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Endocannabinoids affect innate immunity of Muller glia during HIV-1 Tat cytotoxicity

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ABSTRACT

In the retina, increased inflammatory response can cause visual impairment during HIV infection in spite of successful anti-retroviral therapy (HAART). The HIV-1 Tat protein is implicated in neurodegeneration by eliciting a cytokine response in cells of the CNS, including glia. The current study investigated whether innate immune response in human retinal Muller glia could be immune-modulated to combat inflammation. Endocannabinoids, N-arachidonoylethanolamide and 2-arachidonoylglycerol are used to alleviate Tat-induced cytotoxicity and rescue retinal cells. The neuroprotective mechanism involved suppression in production of pro-inflammatory and increase of anti-inflammatory cytokines, mainly through the MAPK pathway. The MAPK regulation was primarily by MKP-1. Both endocannabinoids regulated cytokine production by affecting at the transcriptional level the NF-κB complex, including IRAK1BP1 and TAB2. Stability of cytokine mRNA is likely to have been influenced through tristetraprolin. These findings have direct relevance in conditions like immune-recovery uveitis where anti-retroviral therapy has helped immune reconstitution. In such conditions drugs to combat overwhelming inflammatory response would need to supplement HAART. Endocannabinoids and their agonists may be thought of as neurotherapeutic during certain conditions of HIV-1 induced inflammation.

Introduction

Altered immune responses have been documented as pathophysiology of HIV associated dementia (HAD) (Miller and Meucci, 1999). Increased immune reactions can contribute to neuronal and glial dysfunction and subsequent cell death. Individuals with HIV-1 often suffer from visual impairment and opportunistic retinitis (McCluskey, 1993). The advent of highly active anti-retroviral therapy (HAART) has decreased the incidence of opportunistic infections in HIV-1 positive patients. Intense inflammatory response however occurs in spite of anti-retroviral therapy as in immune recovery uveitis (IRU) and CMV retinitis. This is a consequence of recovering CD4+ levels (Holland, 2008). Early in infection, HIV-1 trans-activating (Tat) proteins, and other regulatory proteins, are released from HIV-1-infected cells (Ensoli et al., 1993). It can interact with receptors of other uninfected cells in the CNS. Significantly, its production is not impacted by the use of antiretroviral drugs (Ma and Nath, 1997). HIV-1 Tat can be taken up by cells and modulate expression of genes that regulate cellular activities such as survival, growth, and angiogenesis. Notably, certain HIV-1 Tat variants are active neurotoxic cytokine generators thus leading to inflammation (Williams et al., 2009a; Zhou et al., 2004). Normally the retina as part of the central nervous system is immune-suppressive; this however makes it peculiarly susceptible to inflammatory response. Like the rest of the CNS, the retina employs its resident glial cells (microglia, Muller glia, and astrocytes) in response to inflammation. Muller glia, the predominant retinal glia are actively involved in many inflammatory conditions of the retina. Activation of Muller glia also has an innate immune component (Shamsuddin and Kumar, 2011). Previous work from the authors show that R28 retinal cells and HIV-1 Tat transgenic mice show pronounced activation on exposure to HIV-1 Tat protein (Chatterjee et al., 2011). Endocannabinoids, their receptors (CB), the proteins responsible for synthesis, reuptake and degradation of these endogenous ligands, together form the endocannabinoid system. Endocannabinoid as an immune-modulator has been well documented in cells of the immune system (Walter and Stella, 2004). Increasingly, it is also seen that resident immune cells of the CNS, such as microglia are influenced by endocannabinoids (Eljaschewitsch et al., 2006; Docagne

Abbreviations: (AEA), N-arachidonoylethanolamide; (2-AG), 2-arachidonoylglycerol; (CB1), cannabinoid receptor type 1; (CB2), cannabinoid receptor type 2; (AM-251), N-(piperidin-1-yl)-5-(4-iodophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3 carboxamide; (AM-630), 6-iodo-2-methyl-1-[2-(4-morpholinyl)ethyl]-1H-indol-3-yl] (4-methoxyphenyl)methanone); (MAP-2), microtubule-associated protein 2; (FR180204), 5-(2-phenyl-pyrazolo[1,5-a]pyridin-3-yl)-1H-pyrazolo[3,4-c]pyridazin-3-ylamine); (SP600125), anthra[1-9-cd]pyrazol-6(2H)-one); (SB203580), 4-[5-(4-fluorophenyl)-2-[4 (methylsulfonyl)phenyl]-1H-imidazol-4-yl]pyridine); (Ly294002), 2-(4-morpholinyl)-8-phenyl-4H-1-benzopyran-4-onehydrochloride); (Ro-318220), 3-[3-[2,5-dihydro-4-(1-methyl-1H-indol-3-yl)-2,5-dioxo-1H-pyrrol-3-yl]-1H-indol-1-yl]propyl carbamimidothioic acid ester mesylate); (U0126), 1,4-diamino-2,3-dicyano-1,4-bis[2-aminophenylthio] butadiene); (MKP-1), mitogen-activated protein kinase phosphatase-1; (MKP-2), mitogen-activated protein kinase phosphatase-2; (IRAK1BP1), IL-1R-associated kinase 1 binding protein 1; (TTP), tristetraprolin.

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et al., 2007). While cannabinoid therapy has been shown to alleviate neurodegeneration in diabetic retinopathy and glaucoma, little is known towards its mechanism of action and its efficacy against HIV-1 infection induced inflammation. In the present study we demonstrate that HIV-1 Tat exposed Muller cells show elevated production of pro-inflammatory factors, potential neurotoxins that can cause retinal degeneration. We further explore the mechanisms by which endocannabinoids can reduce the inflammatory cytokines. We have dissected the signalling pathways involved in this switch towards an anti-inflammatory milieu and demonstrate the critical roles of Mitogen-activated protein kinases (MAPKs) and the canonical NF-κB signalling in this process. Understanding the pathways involved in immune-modulation by endocannabinoids is essential for future development of cannabinoid based therapeutics aimed at abrogation of inflammation in the retina of HIV patients. This is particularly relevant since elsewhere in the CNS, severity of HIV-1 associated neurodegeneration caused by inflammation correlates better with the presence of activated glial cells than with the presence and number of HIV-infected cells (Glass et al., 1995; González-Scarano and Martín-García, 2005).

Results

Endocannabinoids AEA and 2-AG suppress Muller cell activation and protect against HIV-1 Tat induced retinal cell death by repressing inflammatory cytokines

Earlier work from the authors suggested that in the retina constitutive expression of HIV-1 Tat in transgenic mice causes activation in the form of increased GFAP staining (Chatterjee et al., 2011). It has been reported that Tat induces cytotoxicity in glioma cells (Esposito et al., 2002). We therefore checked whether addition of Tat induces Muller cell activation. GFAP expression increases in activated Muller glia (Brahmachari et al., 2006). Tat induced GFAP mRNA increases significantly 2 h onwards. AEA/2-AG co-incubation with Tat reduces GFAP expression at all time points (Fig. 1A). At protein levels there is a significant increase from 8 h onwards (Fig. 1B). Tat-treated cells showed only 55% cell viability. Addition of AEA/2-AG increased cell viability to 82% and the effect was reversed by the addition of CB1/CB2 antagonists (Fig. 1C). CB1 and CB2 cannabinoid receptors are members of the GPCR super family. These receptors act through heterotrimeric $G_{i/o}$ type proteins by inhibiting adenylyl cyclase. CB1/CB2 receptors can regulate MAPK phosphorylation and intracellular Ca^{2+} elevations (Bosier et al., 2010; Herkenham et al., 1990). Tat treatment increased the mRNA expression levels of CB1 and CB2 receptors. Addition of AEA or 2-AG reduced the levels of CB receptors significantly (Fig. 1D) at 8 h. Cleaved caspase-3 an indicator of apoptosis (Kamada et al., 2005) and PARP which gets cleaved on activation of the necrotic pathway (Boulares et al., 1999) were expressed in Tat treated cells alone (Fig. 1E). Addition of AEA/2-AG blocked the expression of activated PARP and cleaved caspase-3. CB1/2 inhibitor reversed the effect of AEA/2-AG with concomitant expression of these indicators. We went on to look at the effect of endocannabinoids on cell survival in ORE during Tat inflammation. Cell specificity was adjudged with Vimentin for Muller glia and MAP2 for retinal neurons. Explants treated with Tat alone showed widespread necrotic cell death, both glial and neuronal (Fig. 1F). In ORE treated with Tat + AEA/2-AG, TUNEL staining was absent from both glial and neuronal cells. AEA and 2-AG are capable of rescuing retinal cells from Tat induced cell death.

AEA and 2-AG regulate pro- and anti-inflammatory cytokines differentially in HIV-1 Tat activated Muller glia

HIV-1 pathology is correlated with cytokine/chemokine deregulation. Increasingly it is seen that the endocannabinoid system affects cytokine generation and can regulate the innate immune system (Yazulla, 2008). The severity of HIV-1 related neurodegeneration as

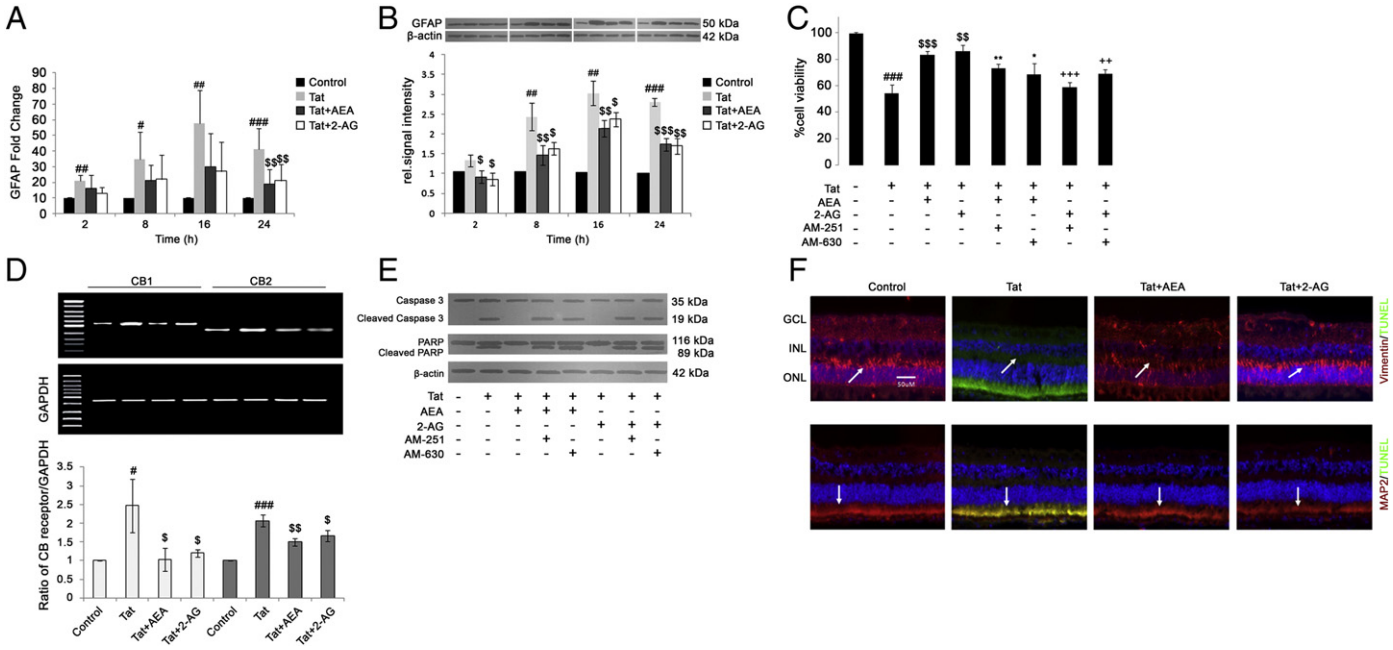

Fig. 1. AEA and 2-AG reduce GFAP activation and increase retinal cell survival against Tat induced inflammation by suppressing inflammatory cytokines. HIV-1 Tat increases GFAP activation significantly in Muller glia at both mRNA (A) and protein (B) levels; AEA and 2-AG repressed GFAP. MTT cell viability assay on *21 div* Muller cells shows an increase in percentage cell viability on the addition of AEA or 2-AG in Tat treated Muller glia (C). Blockage with specific antagonists to CB1 (AM-251) and CB2 (AM-630) reversed the effect of AEA or 2-AG. Results are given as percentage cell viability in Muller glia after AEA/2-AG treatment relative to Tat induced inflammation. Tat alone can increase CB1 and CB2 mRNA levels. AEA and 2-AG can suppress Tat-induced increase in cannabinoid receptors at 8 h (D). Western blot of activated caspase 3 and full length PARP show apoptosis and necrosis respectively, induced under Tat treatment. Addition of CB1/2 antagonists induced cell death under Tat + AEA/2-AG treatment (E). Cell specific markers, Vimentin (Muller glia, arrow) and MAP2 (neuron, arrow) show that Tat-induced cell death can be rescued by both AEA and 2-AG (F). Tat only induced cell death as seen by TUNEL (arrow). Scale bar 50 μm. Histograms are given as mean ± S.D. (n = 3); #p < 0.05, ###p < 0.001 vs. control, $$p < 0.01, $p < 0.001 vs. Tat, *p < 0.05, **p < 0.01 vs. Tat + AEA, ++p < 0.01, +++ < 0.001 vs. Tat + 2-AG. Results are given as mean ± S.D. (n = 3).