be targets of regulation by CB₂ activation. Screening results were confirmed in functional assays, further reinforcing the significance of the development of CB₂ selective agonists with improved bioavailability.

Materials and Methods

Cells and Reagents

Primary human BMVEC were isolated from the temporal cortex of brain tissue obtained during surgical removal of epileptogenic foci in adult patients (Persidsky et al. 1997). BMVEC were cultured as described (Persidsky et al. 2006; Ramirez et al. 2008). Peripheral blood mononuclear cells were obtained from HIV-1, -2, and hepatitis B seronegative donors by leukopheresis and then purified by counter current centrifugation to generate pure (>98 %) populations of monocytes (Gendelman et al. 1988). Monocytes were obtained from the Human Immunology Core of the University of Pennsylvania (Philadelphia, PA). Monocytes were incubated for 7 days in media containing macrophage colony stimulating factor (M-CSF) and allowed to differentiate into monocyte-derived macrophages (MDM).

PCR Arrays (Inflammatory Response and Autoimmunity)

PCR-based microarrays for evaluating the expression of genes commonly involved in inflammatory response were performed using the Taqman Array Human Immune Response (Invitrogen, Carlsbad, CA). The arrays used to assess the anti-inflammatory effects of CB₂ agonists on BMVEC and MDM inflammatory responses were configured in a 96-well plate consisting of a focused panel of 92 targets and 4 endogenous control genes. qPCR was performed on a StepOnePlus Real Time PCR system (Invitrogen). BMVEC were exposed to

TNF α (20 ng/ml, for 24 h, purchased from R&D Systems, Minneapolis, MN) in the presence of CB₂ agonist (O-1966 at 10 μ M acquired from Organix, Woburn, MA or JWH-133 at 10 μ M purchased from Tocris Bioscience, Ellisville, MO). Primary human monocytes were differentiated into MDM for 7 days in the presence of GM-CSF and then exposed to either LPS (50 ng/ml) alone or in combination with 10 μ M of the selective CB₂ agonist, JWH133. After 24 h, the cells were lysed and the total RNA from each sample was isolated. Using reverse transcription for first-strand cDNA synthesis, the converted cDNA was then mixed with Taqman universal PCR master mix (ABI) and added to array plates containing probes to immune response-associated genes (Taqman Array Human Immune Response, ABI). The raw data were analyzed with DataAssist software (ABI) using the delta-delta Ct method (Relative Quantification). The data were expressed as relative gene expression (fold) compared to the untreated control.

Transendothelial Migration Assay

Transendothelial migration assays were performed as described (Rom et al. 2013, 2014). MCP-1/CCL2 (50 ng/mL, purchased from R&D Systems) was used as a relevant chemokine. BMVEC were pretreated with CB₂ agonist (O-1966 at 10 μ M). Treatments were removed prior to monocyte introduction. Chemotaxis was allowed for 2 h. The data are shown as fold difference in migration (mean \pm SEM) from triplicate determinations, calculated from the number of migrated monocytes for each experimental condition divided by the number of migrated monocytes in the untreated, no chemoattractant control.

Transendothelial Electrical Resistance (TEER)

BMVEC were plated on collagen type I coated 96W20idf electrode arrays (ABI) and were treated with recombinant human soluble (s) CD40L (purchased from ProSpec, East Brunswick, NJ) in the presence of CB₂ agonist (JHW-133, O1966) or lysophosphatidic acid (LPA, 10 μ mol, purchased from Santa Cruz Biotechnology, Santa Cruz, CA). TEER measurements were performed using the 1600R ECIS System (Applied Biophysics) as described (Ramirez et al. 2010a, 2012). The results are presented as the average percent change from baseline TEER (expressed as average \pm SEM) from at least three independent experiments consisting of four to six replicates each.

ELISA

Genes identified from the PCR array to be affected by CB₂ activation during inflammatory insult were evaluated for protein expression using ELISA. After MDM were treated as indicated in the figure, conditioned medium was collected to measure IFN γ -inducible protein of 10 kDa (IP-10) or CXCL10, and TNF α using conventional double sandwich ELISA from R&D Systems. Assays were performed according to the manufacturer's instructions.

Statistical Analysis

Data are expressed as the mean \pm SEM of experiments conducted multiple times. Multiple group comparisons were performed by one-way analysis of variance with Dunnet's posthoc

tests (migration, ELISA). Statistical analyses were performed utilizing Prism v6.0c software (GraphPad Software, San Diego, CA). Differences were considered significant at $p < 0.05$.

Results

HIV infection is known to be associated with chronic inflammation and activation of endothelium in different organs (Graham et al. 2013). Such endothelial cell responses can lead to end-organ injury, and interventions diminishing the state of activation (like CB₂ activation) should prevent tissue injury occurring during infection (Ramirez et al. 2012). To evaluate whether the anti-inflammatory effect of CB₂ activation applies to brain endothelial cells, we profiled the expression of genes commonly involved in the regulation of inflammatory response and autoimmunity. Using a commercial PCR-based focused array, 92 genes relevant to inflammatory responses were analyzed in untreated BMVEC (control), BMVEC activated with TNF α (20 ng/ml, 24 h) and TNF α -activated BMVEC in the presence of CB₂ agonists (O-1966 or JWH-133). The array analyses revealed that 33 gene targets were upregulated more than 2-fold in the TNF α -treated BMVEC when compared to untreated control cells. The addition of either O-1966 or JWH-133 resulted in the suppression of 31 and 32 genes, respectively, out of the 33 upregulated by TNF α . Fifteen genes belong to cytokine/chemokine group, 8 are adhesion molecule and receptors; the rest are from a group of cellular response and transcription factors. Table 1 shows the pro-inflammatory mediators that were most affected by CB₂ activation. When compared to BMVEC that were treated with TNF α only, cytokine-stimulated BMVEC exposed to O-1966 showed a 97 % attenuation in iNOS, a 88 % reduction in CXCL10 and CXCL11, and a 40–50 % decrease in ICAM-1, IL-8, CCL2 and CD40 expression among others. Similarly, JWH-133 reduced pro-inflammatory gene expression in TNF α -stimulated BMVEC: 98 % for iNOS, 97 % for CXCL10 and CXCL11, 88 % for ICAM-1 and IL-15, and 75 % for CCL2, MCSF-1 and IL-18 and CD40.

Changes in CD40 expression by CB₂ agonists are of interest because the functional relevance of CD40 expression in BMVEC was previously established in the context of HIV-1 infection (Ramirez et al. 2010b). We showed that exposure of primary human BMVEC to soluble CD40L (sCD40L) upregulated the expression of adhesion molecules ICAM-1 and VCAM-1, which caused a four-fold increase in monocyte adhesion to BMVEC and stimulated migration across an in vitro BBB model. It has been suggested that increased levels of sCD40L are associated with cognitive impairment in HIV infection (Sui et al. 2007). Now, we have assessed the effect of sCD40L on barrier tightness and found that average TEER measurements showed a rapid 5–20 % drop in resistance in a dose-dependent manner (Fig. 1a). The positive control, LPA (a known activator of RhoA) (Yamamoto et al. 2008), caused significant decrease of TEER indicating an appropriate path-ophysiologic response of BBB models. To test the protective effects of CB₂ agonists, O-1966 and JWH-133 (at 10 μ M), prevented a TEER drop caused by sCD40L (Fig. 1b) indicating that CB₂ activation can protect the BBB under inflammatory conditions.

Next using migration assays in an in vitro BBB model, we tested whether CB₂ activation in endothelial cells could prevent monocyte passage across BMVEC monolayers. We used CCL2 as a relevant cytokine that is upregulated in the CNS under neuroinflammatory

conditions. Application of CCL2 to the lower chamber of BBB constructs increased monocyte migration 6-fold, as compared to models without chemokine addition. Pre-treatment of BMVEC with O-1966 attenuated monocyte migration across endothelial monolayers by 50 % (Fig. 2).

Activation of macrophages/microglia in the CNS and secretion of pro-inflammatory factors is believed to cause neuronal dysfunction and HAND (Kaul and Lipton 2006). Selective CB₂ activation was shown to diminish HIV replication in macrophages (Ramirez et al. 2013) and prevent monocyte migration across the BBB (Rom et al. 2013). To evaluate whether the anti-inflammatory effect of CB₂ activation applies to monocyte-derived macrophages (MDM), we profiled the expression of genes commonly involved in the regulation of inflammatory responses (Table 2). Using a commercial PCR-based focused array, 92 genes (including cytokines and cytokine receptors; chemokines and chemokine receptors) relevant to inflammatory responses were analyzed in untreated MDM (control), MDM activated with LPS (50 ng/ml, 24 h) and LPS-activated MDM in the presence of the CB₂ agonist, JWH133. The array analyses revealed that 50 genes were up-regulated more than 2-fold in LPS-treated MDM when compared to untreated control cells. The addition of JWH133 resulted in the suppression of 35 genes out of the 50 genes that were up-regulated by LPS (13 from cytokine/chemokine group, 10 belonging to adhesion molecule/receptors, and 8 to cell response/transcription factors). These included CXCL10, CXCL11, CCL19, CCL5, CCR7 (99–95 % attenuation), TNF α , ICAM-1, and CCL3 (64 % reduction of expression) among others.

Next, we measured the inflammatory molecules corresponding to genes affected by CB₂ stimulation in MDM. ELISA assays for TNF α and CXCL10/IP-10 were performed to validate the gene expression results acquired from the gene array immune response panel. LPS stimulation resulted in a 90-fold increase of TNF α production and CB₂ activation by JWH133 resulted in a 98 % suppression of TNF α secretion (Fig. 3a). Similarly, LPS activation of MDM led to a 242-fold increase in IP-10 production, and JWH-133 treatment reduced IP-10 secretion by 97.5 % (Fig. 3b). These results indicate that CB₂ agonist effectively suppresses potent inflammatory mediators secreted by activated MDM.

Discussion

The rapid move towards legalization of marijuana (now accepted for medicinal or recreational use in 20 states in the US) poses a significant challenge for biomedical research, namely identifying what compounds in the cannabis plant may possess beneficial effects in diseases (2014). Identification of CB₁ and CB₂ receptors with clearly distinct cellular distribution patterns and functions offers this opportunity. High levels of CB₂ expression on immune cells and endothelial cells and its upregulation by inflammatory mediators suggest its participation in immune responses and resolution of inflammation (Buch 2013).

Such effects are very pertinent for HIV-1 infection in and outside of the CNS. HAND (Letendre et al. 2011; Spudich 2014) continues to be highly prevalent and the progression of HAND is associated with biomarkers of chronic immune activation driven by low levels of virus infection in monocytes and macrophages (Cross et al. 2013; Marcotte et al. 2013).

Additional interventions further dampening HIV replication and diminishing chronic neuroinflammation are critically needed. Data presented in this report indicate that a number of pro-inflammatory molecules (playing an important role in HAND progression) have been decreased by CB₂ agonists in brain endothelium and human macrophages. Diminished gene expression has been accompanied by attenuated protein secretion (by MDM) and functional assays mimicking BBB injury (monocyte migration, TEER). These molecules (such as CCL2, TNF α , CXCL10, CCL5) are among those shown to be increased in HAND and are considered as neurotoxins driving neuronal dysfunction. Similarly, a number of such molecules (ICAM-1, CXCL10, CXCL11, CD40, VEGF) were downregulated in BMVEC and such changes were accompanied by decreased monocyte migration across BBB models and preservation of BBB integrity after application of sCD40L. These observations further confirm previously published data in BMVEC (attenuation of adhesion molecule expression, prevention of leukocyte/BMVEC interactions in vitro and in vivo, prevention of BBB leakiness, etc.) (Ramirez et al. 2012) and monocytes/macrophages (decreased migration/adhesion, attenuation of integrin expression, decreased lamellipodia formation, HIV replication) (Ramirez et al. 2013; Rom et al. 2013).

Overall, our data parallel previous work indicating the therapeutic potential of CB₂ activation. In immune cells, CB₂ stimulation decreased production of pro-inflammatory factors (Puffenbarger et al. 2000; Facchinetti et al. 2003) that are implicated in neuronal injury during HIV-1 CNS infection (Kraft-Terry et al. 2010). Neuroprotective effects of CB₂ agonists are associated with suppression of microglia activation (Klegeris et al. 2003; Eljaschewitsch et al. 2006) via inhibition of the release of neurotoxic factors. In vitro and in vivo studies have shown that cannabinoids can act on glia, enhancing the release of the anti-inflammatory cytokines, IL-4 and IL-10 (Molina-Holgado et al. 1998). CB₂ signaling interfered with the enhanced expression of iNOS and CCR2 induced by IFN γ in mouse microglial cells (Racz et al. 2008). Selective CB₂ agonist diminished neuroinflammation in a rodent model of HIVE (Gorantla et al. 2010). Chronic neuroinflammation documented in HIV infection (Suh et al. 2014) requires adjunctive therapies in addition to ART. Highly selective CB₂ agonists with better pharmacologic properties could be one such therapy.

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References

- Cross S, Onen N, Gase A, Overton ET, Ances BM. Identifying risk factors for HIV-associated neurocognitive disorders using the international HIV dementia scale. *J Neuroimmune Pharmacol.* 2013; 8:1114–1122. [PubMed: 24114509]
- Marcotte TD, Deutsch R, Michael BD, Franklin D, Cookson DR, Bharti AR, Grant I, Letendre SL, Group C. A concise panel of biomarkers identifies neurocognitive functioning changes in HIV-infected individuals. *J Neuroimmune Pharmacol.* 2013; 8:1123–1135. [PubMed: 24101401]
- Buch SJ. Cannabinoid receptor 2 activation: a means to prevent monocyte-endothelium engagement. *Am J Pathol.* 2013; 183:1375–1377. [PubMed: 24055258]
- Purohit V, Rapaka RS, Rutter J. Cannabinoid receptor-2 and HIV-associated neurocognitive disorders. *J Neuroimmune Pharmacol.* 2014; 9:447–453. [PubMed: 25015040]

- Molina PE, Amedee A, LeCapitaine NJ, Zabaleta J, Mohan M, Winsauer P, Vande Stouwe C. Cannabinoid neuroimmune modulation of SIV disease. *J Neuroimmune Pharmacol*. 2011a; 6:516–527. [PubMed: 21830069]
- Molina PE, Winsauer P, Zhang P, Walker E, Birke L, Amedee A, Stouwe CV, Troxclair D, McGoey R, Varner K, Byerley L, LaMotte L. Cannabinoid administration attenuates the progression of simian immunodeficiency virus. *AIDS Res Hum Retrovir*. 2011b; 27:585–592. [PubMed: 20874519]
- Molina PE, Amedee AM, Lecapitaine NJ, Zabaleta J, Mohan M, Winsauer P, Vande Stouwe C, McGoey R, Auten MW, Lamotte L, Chandra LC, Birke L. Modulation of Gut-Specific Mechanisms by Chronic Delta9-THC Administration in Male Rhesus Macaques Infected with Simian Immunodeficiency Virus: A Systems Biology Analysis. *AIDS Res Hum Retroviruses*. 2014
- Persidsky Y, Ho W, Ramirez SH, Potula R, Abood ME, Unterwald E, Tuma R. HIV-1 infection and alcohol abuse: neurocognitive impairment, mechanisms of neurodegeneration and therapeutic interventions. *Brain Behav Immun*. 2011; 25(Suppl 1):S61–S70. [PubMed: 21397004]
- Ramirez SH, Reichenbach NL, Fan S, Rom S, Merkel SF, Wang X, Ho WZ, Persidsky Y. Attenuation of HIV-1 replication in macrophages by cannabinoid receptor 2 agonists. *J Leukoc Biol*. 2013; 93:801–810. [PubMed: 23463725]
- Ramirez SH, Hasko J, Skuba A, Fan S, Dykstra H, McCormick R, Reichenbach N, Krizbai I, Mahadevan A, Zhang M, Tuma R, Son YJ, Persidsky Y. Activation of cannabinoid receptor 2 attenuates leukocyte-endothelial cell interactions and blood–brain barrier dysfunction under inflammatory conditions. *J Neurosci*. 2012; 32:4004–4016. [PubMed: 22442067]
- Rom S, Zuluaga-Ramirez V, Dykstra H, Reichenbach N, Pacher P, Persidsky Y. Selective activation of cannabinoid receptor 2 (CB2) in leukocytes suppresses their engagement of the brain endothelium and protects the Blood Brain Barrier (BBB). *Am J Pathol*. 2013; 183:1548–1558. [PubMed: 24055259]
- Persidsky Y, Stins M, Way D, Witte MH, Weinand M, Kim KS, Bock P, Gendelman HE, Fiala M. A model for monocyte migration through the blood–brain barrier during HIV-1 encephalitis. *J Immunol*. 1997; 158:3499–3510. [PubMed: 9120312]
- Persidsky Y, Heilman D, Haorah J, Zelivyanskaya M, Persidsky R, Weber GA, Shimokawa H, Kaibuchi K, Ikezu T. Rho-mediated regulation of tight junctions during monocyte migration across the blood–brain barrier in HIV-1 encephalitis (HIVE). *Blood*. 2006; 107:4770–4780. [PubMed: 16478881]
- Ramirez SH, Heilman D, Morse B, Potula R, Haorah J, Persidsky Y. Activation of peroxisome proliferator-activated receptor gamma (PPARgamma) suppresses Rho GTPases in human brain microvascular endothelial cells and inhibits adhesion and transendothelial migration of HIV-1 infected monocytes. *J Immunol*. 2008; 180:1854–1865. [PubMed: 18209083]
- Gendelman HE, Orenstein JM, Martin MA, Ferrua C, Mitra R, Phipps T, Wahl LA, Lane HC, Fauci AS, Burke DS, et al. Efficient isolation and propagation of human immunodeficiency virus on recombinant colony-stimulating factor 1-treated monocytes. *J Exp Med*. 1988; 167:1428–1441. [PubMed: 3258626]
- Rom S, Zuluaga-Ramirez V, Dykstra H, Reichenbach NL, Ramirez SH, Persidsky Y. Poly(ADP-ribose) polymerase-1 inhibition in brain endothelium protects the blood–brain barrier under physiologic and neuroinflammatory conditions. *J Cereb Blood Flow Metab*. 2014
- Ramirez SH, Fan S, Zhang M, Papugani A, Reichenbach N, Dykstra H, Mercer AJ, Tuma RF, Persidsky Y. Inhibition of Glycogen Synthase Kinase 3{beta} (GSK3{beta}) decreases inflammatory responses in brain endothelial cells. *Am J Pathol*. 2010a; 176:881–892. [PubMed: 20056834]
- Graham SM, Mwilu R, Liles WC. Clinical utility of biomarkers of endothelial activation and coagulation for prognosis in HIV infection: A systematic review. *Virulence*. 2013; 4
- Ramirez SH, Fan S, Dykstra H, Reichenbach N, Del Valle L, Potula R, Phipps RP, Maggirwar SB, Persidsky Y. Dyad of CD40/CD40 ligand fosters neuroinflammation at the blood–brain barrier and is regulated via JNK signaling: implications for HIV-1 encephalitis. *J Neurosci*. 2010b; 30:9454–9464. [PubMed: 20631174]

- Sui Z, Sniderhan LF, Schifitto G, Phipps RP, Gelbard HA, Dewhurst S, Maggirwar SB. Functional synergy between CD40 ligand and HIV-1 Tat contributes to inflammation: implications in HIV type 1 dementia. *J Immunol.* 2007; 178:3226–3236. [PubMed: 17312171]
- Yamamoto M, Ramirez SH, Sato S, Kiyota T, Cerny RL, Kaibuchi K, Persidsky Y, Ikezu T. Phosphorylation of claudin-5 and occludin by rho kinase in brain endothelial cells. *Am J Pathol.* 2008; 172:521–533. [PubMed: 18187566]
- Kaul M, Lipton SA. Mechanisms of neuroimmunity and neurodegeneration associated with HIV-1 infection and AIDS. *J Neuroimmune Pharmacol.* 2006; 1:138–151. [PubMed: 18040780]
- High time for advancing marijuana research. *Nat Neurosci.* 2014; 17:481. [PubMed: 24671059]
- Letendre SL, Zheng JC, Kaul M, Yiannoutsos CT, Ellis RJ, Taylor MJ, Marquie-Beck J, Navia B. Chemokines in cerebrospinal fluid correlate with cerebral metabolite patterns in HIV-infected individuals. *J Neurovirol.* 2011; 17:63–69. [PubMed: 21246320]
- Spudich SS. CROI 2014: neurologic complications of HIV infection. *Top Antivir Med.* 2014; 22:594–601. [PubMed: 24901885]
- Puffenbarger RA, Boothe AC, Cabral GA. Cannabinoids inhibit LPS-inducible cytokine mRNA expression in rat microglial cells. *Glia.* 2000; 29:58–69. [PubMed: 10594923]
- Facchinetti F, Del Giudice E, Furegato S, Passarotto M, Leon A. Cannabinoids ablate release of TNFalpha in rat microglial cells stimulated with lypopolysaccharide. *Glia.* 2003; 41:161–168. [PubMed: 12509806]
- Kraft-Terry SD, Stothert AR, Buch S, Gendelman HE. HIV-1 neuroimmunity in the era of antiretroviral therapy. *Neurobiol Dis.* 2010; 37:542–548. [PubMed: 20044002]
- Klegeris A, Bissonnette CJ, McGeer PL. Reduction of human monocytic cell neurotoxicity and cytokine secretion by ligands of the cannabinoid-type CB2 receptor. *Br J Pharmacol.* 2003; 139:775–786. [PubMed: 12813001]
- Eljaschewitsch E, Witting A, Mawrin C, Lee T, Schmidt PM, Wolf S, Hoertnagl H, Raine CS, Schneider-Stock R, Nitsch R, Ullrich O. The endocannabinoid anandamide protects neurons during CNS inflammation by induction of MKP-1 in microglial cells. *Neuron.* 2006; 49:67–79. [PubMed: 16387640]
- Molina-Holgado F, Molina-Holgado E, Guaza C. The endogenous cannabinoid anandamide potentiates interleukin-6 production by astrocytes infected with Theiler's murine encephalomyelitis virus by a receptor-mediated pathway. *FEBS Lett.* 1998; 433:139–142. [PubMed: 9738948]
- Racz I, Nadal X, Alferink J, Banos JE, Rehnelt J, Martin M, Pintado B, Gutierrez-Adan A, Sanguino E, Bellora N, Manzanares J, Zimmer A, Maldonado R. Interferon-gamma is a critical modulator of CB(2) cannabinoid receptor signaling during neuropathic pain. *J Neurosci.* 2008; 28:12136–12145. [PubMed: 19005078]
- Gorantla S, Makarov E, Roy D, Finke-Dwyer J, Murrin LC, Gendelman HE, Poluektova L. Immunoregulation of a CB2 receptor agonist in a murine model of neuroAIDS. *J Neuroimmune Pharmacol.* 2010; 5:456–468. [PubMed: 20549374]
- Suh J, Sinclair E, Peterson J, Lee E, Kyriakides TC, Li FY, Hagberg L, Fuchs D, Price RW, Gisslen M, Spudich S. Progressive increase in central nervous system immune activation in untreated primary HIV-1 infection. *J Neuroinflammation.* 2014; 11:199. [PubMed: 25465205]

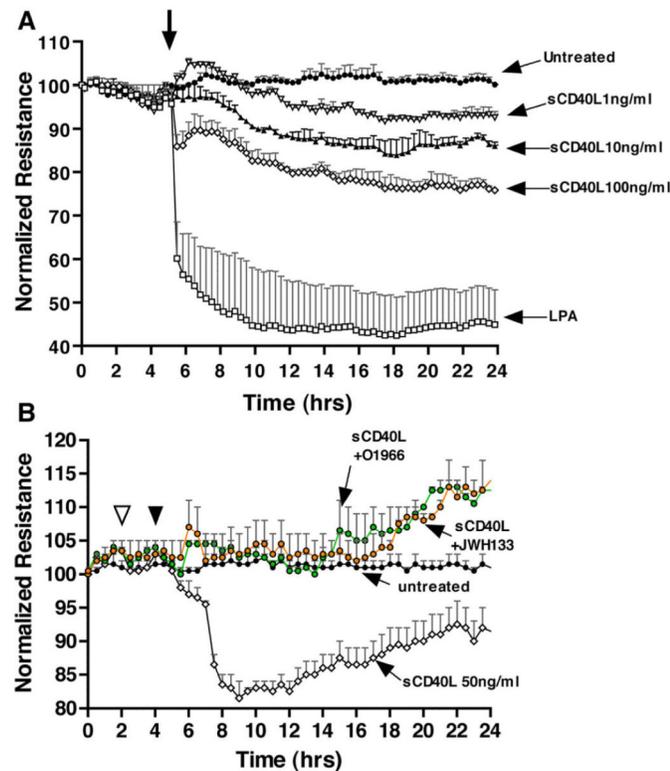


Fig. 1. Barrier function is compromised in sCD40L-treated BMVEC. **a** TEER, an indicator of barrier integrity, was measured (by ECIS) in BMVEC monolayers untreated or treated with 1, 10 and 100 ng/ml sCD40L or LPA (10 μ mol). The resistance was measured at 4000 Hz in 10 min intervals for the duration of the time shown. Treatments were initiated (*arrow*) after stable resistance was reached. **b** TEER was measured after sCD40L application (50 ng/ml) with or without CB₂ agonists (O-1966 and JWH-133 at 10 μ M). BMVEC monolayers were treated with CB₂ agonists for 2 h prior to the addition of sCD40L. Both agonists prevented TEER decrease. Each data point is represented as the percent of the mean value \pm SEM ($n=3$). Open (*white*) *arrowhead* indicates time of CB₂ agonist application; closed (*black*) *arrowhead* indicates time of sCD40L addition

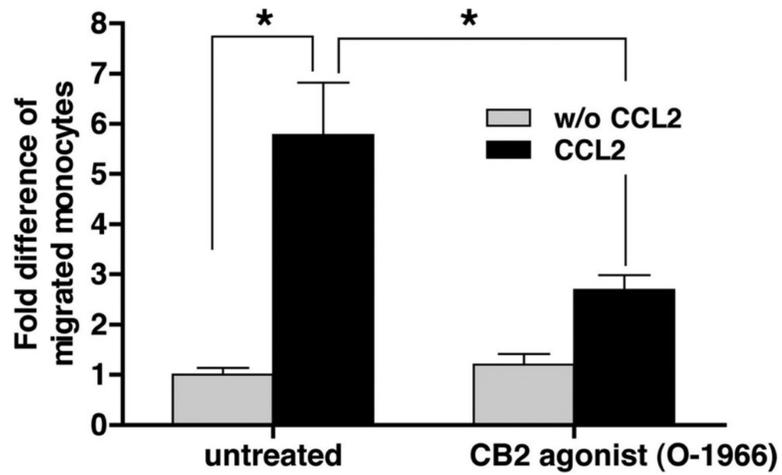


Fig. 2.

CB₂ stimulation blocked monocyte migration across BMVEC monolayers. BMVEC were pretreated with O-1966 (10 μM) that was removed prior to monocyte introduction. The migration assay was performed using 2.5×10⁴ BMVEC/insert with calcein-AM labeled monocytes added to BMVEC seeded on collagen-coated FluoroBlok BD inserts. CCL2 (MCP-1, 30 ng/ml) was used as a relevant chemokine. Chemotaxis was allowed for 2 h. Data are shown as mean±SEM. * *p*<0.01

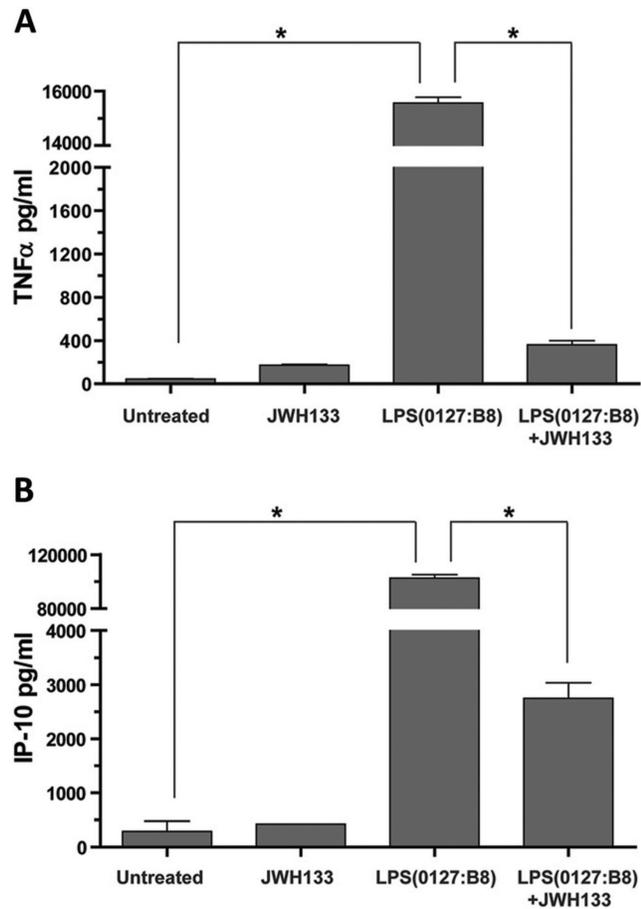


Fig. 3. The selective CB₂ agonist, JWH133, attenuated secretion of inflammatory mediators from LPS-activated MDM. Conditioned medium from MDM was collected after 24 h of treatment as indicated in panels **a** and **b**. TNF α and CXCL10/IP-10 levels were evaluated using ELISA (R&D Systems). **a** LPS exposure increased TNF α secretion by 90-fold when compared to the untreated control, whereas LPS with CB₂ agonist down-regulated the secretion by 98 % when compared to LPS-treated MDM. **b** CXCL10/IP-10, when compared to untreated control, showed a 242-fold induction by LPS that was decreased by 97.5 % by JWH133. The results are expressed as mean values \pm SD with *asterisks* indicating statistical significance ($p < 0.05$)

Table 1CB₂ agonists decreased expression of pro-inflammatory genes in activated BMVEC

Gene/Class	TNF α /control (fold change)	TNF α +O-1966/control (fold change)	TNF α +JWH133/control (fold change)
Chemokine and cytokines			
CXCL11	4148.51	610.38	259.14
CCL19	32.93	10.14	67.45
CXCL10/IP10	1029.68	153.86	32.78
IL18	2	0.6	0.5
TNF α	131.75	9.82	16.8
IL18	266.37	158.95	134.21
CCL2/MCP-1	63.92	38.34	16.13
IL7	31.81	9.54	8.06
IL15	16.25	4.18	2.04
MCSF/CSF1	8.28	2.46	2.09
CCL3	7.99	4.46	4.19
CD40	4.08	2.45	1.03
CXCL4/PF4	4.06	1.22	8.32
Adhesion molecules and receptors			
ICAM1	16.6	9.83	2.1
LRP2	1.99	0.07	0.03
Cell response and transcription factors			
NFKB2	8.1	4.86	2.04
NOS2A	15.82	0.59	0.25
C3	15.98	4.74	8.21
CD38	32.41	9.64	16.49
VEGF	1.01	0.6	0.25

A PCR-based focused array consisting of 92 genes relevant to inflammatory responses analyzed anti-inflammatory responses of CB₂ agonists. Gene expression was assessed in untreated BMVEC (*control*), BMVEC activated with TNF α (20 ng/ml, 24 h) and TNF α -activated BMVEC in the presence of CB₂ agonist (O-1966 or JWH-133). The data are expressed as relative gene expression (fold) compared to the untreated control

Table 2

Identification of genes commonly involved in inflammatory responses that were regulated by CB₂ agonist in LPS-stimulated MDM

Gene/Class	LPS/control (fold change)	LPS+JWH133/control (fold change)	JWH133/control (fold change)
Chemokine and cytokines			
CXCL11	47,746.26	0.26	1.45
CCL19	23,184.93	1033.56	1.40
CXCL10/IP10	5871.45	0.02	0.36
CCL5	362.14	16.01	0.70
IL15	22.53	1.00	0.70
CCL3	22.49	8.03	0.70
IL18	11.67	2.07	1.48
CD40	11.34	2.03	1.41
IL13	10.32	1.01	0.71
IL3	10.04	1.01	0.71
TNF α	5.69	2.03	0.71
IL12A	5.62	0.50	0.69
Adhesion molecules and receptors			
ICAM1	11.43	4.03	2.86
CCR7	11,622.24	519.65	11.47
IL2RA	2923.13	1031.91	0.18
AGTR1	23.21	7.80	0.71
FAS	23.08	1.00	2.86
CXCR3	11.29	1.01	0.71
CD80	90.78	4.04	1.42
Cell response and transcription factors			
EDN1	180.36	31.91	1.41
CD38	90.17	4.02	2.85
NFKB	11.37	2.01	1.43
NFATC4	10.52	7.61	5.34
FASLG	5.62	2.02	1.40

Primary human monocytes were differentiated into MDM for 7 days in the presence of GM-CSF1 and then exposed to either LPS (50 ng/ml) alone or in combination with 10 μ M of the selective CB₂ agonist, JWH133. The raw data were analyzed with DataAssist software (ABI) using the delta-delta Ct method (Relative Quantification). Twenty-four genes were markedly inhibited after exposure to JWH133 in LPS-stimulated MDM. The data are expressed as relative gene expression (fold) compared to the untreated control