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2-Arachidonoylglycerol, an Endogenous Cannabinoid Receptor Ligand, Induces Accelerated Production of Chemokines in HL-60 Cells

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2-Arachidonoylglycerol is an endogenous ligand for the cannabinoid receptors (CB1 and CB2). Previously, we provided evidence that 2-arachidonoylglycerol, but not anandamide (*N*-arachidonoylethanolamine), is the true natural ligand for the cannabinoid receptors. In the present study, we examined in detail the effects of 2-arachidonoylglycerol on the production of chemokines in human promyelocytic leukemia HL-60 cells. We found that 2-arachidonoylglycerol induced a marked acceleration in the production of interleukin 8. The effect of 2-arachidonoylglycerol was blocked by treatment of the cells with SR144528, a cannabinoid CB2 receptor antagonist, indicating that the effect of 2-arachidonoylglycerol is mediated through the CB2 receptor. Augmented production of interleukin 8 was also observed with CP55940, a synthetic cannabinoid, and an ether-linked analog of 2-arachidonoylglycerol. On the other hand, neither anandamide nor the free arachidonic acid induced the enhanced production of interleukin 8. A similar effect of 2-arachidonoylglycerol was observed in the case of the production of macrophage-chemotactic protein-1. The accelerated production of interleukin 8 by 2-arachidonoylglycerol was observed not only in undifferentiated HL-60 cells, but also in HL-60 cells differentiated into macrophage-like cells. Noticeably, 2-arachidonoylglycerol and lipopolysaccharide acted synergistically to induce the dramatically augmented production of interleukin 8. These results strongly suggest that the CB2 receptor and its physiological ligand, *i.e.*, 2-arachidonoylglycerol, play important regulatory roles such as stimulation of the production of chemokines in inflammatory cells and immune-competent cells. Detailed studies on the cannabinoid receptor system are thus essential to gain a better understanding of the precise regulatory mechanisms of inflammatory reactions and immune responses.

Key words: anandamide, 2-arachidonoylglycerol, cannabinoid, CB2 receptor, chemokine.

Abbreviations: 2-AG, 2-arachidonoylglycerol; FBS, fetal bovine serum; IL, interleukin; LPS, lipopolysaccharide; MAP kinase, mitogen-activated protein kinase; MCP-1, macrophage-chemotactic protein-1; Δ^9-THC, Δ^9-tetrahydrocannabinol; 1,25-$(OH)_2$vitamin D_3, 1α,25-dihydroxyvitamin D_3.

Δ^9-Tetrahydrocannabinol (Δ^9-THC) is a major psychoactive component of marijuana and is known to elicit a variety of pharmacological responses such as heightened sensory awareness, euphoria, hallucinations, reduced motor activity and the suppression of the immune response (*1*). The mechanisms of these actions of Δ^9-THC were long remained elusive. In 1988, Devane *et al*. (*2*) demonstrated the occurrence of a specific binding site for cannabinoids in rat brain synaptosomes using [^{3}H]CP55940, a radiolabeled synthetic cannabinoid. Matsuda *et al*. (*3*) then cloned a cDNA encoding the brain-type receptor for cannabinoids (the CB1 receptor). The CB1 receptor is a G protein-coupled seven-transmembrane receptor and is assumed to play an important role in the regulation of neurotransmission (*4*). On the other hand, Munro *et al*. (*5*) cloned another type of cannabinoid receptor (the CB2 receptor), which is expressed abundantly in the immune system. The CB2 receptor is also a G protein-coupled seven-transmembrane receptor and is suggested to be involved in inflammation and the immune response, yet details of the physiological functions of the CB2 receptor remain unknown.

The discovery of specific receptors for cannabinoids prompted the search for their endogenous ligands. In 1992, Devane *et al*. (*6*) identified *N*-arachidonoylethanolamine (anandamide) as an endogenous cannabinoid receptor ligand. Later, we (*7*) and Mechoulam *et al*. (*8*) isolated 2-arachidonoylglycerol (2-AG), an arachidonic acid-containing 2-monoacylglycerol, as another endogenous ligand for the cannabinoid receptors. We provided evidence that both the CB1 receptor and CB2 receptor strictly recognize the structure of 2-AG (*9–12*). Based on these experimental results, we concluded that 2-AG is the true endogenous ligand for the cannabinoid receptors

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and that the cannabinoid receptors are primarily 2-AG receptors (*10–12*).

Concerning the physiological significance of 2-AG, we have previously proposed that 2-AG, derived from the postsynapse and/or presynapse following neural excitation, plays an important role in the attenuation of neurotransmitter release by acting at the CB1 receptor mainly expressed in the presynapse (*13*, *14*). The regulatory roles of 2-AG in neurotransmission have also been reported by several investigators (*15–17*). On the other hand, the exact physiological roles of 2-AG in the immune system have not yet been clarified. It is essential to investigate this issue in detail in order to gain a better understanding of the precise regulatory mechanism of inflammation and immune response for the following reasons: (i) The cannabinoid CB2 receptor is abundant in several types of immune-competent cells and inflammatory cells such as macrophages/monocytes, B lymphocytes and natural killer cells (*4*, *18*, *19*). (i) The administration of CB2 receptor antagonists and Δ^9-THC suppresses the inflammation and immune response *in vivo* (*20*, *21*). 3) Unlike anandamide, 2-AG is a rather common molecule, and can easily be formed from membrane phospholipids such as inositol phospholipids in various types of cells after stimulation (*15*, *22–27*).

Several years ago, Lee *et al*. (*28*) reported that 2-AG affects lymphocyte proliferation. Ouyang *et al*. (*29*) also demonstrated that 2-AG suppresses interleukin 2 (IL-2) gene expression in murine T lymphocytes through the down-regulation of the nuclear factor. Furthermore, several investigators have demonstrated that 2-AG inhibits the production of tumor necrosis factor α (TNF-α) in lipopolysaccharide (LPS)-stimulated mouse macrophages (*30*) and the production of IL-6 in LPS-stimulated J774 cells (*31*). However, it remains unclear whether these effects of 2-AG are attributable to 2-AG itself and mediated through the cannabinoid receptors, or attributable to its metabolites such as prostaglandins and leukotrienes. It also remains ambiguous whether 2-AG plays a stimulative or suppressive role during the course of inflammation and the immune response.

In this study, we investigated in detail the effects of 2-AG on the production of chemokines in HL-60 cells. We obtained clear evidence that 2-AG induces the accelerated production of IL-8, a chemokine for neutrophils, through a CB2 receptor-dependent mechanism. We also found that 2-AG markedly potentiates the LPS-induced augmented production of IL-8 in these cells.

MATERIALS AND METHODS

Reagents—Arachidonic acid, 1α,25-dihydroxyvitamin D_3 [1,25-$(OH)_2$vitamin D_3] and LPS (*E. coli*, Serotype 026: B6) were purchased from Sigma (St. Louis, MO, USA). SR141716A was acquired from Biomol (Plymouth Meeting, PA, USA). CP55940 was purchased from Tocris (Bristol, UK). Ethanolamine (2-aminoethanol) was obtained from Tokyo Kasei Kogyo (Tokyo). Dimethylsulfoxide (DMSO) was obtained from Wako Pure Chem. (Osaka). Anandamide was synthesized from arachidonic acid and ethanolamine as previously described (*11*). SR144528 was a generous gift from Sanofi (Montpellier, France). 1,3-Benzylideneglycerol was synthesized from glycerol and benzaldehyde. 2-AG was prepared from 1,3-benzylideneglycerol and arachidonic acid as described earlier (*11*). An ether-linked analog of 2-AG (2-AG ether) (2-eicosa-5′,8′,11′,14′-tetraenylglycerol) was synthesized from 1,3-benzylideneglycerol and eicosatetraenyl iodide as previously described (*11*).

Cells—Human promyelocytic leukemia HL-60 cells were grown at 37°C in RPMI 1640 medium (Asahi Technoglass, Chiba) supplemented with 10% fetal bovine serum (FBS) (Sigma, St. Louis, MO, USA) in an atmosphere of 95% air and 5% CO_2. In some experiments, the HL-60 cells were differentiated into macrophage-like cells by treatment with 100 nM 1,25-$(OH)_2$vitamin D_3 for 5 days.

Northern Blot Analysis of the IL-8 mRNA—The poly A^+ RNAs were isolated from three groups of undifferentiated HL-60 cells (A, before treatment; B, treated with 2-AG for 2 h; C, treated with the vehicle alone (0.2% DMSO) for 2 h). Briefly, the cells (9×10^7) were incubated in the presence or absence of 2-AG (1 μM) in 60 ml of RPMI 1640 medium containing 0.5% FBS, penicillin (100 U/ml) and streptomycin (100 μg/ml) at 37°C for 2 h. The total RNA was obtained using ISOGEN (Nippon Gene, Tokyo). Poly A^+ RNA was isolated from the total RNA using Oligotex-dT30 Super (Takara Bio, Shiga). The poly A^+ RNA (3 μg) was then electrophoresed in a 1.0% agarose-formaldehyde gel and transferred onto a Hybond-N^+ Nylon membrane (Amersham Biosciences, Piscataway, NJ, USA). The human IL-8 cDNA probe (prepared by digestion of an EST clone BE788958 with *Sal*I and *Eco*RI) and a human β-actin cDNA probe (Wako Pure Chem., Osaka) were labeled with [α-^{32}P]dCTP (222 TBq/mmol, PerkinElmer Japan, Kanagawa) using the Megaprime DNA labeling system (Amersham Biosciences, Piscataway, NJ, USA). Hybridization was performed at 60°C for 20 h in QuikHyb solution (Stratagene, Cambridge, UK). 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 bio-imaging analyzer BAS 1500 (Fuji Photo Film, Tokyo).

Measurements of IL-8 and Macrophage-Chemotactic Protein-1 (MCP-1) Produced by HL-60 Cells and U937 Cells by ELISA—Differentiated and undifferentiated HL-60 cells were suspended in RPMI 1640 medium containing 0.5% FBS, penicillin (100 U/ml) and streptomycin (100 μg/ml) at a density of 1×10^6 cells/ml. The cells (1.5×10^6) were then incubated with 2-AG (1 μM) or various cannabinoid receptor ligands (1 μM) in tissue culture dishes (diameter, 35 mm) (Becton Dickson, Franklin Lakes, NJ, USA) at 37°C for 24 h in an atmosphere of 95% air and 5% CO_2. In the experiment where the effect of the cannabinoid receptor antagonist SR144528 was examined, cells were pretreated with SR144528 (1 μM) for 5 min prior to the addition of 2-AG (1 μM). LPS (100 ng/ml) was simultaneously added to the cells with 2-AG and other cannabinoid receptor ligands. Following the incubation, the cells were centrifuged at 750 ×*g* for 5 min at 4°C and the supernatant was aspirated. The supernatant was stored at –80°C until use. The amounts of IL-8 and MCP-1 in the supernatant were estimated using ELISA kits, OptEIA Human IL-8 Set (BD PharMingen, San Diego, CA, USA) (for IL-8) and Human MCP-1

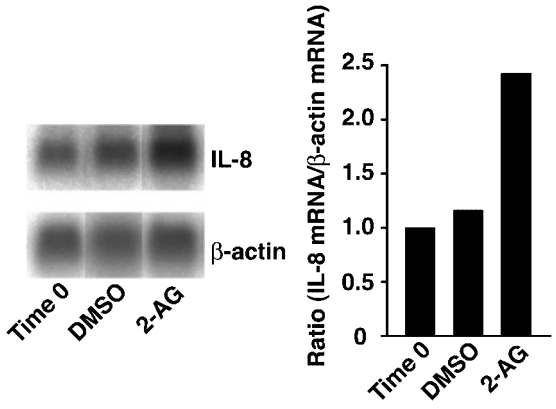

Fig. 1. **Northern blot analysis of the IL-8 mRNAs obtained from 2-AG-stimulated and unstimulated HL-60 cells.** HL-60 cells were incubated in the presence of 2-AG (1 μM) or vehicle (0.2% DMSO) at 37°C for 2 h. The poly A$^+$ RNA was electrophoresed in a 1.0% agarose-formaldehyde gel and then transferred onto a Hybond-N$^+$ Nylon membrane. The human IL-8 cDNA probe and human β-actin cDNA probe were labeled with [α-^{32}P]dCTP using the Megaprime DNA labeling system. Hybridization was performed at 60°C for 20 h. The filter was washed and then analyzed by a bio-imaging analyzer as described in "MATERIALS AND METHODS." The values are the ratios of the level of IL-8 mRNA to that of β-actin mRNA. The data are representative of two separate experiments which gave similar results.

Immunoassay Kit (BioSource International, Camarillo, CA, USA) (for MCP-1).

Statistical Analysis—The statistical analysis was performed using the Student's *t* test. A P value < 0.05 was considered to be significant.

RESULTS

Effect of 2-AG on IL-8 mRNA Level in HL-60 Cells—First, we examined the effect of 2-AG on the expression of the mRNA for IL-8, a chemokine for neutrophils, in HL-60 cells by Northern blot analysis. As shown in Fig. 1, an appreciable amount of IL-8 mRNA was detected in HL-60 cells. We found that the level of IL-8 mRNA was elevated by 2.5-fold following stimulation with 2-AG (1 μM) for 2 h. On the other hand, no appreciable change was observed when the cells were treated with the vehicle alone (DMSO).

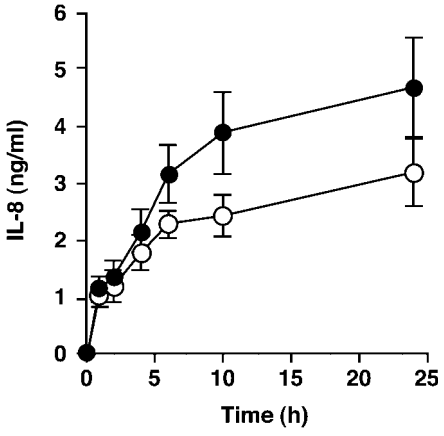

Fig. 2. **Effect of 2-AG on the production of IL-8 in HL-60 cells.** The HL-60 cells (1.5 × 10^6) were incubated in 1.5 ml of culture medium in the presence of 2-AG (1 μM) (closed circles) or vehicle (0.2% DMSO) (open circles) at 37°C for the indicated periods of time. The amount of IL-8 in the supernatant was estimated by ELISA as described in "MATERIALS AND METHODS." The values are the means ± SD of four determinations.

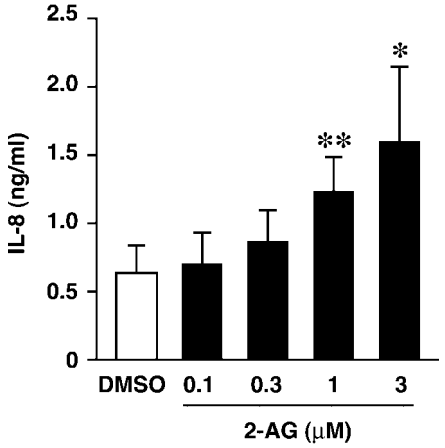

Fig. 3. **Dose-dependency of the effect of 2-AG on the production of IL-8 in HL-60 cells.** HL-60 cells (1.5 × 10^6) were incubated in 1.5 ml of culture medium in the presence of various concentrations of 2-AG (closed bars) or vehicle (0.2% DMSO) (open bar) at 37°C for 24 h. The amount of IL-8 in the supernatant was estimated by ELISA as described in "MATERIALS AND METHODS." The values are the means ± SD of four determinations. **P < 0.01, *P < 0.05 [compared to the control (DMSO)].

Effect of 2-AG on the Production of IL-8 in HL-60 Cells—We next examined the effect of 2-AG on the production of IL-8 by HL-60 cells using ELISA. HL-60 cells were found to produce a substantial amount of IL-8 during incubation even in the absence of any stimuli (Fig. 2). The amount of IL-8 gradually increased with time. Noticeably, the addition of 2-AG (1 μM) to the HL-60 cells markedly enhanced the production of IL-8: 1.6-fold at 10 h and 1.5-fold at 24 h, compared to the control (Fig. 2).

The acceleration of the production of IL-8 in HL-60 cells by 2-AG took place in a dose-dependent manner. The effect was detectable from 0.3 μM and the maximal effect was observed with 3 μM 2-AG (Fig. 3). Higher concentrations of 2-AG (≥10 μM) were not employed in this study, because cytotoxic effects were observed at higher concentrations of 2-AG (≥10 μM) under the present experimental conditions.

We then examined whether the effect of 2-AG is mediated through the cannabinoid CB2 receptor. In this experiment, we added SR144528, a CB2 receptor-specific antagonist, to the cells 5 min prior to the addition of 2-AG. We found that the addition of 1 μM SR144528 markedly suppressed the effect of 1 μM 2-AG (Fig. 4), indicating that the CB2 receptor is involved in the 2-AG-induced accelerated production of IL-8.

The effect of 2-AG on the production of IL-8 was next compared with that of LPS, a potent stimulant of inflammatory cells. As demonstrated in Fig. 5, the addition of either 2-AG or LPS augmented the production of IL-8, with the effect of 1 μM 2-AG comparable to that of 100 ng/ml of LPS.

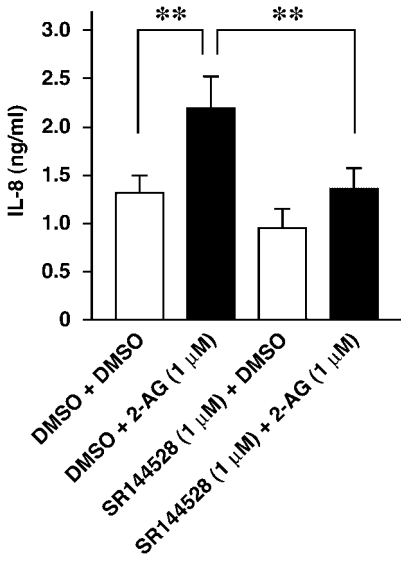

Fig. 4. **Effect of SR144528, a CB2 receptor antagonist, on the 2-AG-induced accelerated production of IL-8 in HL-60 cells.** HL-60 cells (1.5×10^6) were incubated in 1.5 ml of culture medium in the presence of 2-AG (1 μM) or vehicle (DMSO) at 37°C for 24 h. SR144528 (1 μM) or vehicle (DMSO) was added 5 min before the addition of 2-AG. The final concentration of DMSO was 0.4%. The amount of IL-8 in the supernatant was estimated by ELISA as described in "MATERIALS AND METHODS." The values are the means ± SD of four determinations. $^{**}P < 0.01$.

Effects of Various Cannabinoid Receptor Ligands on the Production of IL-8 in HL-60 Cells—We then compared the activity of 2-AG with those of other cannabinoid receptor ligands. Fig. 6 shows the results of such experiments. The addition of 1 μM 2-AG augmented the production of IL-8 by 1.7-fold. CP55940 (1 μM), a potent synthetic cannabinoid, and 2-AG ether (1 μM), a metabolically stable analog of 2-AG, also potentiated the production of IL-8 when added to cells (1.6-fold and 1.8-fold, respectively). In contrast, the addition of anandamide (1 μM) did not enhance the production of IL-8. We also found that free arachidonic acid (1 μM) failed to stimulate the production of IL-8. On the other hand, the addition of 100 ng/ml of LPS enhanced the production of IL-8. The enhanced production of IL-8 in the LPS-stimulated cells was further potentiated in the presence of 2-AG (1 μM), CP55940 (1 μM) or 2-AG ether (1 μM), but not in the presence of anandamide (1 μM) or free arachidonic acid (1 μM).

Effects of Various Cannabinoid Receptor Ligands on the Production of MCP-1 in HL-60 Cells—We next examined the effects of 2-AG, CP55940, 2-AG ether, anandamide and free arachidonic acid on the production of MCP-1, a chemokine for macrophages, in HL-60 cells (Fig. 7). We found that the addition of 1 μM 2-AG augmented the production of MCP-1 by 2.7-fold. Similar effects were observed with CP55940 (1 μM) and 2-AG ether (1 μM) (3.5-fold and 2.7-fold, respectively), whereas anandamide (1 μM) and free arachidonic acid (1 μM) were inactive. The addition of 100 ng/ml of LPS augmented the production of MCP-1. The augmented production of MCP-1 in LPS-stimulated cells was further accelerated in the presence of 2-AG (1 μM), CP55940 (1 μM) or 2-AG ether (1 μM), but not in the presence of anandamide (1 μM) or free arachidonic acid (1 μM), as in the case of IL-8.

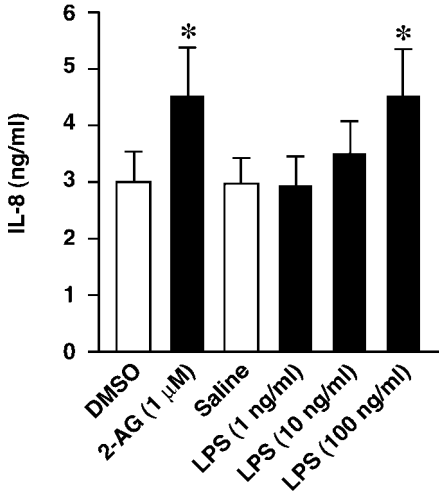

Fig. 5. **Comparison of the effects of 2-AG and LPS on the production of IL-8 in HL-60 cells.** The HL-60 cells (1.5×10^6) were incubated in 1.5 ml of culture medium in the presence of 2-AG (1 μM) or various concentrations of LPS at 37°C for 24 h (closed bars). The amount of IL-8 in the supernatant was estimated by ELISA as described in "MATERIALS AND METHODS." The values are the means ± SD of four determinations. $^{*}P < 0.05$ [compared to the control (DMSO or saline) (open bars)].

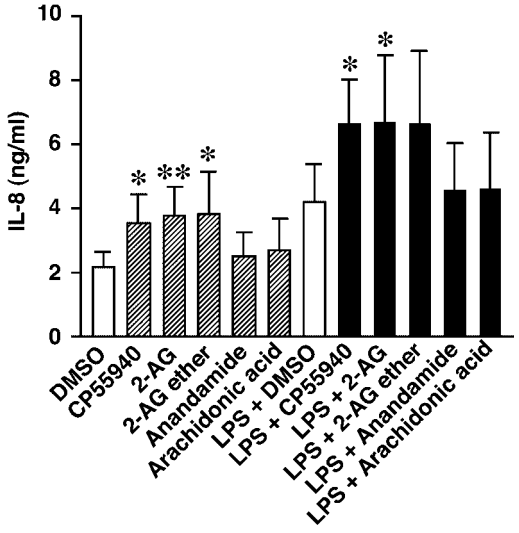

Fig. 6. **Effects of various cannabinoid receptor ligands and arachidonic acid on the production of IL-8 in HL-60 cells.** HL-60 cells (1.5×10^6) were incubated in 1.5 ml of culture medium in the presence of various cannabinoid receptor ligands (1 μM), arachidonic acid (1 μM) or vehicle (0.2 % DMSO) at 37°C for 24 h (hatched bars). In some cases, LPS (100 ng/ml) was added simultaneously with various cannabinoid receptor ligands or arachidonic acid (closed bars). The amount of IL-8 in the supernatant was estimated by ELISA as described in "MATERIALS AND METHODS." The values are the means ± SD of five determinations. $^{**}P < 0.01$, $^{*}P < 0.05$ [compared to the control (DMSO or LPS + DMSO) (open bars)].

Effects of 2-AG and LPS on the Production of IL-8 in HL-60 Cells Differentiated into Macrophage-Like Cells—Finally, we examined the effects of 2-AG and LPS on the

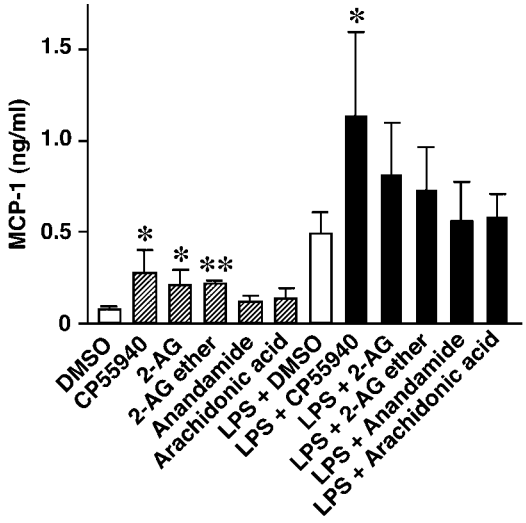

Fig. 7. **Effects of various cannabinoid receptor ligands and arachidonic acid on the production of MCP-1 in HL-60 cells.** HL-60 cells (1.5×10^6) were incubated in 1.5 ml of culture medium in the presence of various cannabinoid receptor ligands (1 μM), arachidonic acid (1 μM) or vehicle (0.2% DMSO) at 37°C for 24 h (hatched bars). In some cases, LPS (100 ng/ml) was added simultaneously with various cannabinoid receptor ligands or arachidonic acid (closed bars). The amount of MCP-1 in the supernatant was estimated by ELISA as described in "MATERIALS AND METHODS." The values are the means ± SD of four determinations. $^{**}P < 0.01$, $^{*}P < .05$ [compared to the control (DMSO or LPS + DMSO) (open bars)].

production of IL-8 in HL-60 cells differentiated into macrophage-like cells. As demonstrated in Table 1, the addition of 2-AG (1 μM) to the differentiated cells induced the augmented production of IL-8 (2.1-fold) similar to the case of undifferentiated cells. The responsiveness to LPS of the differentiated cells was much more prominent compared to undifferentiated cells. The addition of 100 ng/ml of LPS to the differentiated cells induced a marked elevation of IL-8 production (14.1-fold), which was further accelerated when 2-AG (1 μM) was added together with LPS (18.6-fold).

DISCUSSION

The cannabinoid CB2 receptor is a seven-transmembrane, G protein-coupled receptor that exists abundantly in various lymphoid tissues such as the tonsil, spleen and lymph nodes (*4*, *18*, *19*). Several types of leukocytes, such as macrophages/monocytes, B lymphocytes and natural killer cells, are known to express the CB2 receptor (*4*, *18*, *19*); it has been assumed that the CB2 receptor is involved in the differentiation and regulation of several cellular functions of these cells (*28*, *29*, *32–37*). Nevertheless, sufficient information has not yet been accumulated as to the physiological significance of the CB2 receptor and it still remains an enigma. Thus, further intensive studies on the physiological roles of the CB2 receptor and its endogenous ligand are essential.

Previously, we found that 2-AG and other cannabinoid receptor ligands induce rapid transient increases in intracelluar free Ca^{2+} concentrations in HL-60 cells

Table 1. **Effects of 2-AG and LPS on the production of IL-8 in HL-60 cells differentiated into macrophage-like cells.**[a]

	DMSO	2-AG	LPS + DMSO	LPS + 2-AG
ng/ml	5.3 ± 0.3	11.0 ± 3.2[b]	74.6 ± 4.8[c]	98.6 ± 2.5[c,d]

[a]HL-60 cells were differentiated into macrophage-like cells by treatment with 1,25-$(OH)_2$ vitamin D_3 for 5 days. HL-60 cells were then treated with 2-AG (1 μM), LPS (100 ng/ml) or vehicle (0.2% DMSO) at 37°C for 24 h. The amount of IL-8 was estimated by ELISA as described in "MATERIALS AND METHODS." The values are the means ± SD of four determinations. The data are representative of two separate experiments with similar results. [b]$P < 0.05$ [compared to the control (DMSO)]. [c]$P < 0.001$ [compared to the control (DMSO)]. [d]$P < 0.001$ [compared to the control (LPS + DMSO)].

through a CB2 receptor-dependent mechanism (*12*). We investigated in detail the structure-activity relationship of the CB2 receptor ligands using this assay system (*12*). We found that 2-AG is the most efficacious agonist so far examined. In contrast to 2-AG, anandamide was found to act as a weak partial agonist. These results indicate that 2-AG, but not anandamide, is the true natural ligand for the CB2 receptor (*12*). Therefore, it is crucial to explore the possible biological activities of 2-AG toward a variety of inflammatory cells and immune-competent cells in order to clarify the physiological functions of the CB2 receptor.

In this study, we examined in detail the effects of 2-AG on the production of chemokines in HL-60 cells. We found that the addition of 2-AG to HL-60 cells induced enhanced production of IL-8, a chemokine for neutrophils (Figs. 2–6). We also found that the production of MCP-1, a chemokine for macrophages/monocytes, was markedly augmented in the presence of 2-AG (Fig. 7). Similar stimulative effects on the production of IL-8 and MCP-1 were also observed with other cannabinoid receptor ligands such as CP55940, a synthetic cannabinoid, and 2-AG ether, a metabolically stable analog of 2-AG (Figs. 6 and 7). This observation strongly suggests that the cannabinoid receptors are involved in this cellular response. In fact, the addition of SR144528, a CB2 receptor antagonist, to the cells abolished the augmented production of IL-8 induced by 2-AG (Fig. 4). Jbilo *et al*. (*33*) previously reported that CP55940 is able to induce the augmented production of IL-8 and MCP-1 when added to HL-60 cells. The effect of CP55940 was blocked by treatment of the cells with SR144528 (*33*). Thus, it is apparent that the CB2 receptor is responsible for the enhanced production of chemokines by the cannabinoid receptor ligands. The lack of ability of anandamide to stimulate the production of chemokines in HL-60 cells (Figs. 6 and 7) is consistent with the previous observation that anandamide acts as a weak partial agonist or antagonist toward the cannabinoid receptors (*12*, *38*).

Noticeably, 2-AG-induced augmented production of IL-8 was observed not only in undifferentiated HL-60 cells but also in HL-60 cells differentiated into macrophage-like cells by treatment with 1,25-$(OH)_2$ vitamin D_3 for 5 days (Table 1). Interestingly, 2-AG and LPS acted synergistically to induce the markedly elevated production of IL-8 (Table 1). These results suggest that 2-AG may play an essential role in the recruitment of inflammatory cells and immune-competent cells, together with other proinflammatory molecules, by inducing the augmented pro-

duction of chemokines such as IL-8 and MCP-1 during the course of inflammation and the immune responses.

The mechanism underlying the 2-AG-induced augmented production of chemokines has not yet been fully elucidated. Previously, we found that 2-AG induced the rapid phosphorylation and activation of p42/44 mitogen-activated protein kinase (MAP kinase) in HL-60 cells through a CB2 receptor-dependent mechanism (*39*). Recently, we obtained evidence that the addition of PD98059, a p42/44 MAP kinase kinase (MEK) inhibitor, markedly reduced the 2-AG-induced augmented production of IL-8 (Kishimoto, S. and Sugiura, T., unpublished results). We also found that the phosphorylation of p38 MAP kinase and c-Jun kinase took place in 2-AG-stimulated HL-60 cells (*40*). The activation of p38 MAP kinase and c-Jun kinase by 2-AG has also been demonstrated by several investigators (*41*, *42*). Therefore, it seems possible that 2-AG exerts its biological activities toward inflammatory cells and immune-competent cells through these intracellular signaling pathway-dependent mechanisms, although the details remain to be clarified.

In contrast to our present results that 2-AG enhances the production of IL-8 and MCP-1, Gallily *et al*. (*30*) previously reported that 2-AG suppresses the production of TNF-α in LPS-stimulated mouse macrophages in vitro and in LPS-administered mice *in vivo*. Chang *et al*. (*31*) also demonstrated that 2-AG reduces the production of IL-6 in LPS-stimulated J774 cells. The reason for such a difference in the effect of 2-AG between our case and their cases is not known. One possibility is that the effects of 2-AG differ depending on the types of mediator molecules produced by the cells. It is noteworthy that Stefano *et al*. (*43*) reported that 2-AG induces an enhanced release of nitric oxide from human monocytes. Another possibility may exist in that the metabolites of 2-AG possibly formed *in vitro* and *in vivo* are implicated in the response. We confirmed that the acceleration of IL-8 production by 2-AG is mediated through the CB2 receptor by using the CB2 receptor antagonist SR144528. We also confirmed that free arachidonic acid itself does not exhibit any activity. On the other hand, it is not clear whether the effect of 2-AG is mediated through the cannabinoid receptors in the cases of Refs 30 and 31. These authors did not examine the effects of the cannabinoid receptor antagonists on the responses; there remains the possibility that arachidonic acid metabolites derived from 2-AG are involved, at least in part, in the suppression.

It should be noted that Δ^9-THC, a psychoactive constituent of marijuana, has been shown to affect inflammatory reactions and the immune response *in vivo* (*21*). For instance, orally administered Δ^9-THC exhibits anti-inflammatory activity in rat paws injected with several stimulants such as carrageenin (*44*), although there are conflicting results as to this issue (*45*). The administration of Δ^9-THC to experimental animals is also known to cause a decreased resistance to viral and bacterial infection (*21*, *34*, *35*). Several investigators have also demonstrated that Δ^9-THC suppresses the diverse cellular functions of inflammatory cells and immune-competent cells such as phagocytosis, antigen presentation, cytotoxicity and the production of nitric oxide and cytokines such as TNF-α (*21*, *34*, *35*). The mechanism by which Δ^9-THC suppresses inflammatory cell and immune cell functions has not been fully elucidated. It has generally been assumed that the effects of Δ^9-THC are mediated at least in part by the cannabinoid receptors. Previously, we demonstrated that Δ^9-THC is a weak partial agonist of the cannabinoid CB2 receptor (*12*). Bayewitch *et al*. (*45*) also reported that Δ^9-THC acts as an antagonist toward the CB2 receptor. Hence, it is conceivable that Δ^9-THC interferes with the actions of the physiological ligand 2-AG, thereby causing the suppression or inhibition of several functions of inflammatory cells and immune-competent cells, at least in some cases. In agreement with this, Iwamura *et al*. (*20*) recently reported that the administration of JTE-907, a CB2 receptor-specific inverse agonist, markedly reduces inflammation of the paw evoked by the injection of carrageenin in mice. Taking these results together, it seems apparent that the endogenous ligand of the CB2 receptor, *i.e.*, 2-AG, is closely involved in various types of inflammatory reactions such as the carrageenin-induced inflammation.

In conclusion, we found that 2-AG induces the augmented production of IL-8 in HL-60 cells through a CB2 receptor-dependent mechanism. A similar acceleration was also observed in the case of the production of MCP-1. LPS and 2-AG act synergistically to induce the enhanced production of IL-8 in differentiated HL-60 cells. These results strongly suggest that the cannabinoid receptor system plays essential roles in the stimulation and acceleration of inflammatory reactions. Thus far, however, not much information is available as to the physiological functions of 2-AG as a CB2 receptor ligand. Further studies are indispensable to the thorough elucidation of the physiological and pathophysiological significance of the cannabinoid CB2 receptor and its endogenous natural ligand 2-AG in the immune system. Such studies would help us to understand better the precise regulatory mechanisms of inflammatory reactions and immune responses.

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