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A role for CB2 receptors in anandamide signalling pathways involved in the regulation of IL-12 and IL-23 in microglial cells

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ABSTRACT

The endocannabinoid system represents a novel therapeutic target for autoimmune and chronic inflammatory diseases. IL-12 and IL-23 are functionally related heterodimeric cytokines that play a crucial role in the pathogenesis of multiple sclerosis (MS). In the present study we investigated the effects of the endocannabinoid anandamide (AEA) on the inducible expression of the biologically active cytokines IL-12p70 and IL-23, and their forming subunits, in activated microglial cells. We also studied the signalling pathways involved in the regulation of IL-12p70/IL-23 expression and addressed the possible interactions of AEA with these pathways. Here, we show that AEA was capable to inhibit the production of biologically active IL-12p70 and IL-23, and their subunits, by activated human and murine microglial cultures. Treatment of activated microglial cells with inhibitors of several mitogen-activated protein kinase (MAPK) reveals that AEA acts through the ERK1/2 and JNK pathways to down-regulate IL-12p70 and IL-23. These effects were partially mediated by CB2 receptor activation. Together, our results provide the first demonstration of a role of AEA in inhibiting IL-12p70/IL-23 axis in human and murine microglial cells via the CB2 receptor and suggest that the pharmacological manipulation of the endocannabinoid system is a potential tool for treating brain inflammatory and autoimmune diseases, like MS.

1. Introduction

The production of interleukin-12 by cells of myeloid lineage, such as antigen-presenting cells (APCs), is critical for host defence against pathogens. IL-12 is a heterodimeric cytokine composed of p35 and p40 subunits, and of special importance since its expression regulates innate immunity and determines the type and duration of adaptive immune response [1]. Besides forming IL-12p70 heterodimer, p35 and p40 may dimerize with alternate partners to form distinct bimolecular complexes. For example, the recently identified p19 subunit, which lacks biological activity *per se*, combines to the p40 subunit to give the cytokine IL-23. Since both IL-12p70 and IL-23 share the common subunit p40, they have overlapping, but

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also distinct, effects on their targets [2,3]. One of the major differences is that while IL-12 preferentially acts by favouring the development of Th1 cells, IL-23 is involved in the maintenance of Th17 cells, which have been related to multiple sclerosis (MS) pathogenesis in a specific way [4,5].

Microglia, the brain-resident immune cells are activated in most pathological conditions of the central nervous system (CNS) and participate in the regulation of innate and adaptive immune responses [6]. Human, mouse, and rat microglial cells mainly express the cannabinoid receptor type 2, CB2 [7,8], and its expression level depends on the activation state of these cells as occurs for other immune cells [9,10]. The endogenous ligands, anandamide (AEA) and 2-arachidonoylglycerol (2-AG) are also synthesized by microglia that produce approximately 20-fold more endocannabinoids than neurons and astrocytes [11]. The endocannabinoid system is highly activated during brain inflammation and AEA, but not 2-AG, has been shown to be increased in active lesions of MS patients and to protect neurons from inflammatory damage [12]. Studies on the role played by the cannabinoid system during neuroinflammation support the interest of this system as a novel target for therapeutic approach [13,14]. In animal models of MS, cannabinoids attenuate the pathological features of the disease [15,16], an effect also observed by pharmacological interventions aimed at increasing the levels of the endocannabinoid AEA [17–21]. Results from different groups show that the cannabinoid system also plays a role in regulating microglial function such as proliferation [22], cellular migration [11] or the generation of inflammatory mediators [23,24].

Theiler's murine encephalomyelitis virus (TMEV) infects glial cells and causes demyelinating disease in certain mouse strains [25]. In our previous studies, we showed that macrophages infected with TMEV increased their production of AEA and that both AEA and the inhibitors of AEA cellular re-uptake, OMDM1 and OMDM2, inhibited IL-12p40 release [20]. In addition, AEA regulated the transcriptional activity of p40 promoter by activating the repressor site GA-12, likely through prostamide E_2 generation [26]. The identification of human microglia as one of the major sources of IL-23 in brain sections from MS patients [27] points out the importance of microglial cells as cellular targets to down-regulate this family of heterodimeric cytokines. To date, however, the mechanisms by which AEA may regulate the IL-12p70/IL-23 axis in microglial cells are unknown. Cannabinoid receptors have been shown to signal through mitogen-activated protein kinase (MAPK) signalling pathways [14]. The MAPK signal transduction pathways are highly conserved cascades implicated in several aspects of the immune response [28]. The family of protein kinases includes Jun N-terminal protein kinase (JNK), ERK1/2 and p38. The MAP kinases phosphorylate transcription factors that are involved in the process of activation of macrophages for the generation of IL-12p70 and other family-related cytokines [29,30]. The importance of the MAPK signal transduction pathway in controlling many aspects of immune-mediated inflammatory responses has made them a priority for research related to many neuroinflammatory diseases. Here we showed that AEA inhibits the expression of the three subunits p19, p35 and p40 subunits as well as the release of IL-12p70 and IL-23 by LPS/IFN-γ activated human and murine microglia. Our results also indicate that AEA down-regulates IL-12p70 and IL-23 production acting through ERK1/2 and JNK by a mechanism that involves the activation of CB2 receptors.

2. Materials and methods

2.1. Reagents and antibodies

The following materials were used: lipopolysaccharide from *E. coli* serotype O127:B8 and anandamide were purchased from Sigma (Madrid, Spain). Murine recombinant interferon-γ (IFNγ) was from PreproTech (London, UK). Cannabinoid antagonists SR141716A (*N*-piperidino-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-3-pyrazol-carboxamide) (SR1) and SR144528 (*N*-[1S)-endo-1,3,3,-trimethylbicyclo[2.2.1]heptan-2-yil-5-(4-chloro-3methylphenyl)-1-(4-methylbenzyl)-pyrazole-3-carboxamide) (SR2) were supplied by Sanofi Recherche (Montpellier, France). JWH-133 was purchased from Tocris Cookson Ltd. (UK). SP600125, PD98059 and SB203580 were purchased from Cell Signalling Technology (Beverly, USA). Anti-phospho-SAPK/JNK, anti-total-SAPK/JNK, anti-phospho-p44/p42 MAPK (phospho-ERK1/2), anti-total-p44/p42 MAPK (total ERK1/2), anti-phospho-p38 MAPK and anti-total-p38 MAPK antibodies were purchased from New England Biolabs (Beverly, USA). Anti-tubulin antibody was obtained from Sigma (Madrid, Spain). Peroxidase-conjugated anti-rabbit secondary antibodies were from Jackson ImmunoResearch Laboratories (West Grove, USA) and anti-mouse secondary antibody was from Bio-Rad (Hercules, USA). All other reagents were obtained from standard supplies.

2.2. Animals

Balb/c mice from our in-house colony (Cajal Institute, Madrid) were used. Animals were housed in cages with filter tops in a laminar flow hood and maintained on food and water *ad libitum* in a 12-h dark–light cycle. Handling of animals was performed in compliance with the guidelines of animal care set by the European Union (86/609/EEC) and the Spanish regulations (BOE67/8509-12; BOE 1201/2005) on the use and care of laboratory animals, and approved by the local Animal Care and Ethics Committee of the Consejo Superior de Investigaciones Científicas (CSIC).

2.3. Isolation of murine microglia and cell culture

Primary mix glial cultures were prepared as described previously [50]. Briefly, after decapitation, forebrains of newborn Balb/c mice were dissociated mechanically, filtered through a 150-μm nylon mesh, resuspended in DMEM containing 10% heat-inactivated FBS, 10% heat-inactivated horse-serum and 1% penicillin/streptomycin and plated on poly-L-lysine (15 μg/mL) 75 cm^2 flasks (Falcon; Le Pont de Claix, France). After 15 days in culture the flasks were shaken at 230 rpm at 37 °C for 3 h to remove loosely adherent microglia. The supernatant was plated on multiwell culture plates for 2 h. After this, medium was changed to remove non-adherent cells. Cells were grown in a humidified environment containing 5% CO_2 and held at a constant temperature of 37 °C. The