2-Arachidonoylglycerol Induces the Migration of HL-60 Cells Differentiated into Macrophage-like Cells and Human Peripheral Blood Monocytes through the Cannabinoid CB2 Receptor-dependent Mechanism

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2-Arachidonoylglycerol is an endogenous ligand for the cannabinoid receptors (CB1 and CB2) and has been shown to exhibit a variety of cannabimimetic activities in vitro and in vivo. Recently, we proposed that 2-arachidonoylglycerol is the true endogenous ligand for the cannabinoid receptors, and both receptors (CB1 and CB2) are primarily 2-arachidonoylglycerol receptors. The CB1 receptor is assumed to be involved in the stimulation of neurotransmission. On the other hand, the physiological roles of the CB2 receptor, which is abundantly expressed in several types of leukocytes such as macrophages, still remain unknown. In this study, we examined the effects of 2-arachidonoylglycerol on the motility of HL-60 cells differentiated into macrophage-like cells. We found that 2-arachidonoylglycerol induces the migration of differentiated HL-60 cells. The migration induced by 2-arachidonoylglycerol was blocked by treatment of the cells with SR144528, a CB2 receptor antagonist, or pertussis toxin, indicating that the CB2 receptor and G proteins are involved in the 2-arachidonoylglycerol-induced migration. Several intracellular signaling molecules such as Rho kinase and mitogen-activated protein kinases were also suggested to be involved. In contrast to 2-arachidonoylglycerol, anandamide, another endogenous cannabinoid receptor ligand, failed to induce the migration. The 2-arachidonoylglycerol-induced migration was also observed for two other types of macrophage-like cells, the U937 cells and THP-1 cells, as well as human peripheral blood monocytes. These results strongly suggest that 2-arachidonoylglycerol induces the migration of several types of leukocytes such as macrophages/monocytes through a CB2 receptor-dependent mechanism thereby stimulating inflammatory reactions and immune responses.

Δ9-Tetrahydrocannabinol (Δ9-THC) is a major psychoactive constituent of marijuana and is known to exert a variety of biological effects in experimental animals and human such as altered perception, inhibition of memory, immobility, analgesia, and the inhibition of immune response, although the mechanism of these actions of Δ9-THC remained elusive until the late 1980's. In 1988, Devane et al. (1) demonstrated the presence of a specific binding site for cannabinoids in rat brain synaptosomes. Later, Matsuda et al. (2) and Munro et al. (3) cloned the cDNAs for the cannabinoid receptors (CB1 and CB2). It has been assumed that the diverse actions of the cannabinoids are mediated in a large part through these receptors. In 1992, Devane et al. (4) isolated N-arachidonoylthanolamine (anandamide) from pig brain as an endogenous cannabinoid receptor ligand. This compound has been shown to exhibit various cannabimimetic activities in vitro and in vivo (5–8). However, the levels of anandamide in various living tissues were very low (9, 10). Furthermore, anandamide was found to act as a partial agonist at the cannabinoid receptors (10). These observations strongly suggested the existence of another endogenous ligand in mammalian tissues.

In 1995, we (11) and Mechoulam et al. (12) reported that 2-arachidonoylglycerol (2-AG) is the second endogenous ligand for the cannabinoid receptors. 2-AG has been shown to exhibit a strong binding activity toward the cannabinoid receptors (11, 12) and a variety of cannabimimetic activities (5–8, 10, 13, 14). Importantly, 2-AG was found to act as a full agonist at the cannabinoid receptors (15–19). Moreover, 2-AG can be rapidly formed from arachidonic acid-containing phospholipids, such as inositol phospholipids, through the combined actions of phospholipase C and diacylglycerol lipase or the combined actions of phospholipase A and phospholipase C in various types of tissues and cells upon stimulation (20–26). Noticeably, the levels of 2-AG in various mammalian tissues are markedly higher than that of anandamide. Based on these results, we proposed that 2-AG, and not anandamide, is the intrinsic natural ligand for the cannabinoid receptors (15, 16, 27).

Despite their potential physiological and pathophysiological importance, the exact functions of the CB1 and CB2 receptors and their endogenous ligand 2-AG have not yet been fully elucidated. As for the CB1 receptor, several lines of evidence strongly suggested that 2-AG suppresses the neurotransmission through acting at the CB1 receptor expressed predominantly in the presynapse (10, 13, 14). It is becoming evident that 2-AG is a novel type of neuromodulator of profound physiological significance. On the other hand, the functions of the CB2 receptor, which is abundantly expressed in the immune system, still remain an enigma. Little is known concerning the biological activities of 2-AG toward inflammatory cells and immune competent cells. It is essential to investigate in detail the functions of the CB2 receptor and 2-AG to better understand...
stand the precise regulatory mechanisms of inflammatory reactions and immune responses.

In this study, we investigated the possible biological activity of 2-AG toward HL-60 cells differentiated into macrophage-like cells. We found that 2-AG induces the differentiation of HL-60 cells through a cannabinoid CB2 receptor-dependent mechanism. A similar effect was also observed with human monocytes. The physiological and pathophysiological meanings of the 2-AG-induced migration of macrophages/monocytes are discussed.

EXPERIMENTAL PROCEDURES

Chemicals—Arachidonic acid (20:4n-6), palmitic acid (16:0), oleic acid (18:1n-9), linoleic acid (18:2n-6), eicosa-5,8,11,14,17-pentaoenoic acid (20:5n-3), docosa-7,10,13,16,19-hexaenoic acid (22:6n-3), respectively fatty acid-free bovine serum albumin, 1,25-dihydroxyvitamin D3 (1,25-(OH)2vitamin D3), phorbol 12-myristate 13-acetate, and LY294002 were purchased from Sigma. Nitroblue tetrazolium (NBT), wortmannin, and herbimycin A were obtained from Wako Pure Chemicals (Osaka, Japan). Eicosa-5,8,11,14,17-pentaoenoic acid (mead acid) (20:5n-3) was purchased from Cayman Chemical Co. (Ann Arbor, MI). SR141716A was acquired from Biomol (Plymouth Meeting, PA). CP55940 and Y-27632 were purchased from Tocris (Bristol, United Kingdom). WIN55212-2 was obtained from RBI (Natick, MA). PD98059 and SB203580 were acquired from Calbiochem. Pertussis toxin (PTX) was purchased from List Biological Laboratories (Campbell, CA). SR144528 was a generous gift from Sanofi (Montpellier, France). 1,3-Benzylideneglycerol was prepared as described in Ref. 15. 2-AG and the other monoacylglycerols were prepared from 1,3-benzylideneglycerol and respective fatty acids as described earlier (15). An ether-linked analog of 2-AG (2-AG ether) (2-eicosa-5,8,11,14-tetraenylglycerol) was synthesized from 1,3-benzylideneglycerol and eicosatetraenoyl iodide as described previously (15).

Cells—Human promyelocytic leukemia HL-60 cells, human monocytic leukemia U937 cells, and THP-1 cells were grown at 37 °C in RPMI 1640 medium (Asahi Techno Glass Co., Chiba, Japan) supplemented with 10% fetal bovine serum in an atmosphere of 95% air and 5% CO2. HL-60 cells were differentiated into macrophage-like cells by treatment with 100 nm 1,25-(OH)2vitamin D3, for 5 days. U937 cells and THP-1 cells were also differentiated by treatment with 100 nm 1,25-(OH)2vitamin D3, for 5 days. Human monocytes were separated from the peripheral blood of young healthy donors as follows: 1/4 volume of dextran T-500 (Amersham Biosciences) in saline was added to heparinized blood to sediment the erythrocytes, the supernatant (leukocyte-rich fraction) was aspirated and centrifuged at 400 × g for 20 min. The mononuclear leukocyte fraction (the interface layer) was collected and washed with Hank’s balanced salt solution. Monocytes were separated from other mononuclear leukocytes by negative selection using a MACS monocyte isolation kit (Miltenyi Biotec Gmbh, Gladbach, Germany). The purity of the monocytes was 93% as assessed by a nonspecific esterase assay described below.

Migration Assay—The migration of the differentiated HL-60 cells, U937 cells, THP-1 cells, and human monocytes was assayed using Transwell inserts (1 mm pore size, 5 μm) and 24-well culture plates (Coming Costar, Cambridge, MA). Briefly, the cells (106 for the differentiated HL-60 cells, 105 for U937 cells, and 105 for human monocytes) suspended in 0.1 ml of RPMI 1640 medium containing 0.1% bovine serum albumin were transferred to the Transwell insert (the upper compartment). 2-AG was dissolved in Me2SO and added to 0.6 ml of the RPMI 1640 medium containing 0.1% bovine serum albumin in the well of the culture plate (the lower compartment) (the final concentration of Me2SO was 0.2%). After the incubation at 37 °C (for 4 h for HL-60 cells, U937 cells, and THP-1 cells and 2 h for human monocytes) in an atmosphere of 95% air and 5% CO2, the number of cells that migrated from the upper compartment to the lower compartment was counted using a hemocytometer.

NBT Reduction Assay—The NBT reduction assay was performed as described previously (28) with some modifications. Cells were suspended in 25 mM HEPES-Tyrode’s solution (pH 7.4) containing 0.05% NBT and incubated at 37 °C for 7 min. Phorbol 12-myristate 13-acetate was added to the cell suspension at a concentration of 4 μM, and the suspension was incubated for 30 min at 37 °C. After the addition of 10 mm EDTA to stop the reaction, the percentage of positive cells (blue-stained cells) was determined using a hemocytometer.

Nonspecific Esterase Assay—Nonspecific esterase activity was assayed using an α-naphthyl acetate esterase assay kit (Sigma).

Northern Blot Analysis—poly(A)+ RNAs (5 μg) from undifferentiated and differentiated HL-60 cells were electrophoresed in a 1.0% agarose-formaldehyde gel and transferred onto a Hybond-N membrane (Amersham Biosciences). The CB2 probe (human CB2 receptor cDNA Sph/USII digest; 524 bp) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) probe (BD Biosciences) were labeled with [α-32P]dCTP (PerkinElmer Life Sciences) using the Megaprime DNA labeling system (Amersham Biosciences). Hybridization was performed at 60 °C for 16 h in QuikHyb solution (Stratagene). The filter was washed in 0.1× SSC (1× SSC = 0.15 M NaCl and 0.015 M sodium citrate) containing 0.1% SDS at 65 °C and analyzed by a bioimaging analyzer BAS 1500 (Fuji Photo Film, Tokyo, Japan).

Estimation of the Amount of Remaining 2-AG Following the Incubation with the Cells—HL-60 cells differentiated into macrophage-like cells (4 × 105) were suspended in 0.4 ml of RPMI 1640 medium. The cells were then incubated with 1 μg 2-AG for 30 min, 1 h, 2 h, and 4 h. Following the incubation, the supernatant was aspirated, and the lipids were extracted by the method of Bligh and Dyer (29). Butylated hydroxytoluene (final, 0.05%) was added to avoid lipid peroxidation, and 2-heptadecanoylglycerol was added as an internal standard. The lipids were fractionated by TLC using development with petroleum ether:diethyl ether:acetic acid (20:80:1, v/v) in a tank sealed with N2 gas. The area corresponding to standard monoacylglycerol was scraped off the TLC plate, followed by extraction from the silica gel by the method of Bligh and Dyer (29). The extraction was conducted in the presence of butylated hydroxytoluene (0.001%) in an N2 gas-sealed tube. The purified monoacylglycerols were converted to their 1-anthroyl derivatives and then analyzed with a high pressure liquid chromatography system equipped with a reverse phase column (CAPCELL PAK C18 SG120, 4.6 mm × 250 mm; Shiseido Co., Tokyo, Japan) and a fluorescence detector (excitation at 370 nm, emission at 470 nm). The mobile phase was acetonitrile:2-propanol:water (90:4:6, v/v), and the flow rate was 1.4 ml/min as described previously (30).

Statistical Analysis—Statistical analysis was performed using the Student’s t test.

RESULTS

1,25-(OH)2vitamin D3 is known to induce the differentiation of HL-60 cells into macrophage-like cells (31). We first examined the effect of 1,25-(OH)2vitamin D3-treatment on several cellular markers of differentiation and the CB2 receptor mRNA level in HL-60 cells. As shown in Fig. 1A, the percentage of

FIG. 1. The proportion of NBT reduction assay-positive cells (A) and CB2 mRNA levels (B) in undifferentiated HL-60 cells and HL-60 cells differentiated into macrophage-like cells by treatment with 1,25-(OH)2vitamin D3. The NBT reduction assay was performed using 0.05% NBT and 4 μg phorbol 12-myristate 13-acetate as described under “Experimental Procedures.” The data are the means ± S.D. of four determinations. ***, p < 0.001 (compared with the control). The Northern blot analysis of the CB2 receptor mRNA was performed using poly(A)+ RNA obtained from undifferentiated cells and those from differentiated cells as described under “Experimental Procedures.” CB2 receptor gene transcripts, 4.4 and 2.5 kb; GAPDH gene transcript, 1.3 kb.
FIG. 2. Time and dose dependences of 2-AG-induced migration of HL-60 cells differentiated into macrophage-like cells. The HL-60 cells were differentiated into macrophage-like cells by treatment with 1,25-(OH)2 vitamin D3. A, differentiated HL-60 cells were added to the TranswellTM (the upper compartment), and 2-AG (1 μM) or vehicle (Me2SO) was added to the well of the culture plate (the lower compartment). The incubation was carried out for the indicated periods of time. Closed circle, 2-AG; open circle, vehicle (Me2SO) alone. B, cells were added to the TranswellTM (the upper compartment), and various concentrations of 2-AG or vehicle (Me2SO) were added to the well of the culture plate (the lower compartment). The incubation was carried out for 4 h. B, the migration from the upper compartment to the lower compartment was determined as described under “Experimental Procedures.” The data are the means ± S.D. of four determinations.

NBT reduction assay-positive cells in undifferentiated HL-60 cells was low (10.0%). On the other hand, the proportion of NBT reduction assay-positive cells was elevated to 65.7% following 2-AG-evoked migration. These results indicate that the 2-AG-induced migration is mediated by the CB2 receptor.

We then examined whether the 2-AG-induced migration is mediated by the CB2 receptor. SR144528, a cannabinoid CB2 receptor antagonist, exerted only a slight effect on the 2-AG-induced migration, indicating that G/Gαi is involved in the 2-AG-induced migration. We further examined the effects of various inhibitors of intracellular signaling pathways on 2-AG-induced cell migration. As shown in Fig. 4, PD98059 (a specific MAP kinase/extracellular signal-regulated kinase kinase (MEK) inhibitor; 20 μM), SB203580 (a p38 MAP kinase inhibitor; 20 μM), and Y-27632 (a Rho kinase inhibitor; 20 μM) suppressed the migration of differentiated HL-60 cells induced by 2-AG. On the other hand, LY294002 (a phosphatidylinositol 3-kinase inhibitor; 20 μM) and herbimycin A (a tyrosine kinase inhibitor; 20 μM) did not affect the migration markedly. We also confirmed that wortmannin (a phosphatidylinositol 3-kinase inhibitor; 200 nM) did not influence the migration at all (data not shown).

We then compared the activities of the various cannabinoid receptor ligands to induce migration. As shown in Fig. 5, the activity of 2-AG (1 μM) was the highest among those of the various cannabinoid receptor ligands examined in the present study. 2-AG ether (1 μM), anandamide (1 μM), and Y-27632 (a Rho kinase inhibitor; 20 μM) did not affect the migration at all (data not shown).

The activities of the various species of the 2-monoaoylglycerols to induce migration were next compared (Fig. 6). The highest activity was observed with 2-AG (1 μM). Appreciable activities were also observed with 2-eicosa-5,8,11-trienoylglycerol (1 μM) and 2-eicosa-5,8,11,14,17-pentaenoylglycerol (1 μM). However, the activities of the other species such as 2-palmitoylglycerol, 2-oleoylglycerol, 2-linoleoylglycerol, and 2-docosa-4,7,10,13,16,19-hexaenoxyglycerol were almost negligible.

We then examined whether the 2-AG-induced migration is because of chemotaxis (the directional movement along a concentration gradient) or chemokinesis (stirred movement in no specific direction). The migration of the cells from the upper compartment to the lower compartment in the absence of 2-AG was 4.4% (Fig. 7A). The proportion of migrated cells was elevated to 21.5% when 2-AG (1 μM) was added to the lower compartment. The migration was slightly reduced when 2-AG (1 μM) was added to both the upper compartment (with cells) and the lower compartment (18.5%). On the other hand, the
presence of 2-AG (1 μM) in the upper compartment alone (with cells) did not evoke cell migration (5.2%). Because 2-AG is known to be rapidly metabolized by a variety of cells (10, 13), we examined whether exogenously added 2-AG exists as an intact molecule following the co-incubation with the cells. We found that more than 99% of the exogenously added 2-AG (1 μM) was metabolized within 30 min of incubation. Thus, it is rather difficult to determine whether the migration is because of chemotaxis or chemokinesis under the present experimental conditions using 2-AG as a stimulant. To settle this problem, we added 2-AG ether, a metabolically stable analog of 2-AG, instead of 2-AG (Fig. 7B). We found that the percentage of migrated cells when 2-AG ether (1 μM) was added to both the upper compartment (with cells) and the lower compartment (5.8%) was significantly lower than that observed when 2-AG ether (1 μM) was added only to the lower compartment (8.3%), the proportion of migrated cells in the former case being close to the level of the control (4.6%). We also found that the addition of 2-AG ether (1 μM) to the upper compartment alone (with cells) did not enhance the migration of cells from the upper compartment to the lower compartment (4.9%). These results strongly suggest that 2-AG ether elicited mainly chemotaxis rather than chemokinesis.

We next investigated whether 2-AG induces the migration of other types of macrophage-like cells. In this study, we employed two types of human monocytic leukemia cells, U937 cells and THP-1 cells, which were differentiated by treatment with 1,25-(OH)2vitamin D3 before use as in the case of the HL-60 cells. We found that 2-AG (1 μM) significantly enhanced the migration of the differentiated U937 cells and THP-1 cells (Fig. 5).
FIG. 8. Effects of 2-AG on the migration of U937 cells and THP-1 cells differentiated by treatment with 1,25-(OH)\textsubscript{2} vitamin D\textsubscript{3}. The effects of 2-AG (1 µM) on the migration of differentiated monocytic U937 cells (A) and THP-1 cells (B) were examined using the Transwell\textsuperscript{TM} as described under “Experimental Procedures.” The data are the means ± S.D. of six determinations. ***, p < 0.001 (compared with the control (open bar)).

8), although the magnitude of augmentation was rather small compared with the case of the differentiated HL-60 cells.

Finally, we examined whether human peripheral blood monocytes respond to 2-AG. As shown in Fig. 9, 2-AG (1 µM) markedly accelerated the migration of human monocytes. The effect of 2-AG was abolished by treatment of the cells with SR144528 (1 µM), a CB2 receptor-specific antagonist, as in the case of differentiated HL-60 cells.

DISCUSSION

The cannabinoid CB2 receptor is a seven transmembrane, G protein-coupled receptor and is expressed abundantly in various types of inflammatory cells and immune competent cells such as macrophages, natural killer cells, and B lymphocytes (32–34). Previously (16), we examined in detail the structure–activity relationship of a number of CB2 receptor ligands using HL-60 cells, which express the CB2 receptor and exhibit a Ca\textsuperscript{2+} response when challenged with the CB2 receptor ligands. We found that the structure of 2-AG is strictly recognized by the CB2 receptor (16). The agonistic activity of 2-AG was most potent among various structural analogs. Noticeably, 2-AG acted as a full agonist at the CB2 receptor whereas anandamide acted as a weak partial agonist. Gonsiorek et al. (18) also demonstrated that 2-AG is a full agonist, and anandamide is a partial agonist using the membrane fraction of Sf9 cells transfected with the human CB2 receptor cDNA. We proposed that 2-AG, but not anandamide, is the intrinsic natural ligand for the cannabinoid CB2 receptor, and the CB2 receptor is primarily a 2-AG receptor (16).

Not much information is currently available concerning the biological activities of 2-AG toward inflammatory cells and immune competent cells. Previously, Kamiński and co-workers (35) reported that 2-AG affects lymphocyte proliferation. They also demonstrated that 2-AG suppresses the interleukin 2 gene expression in murine T lymphocytes through down-regulation of the nuclear factor (36). In addition, Chang et al. (37) demonstrated recently that 2-AG inhibited the production of interleukin 6 in J774 macrophage-like cells. It remains unclear, however, whether these effects of 2-AG are mediated through the cannabinoid receptor. Recently, we found that 2-AG induces rapid phosphorylation and activation of the p42/44 MAP kinase in HL-60 cells (38). 2-AG-induced activation of the p42/44 MAP kinase was abolished when the cells were pretreated with either SR144528 or PTX, indicating that the response was mediated through the CB2 receptor and G\textsubscript{i/Go}. We also found that rapid phosphorylation of the p38 MAP kinase and c-Jun N-terminal kinase takes place in 2-AG-stimulated HL-60 cells.\textsuperscript{2} The 2-AG-induced activation of the p38 MAP kinase and c-Jun N-terminal kinase has also been reported by several investigators (39, 40). These results strongly suggest that 2-AG plays some essential role in the inflammation and immune responses, although the exact physiological functions of 2-AG in inflammatory cells and immune competent cells still remain unclear.

In this study, we explored the effect of 2-AG on the motility of HL-60 cells. We found that 2-AG induces the migration of HL-60 cells differentiated into macrophage-like cells (see Figs. 2–7). Similar effects were observed with other macrophage-like cells of human origin such as U937 cells and THP-1 cells and human peripheral blood monocytes (see Figs. 8 and 9), suggesting that 2-AG-induced migration is a common event in human macrophages/monocytes.

The 2-AG-induced migration of differentiated HL-60 cells was markedly reduced when the cells were pretreated with either SR144528 or PTX (Fig. 3), suggesting that the migration was mediated through the CB2 receptor and G\textsubscript{i/Go}. Arachidonic acid and its metabolites did not participate in the 2-AG-induced migration, because free arachidonic acid was not capable of inducing the migration (data not shown). This was also confirmed by the fact that 2-AG ether was able to induce the migration (Fig. 5), although its activity was rather weak compared with that of 2-AG. On the other hand, the Rho kinase, MEK and p38 MAP kinase, were suggested to be involved in the 2-AG-induced migration of differentiated HL-60 cells, because Y-27632 (a Rho kinase inhibitor), PD98059 (a MEK inhibitor) and SB203580 (a p38 MAP kinase inhibitor) suppressed the migration (Fig. 4). The inhibition of cell migration by Y-27632 (1–100 µM) (41, 42), PD98059 (10–20 µM) (43–45), and SB203580 (10–50 µM) (42, 46) has already been reported for several types of cells stimulated with various chemottractants, although there are conflicting results as to the inhibition by PD98059 (42, 46). The relationship between Rho kinase and p38 MAP kinase, as well as MEK, is known to be complicated. Ashida et al. (42) reported that Rho kinase is upstream of p38 MAP kinase in monocyte chemotactrant protein-1-stimulated THP-1 cells. Details of the intracellular signaling pathways for 2-AG-induced migration of HL-60 cells will be clarified in the future.

The activity of 2-AG was highest among those of the vari-

\textsuperscript{2}T. Sugiura and Y. Kobayashi, unpublished results.
ous cannabinoid receptor ligands (Fig. 5). This is reasonable in view of the fact that 2-AG is the true endogenous ligand of the cannabinoid CB2 receptor. It has already been shown that 2-AG is present in appreciable amounts in various mammalian tissues (10, 13). 2-Monacylglycerols containing saturated, monoenoic, dienoic, and hexaenoic fatty acids did not exhibit any appreciable activity, whereas 2-eicosa-5′,8′,11′-trienoylglycerol and 2-eicosa-5′,8′,11′,14′,17′-pentaenoylglycerol induced the migration to some extent (Fig. 6). These results are in general agreement with the results of the Ca²⁺ transient experiments reported previously (16). We have found that the presence of the double bond at the Δ5-position is important for some characteristic conformation of the agonistic molecules (15, 16).

The migration of HL-60 cells induced by 2-AG was assumed to mainly involve chemotaxis rather than chemokinesis, because 2-AG elicited mainly chemotaxis (Fig. 7). This was also confirmed by the fact that the migration of HL-60 cells observed in the presence of 2-AG in both the upper and lower compartments was markedly reduced compared with the case of the presence of 2-AG only in the lower compartment, when 0.5 mM diisopropylfluorophosphate, a monoaoylglycerol lipase inhibitor, was added to block the hydrolysis of 2-AG: 9.3 ± 0.4, 26.7 ± 2.1, and 19.5 ± 3.3% for vehicle alone, 2-AG present only in the lower compartment, and 2-AG present in both compartments, respectively (the means ± S.D. of four determinations). We confirmed that 2-AG was rapidly metabolized during the co-incubation with the cells when the monoaoylglycerol lipase inhibitor was not included in the incubation mixture as mentioned before. It cannot be ruled out, however, that some part of the migration induced by 2-AG was because of chemokinesis. Whatever the mode and the mechanism of action, the fact that 2-AG induces the migration of macrophage-like cells and monocytes is quite noticeable, because various types of proinflammatory molecules are known to induce the migration and recruitment of inflammatory cells. Very recently, Jorda et al. (48) also reported that 2-AG induces the migration of mouse splenocytes and myeloid cells, yet the elucidation of the detailed mechanism of 2-AG-induced migration of these cells awaits further investigations.

Previously, Gallily et al. (49) reported that 2-AG suppresses the production of tumor necrosis factor α in lipopolysaccharide-stimulated mouse macrophages in vitro and in lipopolysaccharide-administered mice in vivo, although whether these effects of 2-AG are mediated through the CB2 receptor is uncertain. On the other hand, we found that the addition of 2-AG to HL-60 cells enhanced the production of chemokines such as interleukin 8 and MCP-1 through a CB2 receptor-dependent mechanism. Based on the results of a previous investigation on chemokine production and the present study on cell migration, we assume that 2-AG acts as a stimulator or accelerator, rather than as a suppressor, of inflammation reactions and immune responses.

As for Δ⁹-THC, it has been reported that Δ⁹-THC suppresses inflammation and immune responses in vivo (32-34). The mechanism by which Δ⁹-THC suppresses the inflammatory reactions and immune response has long remained obscure. Previously, we demonstrated that Δ⁹-THC is a weak partial agonist of the cannabinoid CB2 receptor (16). Bayewitch et al. (49) also reported that Δ⁹-THC acted as an antagonist toward the CB2 receptor. Noticeably, SR144528 and JTE-907, CB2 receptor antagonists/inverse agonists, inhibited inflammation in vivo (50). It is possible, therefore, that Δ⁹-THC blocks the action of the endogenous natural ligand of the CB2 receptor, that is, 2-AG, thereby inducing the suppression of inflammatory reactions and immune responses.

In conclusion, we found that 2-AG induces the migration of HL-60 cells differentiated into macrophage-like cells through the CB2 receptor, Gi/Gi, and several other signaling molecules-dependent mechanisms. Similar effects were observed with other macrophage-like cells and human monocytes. The migration induced by 2-AG was mainly attributed to chemotaxis rather than chemokinesis. 2-AG is known to be generated from stimulated inflammatory cells and immune competent cells such as macrophages upon stimulation (20, 23–25) through an increased phospholipid metabolism such as inositol phospholipid turnover. It is possible that 2-AG, derived from a variety of stimulated tissues and cells, plays physiologically and pathophysiologically essential roles during the course of inflammatory reactions and immune responses.

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