CB₂ cannabinoid receptor agonist JWH-015 modulates human monocyte migration through defined intracellular signaling pathways

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Montecucco F, Burger F, Mach F, Steffens S. CB₂ cannabinoid receptor agonist JWH-015 modulates human monocyte migration through defined intracellular signaling pathways. Am J Physiol Heart Circ Physiol 294: H1145–H1155, 2008. First published January 4, 2008; doi:10.1152/ajpheart.01328.2007.—Recruitment of leukocytes to inflammatory sites is crucial in the pathogenesis of chronic inflammatory diseases. The aim of this study was to investigate if activation of CB₂ cannabinoid receptors would modulate the chemotactic response of human monocytes. Human monocytes treated with the CB₂ agonist JWH-015 for 12–18 h showed significantly reduced migration to chemokines CCL2 and CCL3, associated with reduced mRNA and surface expression of their receptors CCR2 and CCR1. The induction of ICAM-1 in response to IFN-γ was inhibited by JWH-015. Moreover, JWH-015 cross-desensitized human monocytes for migration in response to CCL2 and CCL3 by its own chemoattractant properties. The CB₂-selective antagonist SR-144528, but not the CB₁ antagonist SR-147778, reversed JWH-015-induced actions, whereas the CB₂ agonist JWH-133 mimicked the effects of JWH-015. The investigation of underlying pathways revealed the involvement of phosphatidylinositol 3-kinase/Akt and ERK1/2 but not p38 MAPK. In conclusion, selective activation of CB₂ receptors modulates chemotaxis of human monocytes, which might have crucial effects in chronic inflammatory disorders such as atherosclerosis or rheumatoid arthritis.

Cannabinoids modulate immune functions and therefore have a therapeutic potential for the treatment of inflammatory diseases (28, 29, 40). It is thought that the immunomodulatory effects of cannabinoids are mediated by the CB₂ cannabinoid receptor expressed on immune cells. A growing body of evidence suggests that endocannabinoid signaling plays a critical role in pathophysiological conditions such as hemorrhagic or endotoxic shock (53, 56), liver cirrhosis (4), fibrosis (24, 52), ischemia-reperfusion (I/R) injury (5, 44), acute heart failure (36), osteoporosis (25, 39, 51), and cutaneous contact hypersensitivity (26). We (49) have recently provided the first experimental evidence for a possible role of CB₂ receptors in atherosclerosis progression.

The recruitment of inflammatory cells into the intima is crucial for the development and progression of atherosclerosis (19, 20, 57). The tethering, rolling, adhesion, and transendothelial migration of leukocytes are triggered by the local production of chemokines and chemokine receptors as well as adhesion molecules (8). Cannabinoids have been reported to modulate the migration of various cell types. Thus, it has been reported that treatment of rat macrophages with the synthetic cannabinoid CP-55,940 reduced both spontaneous and formyl-metionyl-leucine-phenylalnine-induced chemotaxis (48). The nonpsychoactive marijuana component cannabidiol has been shown to inhibit murine macrophage chemotaxis in vitro and in vivo in a CB₂ receptor-dependent manner (47). Both endogenous as well as synthetic agonists 2-AG, CP-55,940 and WIN-55,212-2, as well as the CB₂-selective agonists JWH-015 and JWH-133, caused a significant inhibition of the chemokine stromal cell-derived factor-1/CXCL12-induced and CXCR4-mediated chemotaxis of Jurkat or primary human T cells (11, 15). In addition, in an experimental autoimmune encephalomyelitis mouse model, WIN-55,212-2 attenuated leukocyte rolling and adhesion on endothelial cells through the activation of CB₂ receptors (38). Furthermore, the novel CB₂-selective inverse agonist Sch.336 potently inhibited leukocyte chemotaxis to 2-AG, HU-210, or monocye chemoattractant protein-1/CCL2 in vitro and in vivo (33). In a mouse model of liver I/R injury, the CB₂-selective agonist JWH-133 protected against I/R damage by decreasing inflammatory cell infiltration, tissue and serum TNF-α, macrophage inflammatory protein (MIP)-1α/CCL3 and MIP-2/CXCL2 levels, and expression of adhesion molecule ICAM-1 (5).

In vitro, JWH-133 has been shown to attenuate TNF-α-induced ICAM-1 and VCAM-1 expression in human liver sinusoidal endothelial cells (HLSECs) and the adhesion of human neutrophils to HLSECs. In a different study, anandamide dose dependently attenuated TNF-α-induced ICAM-1 and VCAM-1 expression in human coronary artery endothelial cells (HCAECs) and the adhesion of THP-1 monocytes to HCAECs in a CB₁- and CB₂-dependent manner (6). The role of the CB₂ receptor in this process could be confirmed by additional data obtained with CB₂ agonists, which reduced ICAM-1 and VCAM-1 expression in HCAECs as well as the adhesion and transendothelial migration of THP-1 monocytes (43). Finally, we (49) have shown that tetrahydrocannabinol inhibited murine peritoneal macrophage chemotaxis in response to CCL2 and reduced expression of the chemokine receptor CCR2 on splenocytes. These effects were blocked by the CB₂ receptor antagonist SR-144528 or when cells from CB₂ knockout mice were used.

In this study, we investigated if CB₂-selective agonists would modulate the migration of human monocytes and thus may have a therapeutic potential in inflammatory conditions such as atherosclerosis.

MATERIALS AND METHODS

Reagents. The CB₂-selective agonist JWH-015 was obtained from Cayman Chemical and JWH-133 was obtained from Tocris Bioscience. The CB₁ antagonist SR-147778 and the CB₂ antagonist H1145

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SR-144528 were kindly provided from Sanofi-Synthelabo (France) (45, 46). Stock solutions (10–20 mM) of cannabinoids and antagonists were prepared in DMSO. Appropriate dilutions of DMSO were added as vehicle controls in all experiments. BSA was from Sigma-Aldrich. CCL2, CCL3, IFN-γ, anti-phosphorylated ERK1/2 (T202/Y204, AF1018) polyclonal antibody (Ab), anti-phosphorylated p38 MAPK (T180/Y182, AF869) polyclonal Ab, anti-ERK1/2 polyclonal Ab (AF1576), phycoerythrin (PE)-conjugated anti-human CCR1 Ab (FAB145P), and PE-conjugated anti-human CCR2 Ab (FAB151P) were all purchased from R&D Systems Europe. PE-conjugated anti-human CD54/ICAM-1 Ab (no. 555511) and FITC-conjugated anti-human CD14 Ab (no. 555397) were obtained from BD Pharrmingen. Anti-human CB2 receptor Ab was obtained from Abcam (ab5561) and PE-conjugated goat anti-rabbit IgG antibody was obtained from Molecular Probes. The kinase inhibitor LY-294002 [phosphatidylinositol 3-kinase (PI3K) inhibitor] was obtained from Sigma-Aldrich, and PD-98059 (MEK inhibitor) and SB-203580 (p38 MAPK inhibitor) were obtained from BioMol Research Laboratories. Anti-p38 (H-147, sc-7149) polyclonal Ab, anti-Akt1/2/3 (H-136, sc-8312) polyclonal Ab, and anti-phosphorylated Akt1/2/3 (Thr308, sc-16646-R) polyclonal Ab were obtained from Santa Cruz Biotechnology.

Isolation of human monocytes. Human monocytes were isolated from buffy coats of healthy volunteers under a protocol approved by the local Ethics Committee. All donors provided written, informed consent to participate. Mononuclear cells were collected from buffy coats of healthy volunteers under a protocol approved by the local Ethics Committee. All donors provided written, informed consent to participate. Mononuclear cells were collected from buffy coats of healthy volunteers under a protocol approved by the local Ethics Committee. All donors provided written, informed consent to participate.

Modified Boyd chamber migration assays and checkerboard analysis. To study the effect of cannabinoid treatment on migration, cells were incubated in RPMI medium containing 25 mM HEPES and 500 ng/ml polymyxin B in the presence or absence of different doses of JWH-015 (5, 10, and 20 μM) for 12 h at 37°C in a humidified atmosphere with 5% CO2. Cells were then washed three times in PBS and resuspended in chemotaxis medium (RPMI medium containing 25 mM HEPES and 1% BSA) to test their locomotory responses to medium alone, 10 nM CCL2, or 10 nM CCL3. Monocyte chemotaxis was assessed in a 48-well microchemotaxis chamber (NeuroProbe) using a 5-μm pore size, 5-μm-thick polyvinylpyrrolidone-free polycarbonate filter (NeuroProbe). Cells were seeded in the upper wells while medium or chemoattractant solutions were added to the lower wells.

To test the chemotactic properties of CB2-selective cannabinoids, untreated monocytes were tested for their ability to migrate in response to medium alone, JWH-015 (5, 10, and 20 μM), or JWH-133 (10 μM). In some experiments, cells were pretreated for 10 min with the receptor antagonists SR-147778 (1 μM) or SR-144528 (1 μM) or for 60 min with the kinase inhibitors LY-294002 (50 μM), PD-98059 (50 μM), or SB-203580 (1 μM). After treatment with the kinase inhibitors, cells were washed three times in chemotaxis medium before their locomotory abilities were tested. In a different set of experiments, cells and different doses of JWH-015 (5, 10, and 20 μM) were seeded in the upper well while medium alone or 10 nM CCL2 or 10 nM CCL3 were added to the lower well. Checkerboard analysis was performed by the addition of different doses of JWH-015 (5, 10, and 20 μM) in both the upper (with cells) and lower wells.

Under all conditions, the modified Boyden chamber was incubated for 60 min at 37°C in a humidified atmosphere with 5% CO2. Filters were then removed from the chambers and stained with Diff-Quick (Baxter). Each condition was performed in duplicate. Cells of five random oil-immersion fields were counted at ×1,000 magnification (by a blinded observer), and the chemotaxis index was calculated from the number of cells migrated to the test sample divided by the number of cells migrated to the medium.

Flow cytometry. Monocytes were cultured at a concentration of 5 × 10⁶ cells/ml in serum-free RPMI medium containing 25 mM HEPES...
and 500 ng/ml polymyxin B in the presence or absence of JWH-015 (5, 10, and 20 μM) or JWH-133 (10 μM) with or without receptor antagonists SR-147778 (1 μM) and SR-144528 (1 μM) or kinase inhibitors LY-294002 (50 μM), PD-98059 (50 μM), and SB-203580 (1 μM) for 18 or 24 h at 37°C in a humidified atmosphere with 5% CO₂. In some experiments, cells were stimulated with IFN-γ (100 U/ml). FITC- or PE-labeled anti-human CCR1, CCR2, CD54, and CD14 Ab as well as corresponding isotype controls were used. Surface CB2 receptor staining was performed by Fe blocking with human IgG and a further incubation with a rabbit polyclonal antibody to the CB2 receptor followed by an incubation with PE-conjugated goat anti-rabbit IgG Ab. CellQuest software was used for acquisition and analysis on a FACSCalibur (BD Biosciences). Data are expressed as mean fluorescence intensities (MFIs) compared with baseline expression (defined as 100%).

Cytotoxicity assay. Cell death was determined by the quantification of lactate dehydrogenase (LDH) release (BioVision) in cell culture supernatants after 12, 18, and 24 h of incubation in the presence or absence of JWH-015 (20 μM), JWH-133 (10 μM), SR-147778 (1 μM), or SR-144528 (1 μM) and counts of trypan blue-positive cells.

Apoptosis assay. Apoptosis rates of monocytes after 0, 18, and 24 h of treatment with JWH-015 (20 μM), JWH-133 (10 μM), SR-147778 (1 μM), or SR-144528 (1 μM) were determined via the analysis of phosphatidylserine externalization using an annexin V-FITC apoptosis detection kit (MBL).

Immunoblot analysis. Monocytes were cultured at a concentration of 5 × 10⁶ cells/ml in serum-free RPMI medium containing 25 mM HEPES in the presence or absence of JWH-015 (20 μM), 10 nM CCL2, or DMSO [0.1% for various time points (between 2.5 and 30 min)]. The reaction was stopped on ice, and cells were centrifuged at 4°C to remove culture supernatants. Total protein was extracted in lysis buffer containing 20 mM Tris-HCl (pH 7.5), 150 mM NaCl, 10 mM NaF, 1% Nonidet P-40, 10% glycerol, 1 mM PMSF, 10 μg/ml aprotinin, 10 μg/ml leupeptin, and 0.5 mM Na₃VO₄. Proteins (40 μg) were electrophoresed through polyacrylamide-SDS gels and transferred by electroblotting onto nitrocellulose membranes. Membranes were blocked for 1 h in 5% (wt/vol) nonfat milk before being incubated with appropriate dilutions of primary phospho-specific Ab to ERK1/2, Akt, or p38 MAPK as well as corresponding secondary Ab. Blots were developed using the ECL system (Immobilion, West-Fig. 2. Analysis of cell viability and the apoptosis rate in cultured human monocytes. Monocytes were cultured in serum-free medium with or without 0.1% DMSO [vehicle (v)], 20 μM JWH-015, 10 μM JWH-133, the CB₁ antagonist (αCB₁) 1 μM SR-147778, or the CB₂ antagonist (αCB₂) 1 μM SR-144528 for the indicated time points. A and B: cell death was determined as the percentage of trypan blue-positive cells in a total of 500 counted cells (n = 2). C and D: the apoptosis rate was determined by flow cytometric analysis of annexin V-FITC and propidium iodide (PI) staining. Early apoptotic cells are annexin V single-positive cells, whereas late apoptotic/necrotic cells are annexin V/PI double-positive cells (n = 4). E: representative histograms of flow cytometric analysis of annexin V staining after 12 h (cells were gated to exclude PI-positive cells). CTL, control.
CB2 cannabinoid receptors and monocyte migration

RESULTS

CB2 cannabinoid receptors are expressed on primary human monocytes. To obtain nonactivated primary human monocytes, we performed Ficoll-Percoll density gradient centrifugation. We first confirmed the presence of CB2 cannabinoid receptors on freshly isolated monocytes and found high expression levels on the cell surface (Fig. 1A). In addition, CB2 mRNA expression in monocytes was detected by RT-PCR (Fig. 1B). Interestingly, we observed a significant increase of CB2 surface

A

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Fig. 3. Pretreatment with JWH-015 reduces monocyte migration to CCL2 and CCL3 via inhibition of CCR1 and CCR2 expression in a CB2-dependent manner. A: monocytes were treated with JWH-015 for 12 h, washed, and tested for their ability to migrate to 10 nM CCL2 or CCL3 (n = 6). **P < 0.01 and ***P < 0.001 vs. CCL2 alone; ##P < 0.01 and ###P < 0.001 vs. CCL3 alone. B: FACS analysis of CCR2 and CCR1 expression on monocytes after 18 h of JWH-015 treatment. ***P < 0.001 and ###P < 0.001 for CCR2 vs. untreated (n = 3); #P < 0.05 for CCR1 vs. untreated (n = 4). C: real-time RT-PCR analysis of CCR2 and CCR1 mRNA levels (normalized to the hypoxanthine guanine phosphoribosyl transferase control) in monocytes after 12 h of JWH-015 treatment. #P < 0.01 for CCR2 vs. untreated (n = 5); CCR1 was not significant (n = 4). D: FACS analysis after 18 h with or without 20 μM JWH-015 alone (n = 11) or in the presence of aCB1 SR-147778 (1 μM) or aCB2 SR-144528 (1 μM) (n = 9 for antagonists + JWH-015 and n = 2 for antagonists alone), or the CB1 agonist JWH-133 (10 μM) alone (n = 5). ***P < 0.001 vs. untreated; tP < 0.001 vs. JWH-015; #P < 0.05 vs. untreated; ###P < 0.01 vs. JWH-015. E and F: representative histograms of CCR2 and CCR1 expression of untreated cells (shaded peaks) or treated with 20 μM JWH-015 (solid lines).
expression when monocytes were cultured for up to 24 h (Fig. 1C). The presence of CB2 agonist JWH-015 (20 μM) did not change the observed modulation. To validate our experimental conditions, we tested the cell viability and extent of apoptosis in monocytes treated with cannabinoids or antagonists for up to 24 h. We found an increasing percentage of dead and apoptotic cells in monocyte cultures (Fig. 2), comparable with magnitudes previously reported (21, 37). The increase of cell death was due to the culture conditions in serum-free medium, which was performed to avoid the interference with serum factors. Importantly, treatment with cannabinoids and/or antagonists did not affect cell viability and apoptosis rates (Fig. 2). Under none of the experimental conditions did we observe increased LDH release compared with untreated cells after 12, 18, and 24 h of incubation (data not shown).

Treatment with the CB2 agonist JWH-015 reduces monocyte migration to CCL2 and CCL3 by reducing chemokine receptor expression. We assessed monocyte migration in response to CCL2 and CCL3 with the modified Boyden chamber system. Pretreatment with 5–20 μM of the CB2 agonist JWH-015 for 12 h significantly reduced the chemotactic response to CCL2 and CCL3 in a dose-dependent manner (Fig. 3A). Maximum inhibition was observed at 10–20 μM JWH-015. We next analyzed the effect of JWH-015 on the expression of the chemokine receptors that bind CCL2 and CCL3. Treatment of monocytes with JWH-015 for 18 h inhibited surface expression of chemokine receptor CCR2 and, to a lesser extend, CCR1 at a concentration of 10–20 μM (Fig. 3, B, E, and F). This effect was associated with significantly reduced CCR2 mRNA levels, as determined after 12 h of JWH-015 treatment (Fig. 3C). We also observed a marked, but not significant, reduction of CCR1 mRNA levels. Pretreatment with the CB2 antagonist SR-144528 reversed the effect of JWH-015 on surface CCR2 and CCR1 expression, whereas the CB1 antagonist SR-147778 had no effect (Fig. 3D). It is of note that cotreatment of cells with JWH-015 and the CB2 antagonist SR-144528 induced a marked, but not significant, increase of chemokine receptor expression compared with untreated cells. Treatment with the CB1 antagonist SR-147778 or the CB2 antagonist SR-144528 alone did not have a significant effect. Similar to JWH-015, incubation with the CB2 agonist JWH-133 at 10 μM also reduced CCR2 and CCR1 surface expression (Fig. 3D), whereas the vehicle DMSO alone did not affect mRNA or surface expression of chemokine receptors (data not shown).

Treatment with JWH-015 modulates ICAM-1 expression on human monocytes. We further investigated the effect of JWH-015 on the expression of ICAM-1, which is implicated in monocyte recruitment. The basal expression of ICAM-1, which was strongly expressed on all monocytes, was significantly (1.44-fold) enhanced after 24 h of incubation with IFN-γ (Fig. 4, A and B). When JWH-015 (20 μM) or JWH-133 (10 μM) was added during stimulation, the IFN-γ-induced activation
was significantly inhibited. The CB₂ antagonist SR-144528 reversed the effect of JWH-015, whereas the CB₁ antagonist SR-147778 did not inhibit the effect of JWH-015. Coincubation with JWH-015 and the CB₂ antagonist SR-144528 induced a marked, but not significant, increase of IFN-γ-induced ICAM-1 expression compared with IFN-γ-treated cells. Similarly, the antagonists alone had a marked, but not significant, effect on the IFN-γ-induced activation of ICAM-1. Neither treatment with JWH-015 nor receptor antagonists alone reduced the basal ICAM-1 expression on monocytes (Fig. 4A and data not shown).

**JWH-015 induces chemotaxis of human monocytes.** Because various cannabinoids have been reported to chemoattract different cell types, we next tested the ability of JWH-015 and JWH-133 to induce migration of freshly isolated monocytes. The number of cells migrating to the respective cannabinoid was increased compared with the medium control, with a significant effect observed at 20 µM JWH-015 (chemotaxis index of 1.98 ± 0.26) or 10 µM JWH-133, respectively (chemotaxis index of 1.98 ± 0.2; Fig. 5A). Pretreatment with the CB₂ antagonist SR-144528 inhibited the effect of JWH-015 and JWH-133, whereas the CB₁ antagonist SR-147778 had no effect (Fig. 5B).

To distinguish if the migration was due to the induction of chemotaxis or chemokinesis, we performed a checkerboard analysis with increasing concentrations of JWH-015 above and below the filter. We found a directed migration versus a gradient across the filter, indicating that JWH-015 induced chemotaxis rather than random chemokinesis (Fig. 5C).

**JWH-015 cross-desensitizes monocytes for migration to CCL2 and CCL3.** To investigate the chemotactic effect of JWH-015 in the presence of a chemokine gradient, freshly isolated monocytes were seeded with increasing concentrations of JWH-015 to the upper well and chemoattracted with CCL2 or CCL3, respectively. We found that the migration to both CCL2 and CCL3 was significantly reduced in the presence of 10–20 µM JWH-015, suggesting that JWH-015 cross-desensitizes monocytes for migration to these chemokines (Fig. 5D).

**JWH-015-mediated modulation of monocyte migration involves PI3K/Akt and ERK1/2 but not p38 MAPK signaling pathways.** To investigate if the JWH-015-mediated effects on chemokine receptor expression and migration were dependent on distinct kinase signaling pathways, we performed experiments with selective kinase inhibitors for PI3K and MEK1/2 (the activator of ERK1/2) as well as p38 MAPK. Pretreatment with the PI3K inhibitor LY-294002 or the MEK1/2 inhibitor PD-98059 abolished the inhibition of CCR2 expression in response to JWH-015 (Fig. 6A). Conversely, pretreatment with the p38 MAPK inhibitor SB-203580 had no effect. Similar effects of kinase inhibitors were observed on CCR1 expression; however, the differences were not significant. To study if JWH-015-induced migration was dependent on the same downstream pathways as the above-described effects on chemokine receptor expression, we performed migration assays after pretreatment with kinase inhibitors. We found that both the PI3K inhibitor LY-294002 and the MEK1/2 inhibitor PD-98059 inhibited the chemotactic response to JWH-015, whereas the p38 MAPK inhibitor SB-203580 had no effect (Fig. 6B). To confirm these findings, we determined the effect of JWH-015 on kinase activation by Western blot analysis (Fig. 6, C and D). CCL2 was used as a positive control for kinase activation in parallel experiments. We found increased phosphorylation of the direct downstream kinases of PI3K and MEK1/2, namely, Akt and ERK1/2. However, we also deter-

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Fig. 5. JWH-015 and JWH-133 induce chemotaxis of human monocytes in a CB₂-dependent manner. A: untreated monocytes were tested for their ability to migrate to increasing concentrations of JWH-015 (n = 6) or JWH-133 (n = 3). **P < 0.01 and *P < 0.05 vs. without JWH-015. B: migration versus 20 µM JWH-015 or 10 µM JWH-133 in the presence of aCB₁ SR-147778 (1 µM) or aCB₂ SR-144528 (1 µM) (n = 8 for JWH-015 and n = 4 for JWH-133). ***P < 0.001 vs. JWH-015; **P < 0.01 vs. JWH-133. C: checkerboard analysis showing that monocyte migration depended on a JWH-015 gradient across the filter. Untreated monocytes were seeded with increasing concentrations of JWH-015 to the upper well, and increasing concentrations of JWH-015 were added to the lower well. Bars represent means; n = 10. D: JWH-015 desensitizes monocytes for migration to CCL2 and CCL3 gradients. Untreated monocytes were seeded with increasing concentrations of JWH-015 to the upper well, and 10 nM CCL2 or CCL3 was added to the lower well (n = 6). *P < 0.05 vs. without JWH-015; #P < 0.05 vs. without JWH-015.
mined a significant activation of p38 MAPK, as shown in the representative blots in Fig. 6C and the densitometric quantification of four to five individual experiments in Fig. 6D.

DISCUSSION

Monocytes are circulating precursors of tissue macrophages and dendritic cells (30). Monocyte-derived macrophages and dendritic cells fulfill critical functions in innate and adaptive immunity during inflammation and play a crucial role in various chronic diseases, such as rheumatoid arthritis and atherosclerosis (20, 50). Recruitment of monocytes from the bloodstream into tissues is a complex process, in which adhesion molecules, chemokines, chemokine receptors, and also intracellular proteins are involved (17, 54). Because cannabinoids have been reported to modulate the migration of various cell types, including immune cells, we aimed to investigate if selective activation of CB2 cannabinoid receptors would modulate human monocyte migration.

The major findings of our study are that CB2 receptor activation via JWH-015 modulates the recruitment of human monocytes by various mechanisms: 1) JWH-015 reduces the expression of CCR2 and CCR1, which results in reduced chemotaxis to CCL2 and CCL3; 2) JWH-015 blocks the IFN-γ induced upregulation of adhesion molecule ICAM-1; and 3) JWH-015 induces monocyte migration by its own chemotactic properties and is able to cross-desensitize the migration in response to CCL2 and CCL3.

Our data confirming high levels of CB2 receptor expression on human monocytes (using flow cytometry analysis and RT-PCR) suggest an important role for the CB2 receptor in different monocyte functions. We first studied the effect of JWH-015 on CCL2- and CCL3-induced migration, two major chemokines expressed at inflammatory sites that trigger monocyte recruitment (10). The observed reduction of monocyte migration after 12 h of treatment with JWH-015 is explained by the reduced expression of CCR2 and, to a lesser extent,
CCR1. We focused on the principal receptors involved in monocyte locomotion, such as CCR2, the corresponding receptor for CCL2, and CCR1, the receptor for CCL3. Although CCL3 is also capable of binding CCR5, this receptor is expressed at low levels on human monocytes and is considered less important for monocyte migration to CCL3 (58). We also tested the effect of JWH-015 on ICAM-1 expression. ICAM-1 is a member of the IgG superfamily and represents a specific ligand for integrins, such as CD11a/CD18 (lymphocyte function-associated Ag-1) and CD11b/CD18 (Mac-1) (41). ICAM-1 is expressed on both leukocytes and endothelial cells, and is crucial in monocyte rolling and transendothelial migration (14). Furthermore, ICAM-1 also interacts with its ligands on monocytes, thus generating homotypic aggregation of monocytes (14). In our experiments, JWH-015 was capable of inhibiting the IFN-γ-induced upregulation of ICAM-1. This is in agreement with previously published data demonstrating that CB2 agonists as well as the endocannabinoid anandamide are able to attenuate TNF-α-induced ICAM-1 and VCAM-1 expression in HLSECs or HCAECs (5, 6, 43). However, our data provide the first evidence for the modulation of ICAM-1 expression on monocytes, suggesting a possible double action to reduce monocyte-endothelial cell adherence and monocyte transendothelial migration.

In our experiments, JWH-015 was capable of inhibiting chemokine-induced migration at doses of 5–20 μM. These micromolar concentrations have been well demonstrated as pharmacological concentrations for obtaining a functional activity of the response to JWH-015-mediated CB2 receptor activation in various monotypic cells (15, 16, 27, 31, 34). JWH-015 is considered as a CB2-selective agonist because the binding affinity to CB1 receptors is ~30-fold lower compared with CB2 receptors (Ki values of 13.8 and 383 nM for CB2 and CB1 receptors, respectively) (42). However, the effective doses for inhibition were much higher than the Ki value for CB2 receptors, which may also lead to activation of CB1 receptors, which are also expressed at considerable levels on monocytes (13). To investigate the implication of CB1 and CB2 receptors in the observed modulation of migration and downregulation of chemokine receptor and adhesion molecule expression, we used selective receptor antagonists as well as a more selective CB2 agonist, JWH-133 (Ki values of 3.4 and 680 nM for CB2 and CB1 receptors, respectively) (22). Only the CB2 antagonist SR-144528 reversed the effect of JWH-015, whereas the CB1 antagonist SR-147778 had no effect. In accordance with this, the CB2 agonist JWH-133, at a concentration of 10 μM, exhibited similar effects as JWH-015. These findings suggest that the effects on migration and chemokine receptor and ICAM-1 expression were all dependent on CB2 activation. Interestingly, our experiments performed with the CB2 antagonist indicate that it may not only reverse the effect of JWH-015 on chemokine receptor and ICAM-1 expression but further enhance their expression. However, neither the increase of chemokine receptor expression compared with untreated cells nor the increase of ICAM-1 compared with IFN-γ-treated cells were not significant. A possible underlying mechanism for the observed effects has been previously described by Bouaboula and co-workers (7). In a stably CB2 receptor-transfected rodent cell line, the CB2 antagonist SR-144528 has been shown to block not only the activity of CB2 receptors but also the MAPK activity of other G protein-coupled receptors. Sustained treatment with SR-144528 induced an upregulation of the cellular G protein level, which was associated with a concomitant loss of the ability of SR-144528 to inhibit MAPK activation. It is conceivable that altered G protein levels in response to antagonist treatment might directly affect the expression of chemokine receptors that are G protein-coupled receptors or downstream signals of ICAM-1 and/or chemokine receptors, which may, in turn, alter their expression levels.

In addition to the long-term effects (after 12–24 h) of JWH-015 on monocyte migration, we also investigated short-term actions (after a few minutes) of JWH-015 on monocytes.
We found that JWH-015 itself is chemoattractant for monocytes, an effect that has been previously reported for various cannabinoids on other cell types (11, 15, 33, 38, 47–49). The migration versus 20 μM JWH-015 was dependent on CB2 receptors, as verified by experiments with selective receptor antagonists and the use of the CB2 agonist JWH-133, which was also chemoattractant at a concentration of 10 μM. Moreover, checkerboard analysis confirmed that the locomotion was rather not due to induction of random cellular movement but more likely a directed migration versus a JWH-015 gradient. In an attempt to understand the effect of systemic administration of the CB2 agonist in an inflammatory condition, we further investigated the effect of JWH-015 on monocyte migration in the presence of a chemokine gradient. Increasing concentrations of JWH-015 (10–20 μM) added to the cells were able to inhibit the chemotactic response versus CCL2 and CCL3, indicating that the CB2 agonist desensitizes monocytes for migration to other chemotactants. A similar action has been previously reported for other chemoattractants. Indeed, certain chemoattractants desensitize the cells toward further stimulation with other chemoattractants (1). Consequently, the systemic administration of sufficient amounts of JWH-015 may desensitize cells toward chemokines such as CCL2 and CCL3 and, thus, reduce recruitment of monocytes to inflammatory sites. Likewise, opioids have been shown to inhibit chemokine-induced migration through desensitization of chemokine receptors (18). The underlying mechanisms involve in opiate-induced phosphorylation of chemokine receptors but not receptor internalization or an altered chemokine binding capacity. It has been hypothesized that receptor dimers may exist between CB2 receptors and chemokine receptors, which may explain, at least in part, the observed pleiotropic effects on monocyte migration (35). It is conceivable that binding of CB2 ligands could affect the ability of chemokine receptors to signal properly.

Various studies have investigated the role of CB2 receptor activation on inflammatory cell migration in the past, with both increases and decreases of cell migration being reported, depending on the agonist and cell type used (35). Thus, under different pathogenic conditions, cannabinoids may exhibit either pro- or anti-inflammatory effects, which does not allow general conclusions to be drawn. Our data on both long- and short-term effects of the two CB2 agonists JWH-015 and JWH-133 suggest an anti-inflammatory action in chronic inflammatory conditions where monocyte recruitment plays a prominent role. While reducing chemokine receptor and adhesion molecule expression, and consequently migration versus CCL2 and CCL3, JWH-015 may also recruit monocytes by its intrinsic chemotactic properties. Thus, enhanced plasma cannabinoid levels might counterbalance recruitment to local chemokine gradients at inflammatory sites.

Finally, to study the intracellular signaling pathways triggered by JWH-015, we used selective inhibitors of various kinases involved in cell migration, i.e., PI3K/Akt (LY-294002) and MEK1/2 (PD-98059), which is a kinase activating ERK1/2, as well as p38 MAPK (SB-203580). Inhibitors of PI3K/Akt and MEK1/2 reversed JWH-015-mediated inhibition of chemokine receptor expression (long-term effect) and migration versus JWH-015 (short-term effect), whereas the inhibitor of p38 MAPK was ineffective. Conversely, Western blot analysis revealed that JWH-015 not only induced the activation of Akt and ERK1/2 but also of p38 MAPK.

Similar discrepancies between kinase activation and their involvement in cellular functions have been previously described. Indeed, it has been shown that PI3K/Akt and MEK1/2 pathways were not involved or only partially involved in monocyte chemotaxis in response to CCL2 and CCL3, although these two chemokines were capable of activating both PI3K/Akt and ERK1/2 (2, 3, 12, 55). On the other hand, CCL2 has been shown to activate p38 MAPK, and inhibitors of p38 MAPK induced a substantial inhibition of CCL2-mediated migration (2, 3, 9). Our data suggest two potential targets, namely, PI3K/Akt and ERK1/2, selectively involved in JWH-015-mediated short-term and long-term effects on monocyte migration, without the involvement of classical p38 MAPK-dependent intracellular pathways (Fig. 7). However, while JWH-015 induces p38 MAPK phosphorylation, the potential downstream targets of p38 signaling in our model remain unclear.

In conclusion, we have shown that the cannabinoid JWH-015 modulates the recruitment of human monocytes by various immediate and delayed effects in a CB2-dependent manner. Both short- and long-term effects depend on PI3K/Akt and ERK1/2 signaling; the immediate effect is the induction of monocyte migration by its own potent chemotactic properties, which might inhibit the recruitment to local inflammatory sites by desensitizing cells to chemokine gradients; the delayed effects are reduced monocyte migration versus CCL2 and CCL3 via downregulation of CCR2 and CCR1 and inhibition of IFN-γ-induced ICAM-1 upregulation. Taken together, these anti-inflammatory properties might have crucial effects in chronic inflammatory disorders such as atherosclerosis and rheumatoid arthritis.

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