

Involvement of the Endogenous Cannabinoid 2 Ligand 2-Arachidonyl Glycerol in Allergic Inflammation

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Key Words

Cannabinoid 2 · 2-Arachidonyl glycerol · Allergic inflammation · Contact dermatitis · Pulmonary bronchitis · Microarray

Abstract

Background: Cannabinoid (CB) 2 is expressed on immune and inflammatory cells. Identification of 2-arachidonyl glycerol (2-AG) and anandamide as endogenous CB2 ligands has allowed investigations of the roles of CB2 and its endogenous ligand system in inflammatory cells. However, the roles of this receptor-ligand system in inflammatory and allergic immune responses in vivo have not been fully elucidated.

Methods: Two mouse allergy models, namely ear dermatitis induced by 2,4-dinitrofluorobenzene and allergic bronchitis induced by ovalbumin, were analyzed for 2-AG amounts in allergic tissues, with reference to allergic and inflammatory symptoms. To investigate the gene expression via CB2 in inflammatory cells, human promyelocytic HL-60 cells were stimulated by the CB2 ligand 2-AG ether and analyzed using a DNA microarray. **Results:** In the ear dermatitis model, the 2-AG amount increased upon serial 2,4-dinitrofluorobenzene challenges and was correlated with ear weight gain. The increased ear thickness in this allergy model was clearly suppressed in CB2 knockout mice, suggesting that the

generated endogenous CB2 ligands induce ear thickness through aberrant inflammatory responses and remodeling mediated via CB2. In the allergic bronchitis model, the 2-AG level in bronchoalveolar lavage was increased and sustained during the elevation of inflammatory cell infiltration. The DNA microarray analysis of human HL-60 cells revealed that 2-AG ether induced expressions of not only inflammatory chemokines/cytokines but also of cell growth factors. **Conclusion:** Our data strongly suggest that endogenous CB2 ligands upregulated upon disease progression in allergic models are involved in aberrant alterations of both inflammatory responses and tissue cell growth.

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Introduction

Cannabinoid (CB) 2 was originally identified and cloned from the human promyelocytic leukemia cell line HL-60 [1]. Subsequently, 2-arachidonyl glycerol (2-AG) and anandamide (AEA) were identified as CB2 ligands in the rat brain and dog intestine [2, 3]. This receptor ligand system has been studied in terms of its implications for inflammatory diseases, because CB2 is specifically expressed in monocytes/macrophages, B lymphocytes, natural killer cells and granulocytes, but shows no or lower

expression in T lymphocytes and other tissue cells [4–10]. Thus far, direct effects of 2-AG on HL-60 cells, human monocytes and other inflammatory cells to induce chemotaxis and activation have been reported, and indirect effects of 2-AG mediated by CB2 on these cells have also been reported in terms of the production of interleukin (IL)-8, which enhances chemotaxis and activation of human granulocytes, monocytes and other inflammatory cells [6]. These previous findings of direct and indirect effects of 2-AG suggest that the CB2 ligand system mainly contributes to chemotaxis of proinflammatory cells. However, it has not been fully elucidated how CB2 and its endogenous ligand system are involved in the progression of inflammatory diseases, because the indirect effects occurring in tissues/organs via endogenous CB2 ligands have not been well clarified.

Both 2-AG and AEA are ligands for CB2 as well as for CB1 [6]. In addition to CB1 expression on neuronal cells, CB1 is expressed on peripheral tissues such as mesenchymal fibroblastoid cells and can induce downstream signaling via its ligands including AEA and 2-AG. In fact, a CB1-specific antagonist, and to a lesser extent a CB2-selective antagonist, inhibits extracellular signal-regulated kinase (ERK) phosphorylation, which is induced by endogenous CB ligands in fibroblastoid cells [11]. Since ERK is a key kinase that mediates cell growth signaling, these findings suggest that endogenously produced CB ligands directly induce tissue cell growth, at least in fibroblastoid cells. In the chronic inflammatory disease rheumatoid arthritis, the synovial fluid of patients was reported to contain high levels of AEA and 2-AG and decreased levels of palmitoylethanolamide and oleoylethanolamide, which have been reported to act as antagonists or competitors for the CB ligands 2-AG and AEA. Moreover, AEA and 2-AG can induce ERK activation to promote synovial cell growth [12]. These findings suggest that CB ligand systems can directly modulate the state of tissue cell remodeling in chronic inflammatory diseases via CB1, but to a lesser extent or not at all via CB2. However, to fully elucidate the contributions of the systems involving endogenous ligands for CB1 or CB2 separately in *in vivo* allergy and inflammation models, it is necessary to utilize gene knockout (KO) mice as well as preferentially CB2-expressing cells.

In this study, we conducted experiments to clarify the production of 2-AG during disease progression in experimental inflammation models and to examine the gene expressions induced by the CB2-2-AG system in HL-60 cells, to further understand the direct and indirect effects of 2-AG via CB2 *in vivo*. The obtained results using wild-type

(WT) and CB2-KO mice strongly suggest that the CB2 ligand system induces inflammatory responses as well as alterations in the tissue remodeling processes in allergic disease models. In addition, a DNA microarray analysis revealed that 2-AG induced the expression of genes related to not only proinflammatory cytokines/chemokines but also to cell growth/extracellular matrix (ECM) production. We found that the gene expression levels of IL-8, macrophage inflammatory protein (MIP)-1 α/β and tumor necrosis factor (TNF)- α as cytokines/chemokines were elevated, and those of connective tissue growth factor (CTGF) and growth response genes were clearly enhanced. The direct and indirect implications of elevated 2-AG amounts in the inflammatory response and tissue remodeling in animal models are discussed further in terms of the effects of the 2-AG-mediated gene expressions.

Materials and Methods

Animals and Cells

Female BALB/c mice (8 weeks old) were obtained from Charles River Laboratories Japan Inc. (Yokohama, Japan). Female CB2-KO mice on a C57BL/6 background and their age-matched wild type (WT) littermates were provided by Prof. A. Zimmer (Bonn University, Bonn, Germany), and breeding and genotyping were performed as described previously [13]. They were housed in plastic cages in a room illuminated on a 12-hour light cycle (08.00–20.00) with controlled temperature ($25 \pm 3^\circ\text{C}$) and humidity ($55 \pm 15\%$) and fed a standard laboratory diet and given water *ad libitum*. All procedures related to the use of animals in this study were reviewed and approved by the Institutional Animal Care and Use Committee at Japan Tobacco Inc.

Human promyelocytic leukemia HL-60 cells were grown at 37°C in RPMI-1640 medium (Invitrogen, Carlsbad, Calif., USA) supplemented with 10% fetal bovine serum (FBS; Clontech Laboratories, Palo Alto, Calif., USA) in an atmosphere of 95% air and 5% CO_2 .

Reagents

2,4-Dinitrofluorobenzene (DNFB), oxazolone, acetone, chloroform, methanol, dibutylhydroxytoluene (BHT), 1-anthroyl cyanide and quinuclidine were obtained from Wako Pure Chemical Industries Ltd. (Osaka, Japan). Ovalbumin (OVA) was purchased from Sigma-Aldrich Co. (St. Louis, Mo., USA). Aluminum hydroxide hydrate (alum) was purchased from Cosmo Bio Co. Ltd. (Tokyo, Japan). 2-AG ether was synthesized at Japan Tobacco Inc. (Osaka, Japan). An SV Total RNA Isolation kit was obtained from Promega Corporation (Madison, Wisc., USA). A Mouse Genome Oligo Microarray was purchased from Agilent Technologies Inc. (Palo Alto, Calif., USA).

Mouse Contact Dermatitis Model

Twenty microliters of acetone/olive oil (3:1, v/v) was applied to each side of the left ear, and 20 μl of 1% DNFB in acetone/olive oil (3:1, v/v) was applied to each side of the right ear of female BALB/c

mice (8 weeks old) four times at 7-day intervals. Before the fourth application, the mice were assigned to four groups of 5 mice based on the index body weight. Before application (0 h) and at 1, 4 and 24 h after application, mice were euthanized by cervical dislocation, and the left and right ears were removed and weighed. After weighing the pinna, the ears were dissected and immediately frozen in liquid nitrogen. They were then homogenized in a Bligh and Dyer [14] extraction mixture using a Polytron homogenizer (Central Scientific Commerce Inc., Tokyo, Japan). A mixture of chloroform/methanol (1:2, v/v) was added, followed by addition of an antioxidant, 1% BHT, at a 1/1,000 volume. These samples for measurement of 2-AG were stored at -20°C until analysis.

CB2-KO and WT mice on a C57BL/6 background were sensitized with repeated topical applications of DNFB as described previously [15]. Briefly, 20 μl of 1% DNFB in acetone/olive oil (3:1, v/v) was applied to each side of the right and left ears of mice five times at 7-day intervals. The ear thickness was measured in a blinded manner using a thickness gauge (Mitutoyo Corporation, Kawasaki, Japan) just before and at 24 h after each DNFB challenge and was expressed as the increase in ear thickness relative to the value measured immediately before exposure to DNFB. The ear thickness before the first application was set as 0.

Mouse Pulmonary Bronchitis Model

Female BALB/c mice were administered an intraperitoneal injection of 0.5 ml of saline containing 1 μg of OVA and 4 mg of alum on days 0 and 14. On day 21, the mice were presented with an inhaled challenge of 5% OVA for 25 min to induce airway inflammation. At 24, 48 and 72 h after the OVA challenge, respectively, the mice were euthanized by exsanguination. Subsequently, the mouse tracheae were cannulated and the lungs were lavaged four times with 1 ml of PBS(–) containing 5 mM EDTA. The bronchoalveolar lavage fluid (BALF) samples were analyzed for the numbers of total cells, neutrophils, lymphocytes and eosinophils using an automatic analyzer (Advia 120; Siemens Japan K.K., Tokyo, Japan). The BALF samples were then centrifuged at 1,200 rpm for 5 min at 4°C . Next, 1.875 ml of chloroform/methanol (1:2, v/v) was added to 0.5-ml aliquots of the supernatants, and 2.4 μl of 1% BHT was added to avoid lipid peroxidation. These samples for 2-AG measurement were stored at -20°C until analysis.

Measurement Procedure for Endogenous 2-AG

2-Heptadecanoylglycerol (0.1 nmol) was added as an internal standard. Total lipid was extracted by the method of Bligh and Dyer [16]. The monoacylglycerols were fractionated by thin-layer chromatography (TLC) with development using petroleum ether/diethyl ether/acetic acid (20:80:1, v/v/v) in a sealed tank containing N_2 gas. The area corresponding to the standard monoacylglycerol was scraped off the TLC plate and extracted from the silica gel. The monoacylglycerol extract containing 2-AG was dissolved in dehydrated acetone, added with 1-anthroyl cyanide and quinuclidine, and incubated at 45°C for 60 min. The monoacylglycerols were converted to their 1-anthroyl derivatives and fractionated by TLC with development using petroleum ether/diethyl ether/acetic acid (65:35:1, v/v/v). The fractions containing 1-anthroyl 2-AG were then extracted and analyzed with a high-performance liquid chromatography system equipped with a reverse-phase column (Capcell Pak C18 SG, 4.6×250 mm; Shiseido Co., Tokyo, Japan) and a fluorescence detector (excitation at 370 nm; emission at 470 nm). The mobile phase was acetonitrile/2-propa-

nol/water (90:4:6, v/v/v), and the flow rate was 1.4 ml/min as described previously [17].

Human cDNA Microarray Analyses

HL-60 cells (3×10^5 cells/ml) suspended in RPMI-1640 medium containing 0.5% FBS were transferred to culture flasks and cultured for 12 h. The medium was then replaced with FBS-free RPMI-1640 medium, and added with 2-AG ether (final concentration, 5 μM). After 1 h of 2-AG ether stimulation, the cells were collected and washed in cold PBS(–). Total RNA was extracted from the collected cells according to the protocol of the SV Total RNA Isolation Kit. Total RNA was eluted with 100 μl of RNase-free water. Some of the eluted RNA samples were tested by quantity and quality checks, and the other samples were stored at -80°C until microarray analysis. RNA (5 μg) was labeled with cyanine 3-cytidine triphosphate (Cy3) or cyanine 5-cytidine triphosphate (Cy5), respectively, according to the protocol of the Mouse Genome Oligo Microarray. RNA derived from untreated cells as a control template for the reverse transcription reaction was labeled with Cy3, and RNA derived from 2-AG ether-treated cells as a template for the reverse transcription reaction was labeled with Cy5. The Cy3- and Cy5-labeled cDNA targets were mixed and hybridized with the Mouse Genome Oligo Microarray. The slides were placed into a hybridization chamber (Agilent Technologies Inc.) and hybridization was allowed to proceed at 60°C for 17 h. The slides were then removed from the chamber, and washed and dried using an In Situ Hybridization Kit-Plus (Agilent Technologies Inc.) according to the array manufacturer's protocol. Immediate scanning of the slides was performed with a dual-laser DNA microarray scanner (Agilent Technologies Inc.). The intensity of each hybridization signal was evaluated using Feature Extraction software (Agilent Technologies Inc.). The common logarithm of the Cy5/Cy3 ratio for each sample was calculated by averaging the spots. A cut-off value for each expression level was automatically calculated according to the background fluctuation. The gene corresponding to the selected spot was calculated for its Cy5/Cy3 fluorescence intensity ratio as the gene expression ratio (fold value). The experiments were conducted three times, and the average value was calculated for each fold value.

Statistics

Data were represented as means $\pm$ SD. Two-group data for the variance were analyzed by the F test. If the variance showed a significant difference, Student's t test was performed. If the variance did not show a significant difference, Aspin-Welch's test was performed. Multi-group data for the variance were analyzed by Bartlett's test. If the variance differed significantly, Dunnett's test was performed. If the variance did not differ significantly, the Steel test was performed. Values of $p < 0.05$ were considered significant.

Results

Increases in 2-AG in the Early and Late Phases of the Mouse Contact Dermatitis Model

As a skin allergic model to assess the amount of 2-AG, we evaluated a hapten-induced contact dermatitis model involving serial applications of DNFB. At 1 week after

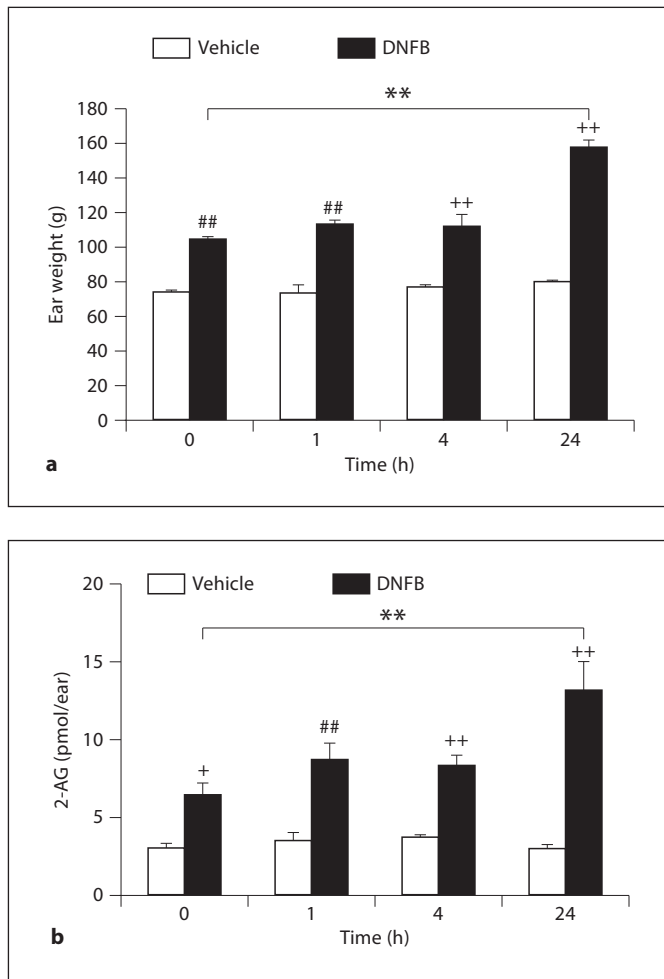


Fig. 1. Increases in ear swelling and 2-AG levels in a mouse contact dermatitis model. The changes in the ear weight (a) and increases in the 2-AG amount (b) before and at 1, 4 and 24 h after the DNFB challenge are shown. Data are expressed as means $\pm$ SD. ^{##} p < 0.01, vs. vehicle control (Student's t test). ⁺⁺ p < 0.01, vs. vehicle control (Aspin-Welch test). ^{**} p < 0.01, vs. before application of DNFB (Student's t test). ⁺ p < 0.05 and ⁺⁺ p < 0.01, vs. vehicle control (Aspin-Welch test).

DNFB priming and three boosts to the ear, the mice were challenged with DNFB to induce allergic dermatitis. The changes in the ear weight at various times after the DNFB challenge are shown in figure 1. A clear increase in the ear weight was observed at 24 h after the challenge. While a common detection method for swelling is to measure the thickness of the ear, the weight of the dissected ear was measured to exactly quantify the 2-AG amounts in the ear in this time course experiment. Incidentally, the correlation between the thickness and weight in ear inflammation was confirmed to show a proportional re-

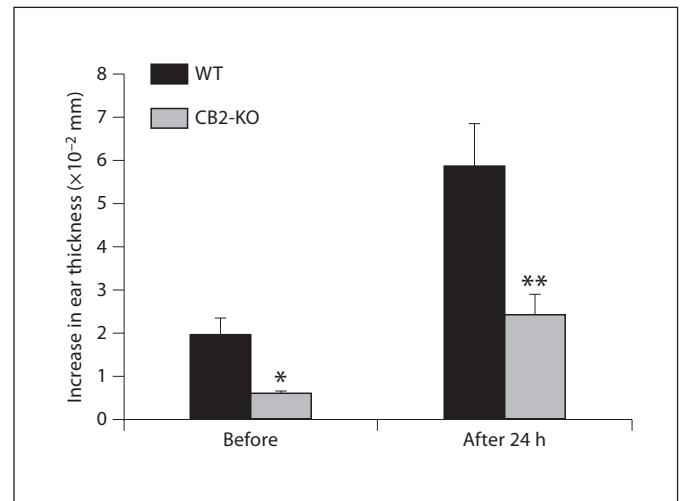


Fig. 2. Suppression of ear dermatitis in CB2-KO mice. After the challenge with DNFB, the ear thickness was measured at 24 h. Data are expressed as means $\pm$ SD. ^{*} p < 0.05 and ^{**} p < 0.01, vs. WT mice (Student's t test).

lationship (data not shown). An increase in the 2-AG amount in the ear was also clearly observed at 24 h, with increases detected at earlier times such as 1 and 4 h after the challenge. Importantly, the amount of 2-AG was increased by up to 5-fold compared with that in vehicle-treated ears. This increase in 2-AG was well correlated with the increase in the ear weight, with the fold-increase in the 2-AG level being higher than that of the ear weight.

Suppression of Ear Dermatitis in CB2-KO Mice

To confirm the involvement of CB2-mediated responses in the DNFB-induced dermatitis model, we employed CB2-KO mice and induced dermatitis using the same procedure. Following the challenge with DNFB, the ear thickness was measured after 24 h (fig. 2). It was found that CB2-KO mice did not accumulate ear thickness after the priming and three boosts of DNFB, and that the swelling induced by the DNFB challenge was markedly reduced but existed in CB2-KO mice. These findings indicate that CB2 and its endogenous ligands contribute not only to enhanced inflammatory responses but also to accumulated aberrant ear thickness.

Increases in 2-AG in a Mouse Pulmonary Bronchitis Model

Next, we investigated how the endogenous CB2 ligand 2-AG increases depending on the progression of inflammatory responses in other models. Since pulmonary

bronchitis features the induction of infiltrating inflammatory cells in the bronchoalveolar space and aberrant changes in the populations of bronchial tissue cells, we set up an OVA-induced pulmonary bronchitis model. Mice that had been primed and boosted once by OVA were challenged with an aerosol of OVA solution, and their BALF was obtained at various time points until 72 h. As shown in figure 3a–d, the infiltration of inflammatory cells composed of granulocytes (neutrophils and eosinophils) and lymphocytes was increased at 48 and 72 h, resulting in an elevation of the total cell number. The amount of 2-AG in BALF was also increased by up to 3-fold compared with BALF of WT mice and mice before the antigen challenge. Such increases in the 2-AG level in BALF were observed at 24 and 48 h, when significant cell infiltrations in BALF were observed and prolonged to 72 h (fig. 3e). These findings demonstrated that 2-AG, as an endogenous CB2 ligand, was increased in BALF after antigen challenge in sensitized mice, and that the numbers of infiltrating inflammatory cells were elevated and correlated with the increase in the amount of 2-AG.

Induction of Gene Expressions of both Inflammatory Cytokines/Chemokines and Growth Factors in Human HL-60 Cells by 2-AG Ether

We further investigated the gene expressions induced by the surrogate CB2 ligand 2-AG ether. Since the human leukemia cell line HL-60 preferentially expresses CB2 but not CB1 at the stage of undifferentiated promyelocytic cells and differentiated granulocytic cells, we used this myeloid lineage of HL-60 cells to examine the gene expression. In addition, previous studies by us and others found that two proinflammatory cytokines, IL-8 and monocyte chemotactic protein (MCP)-1, were induced by CB2 ligands in HL-60 cells [18, 19]. These previous findings in HL-60 cells can be used for reference and comparison in our DNA array experiments to clarify the comprehensive gene expression induced by 2-AG ether. 2-AG ether, a synthetic CB2 ligand with structural similarity to endogenous 2-AG that is resistant to metabolic degradation by hydrolysis, was added to HL-60 cells and the gene expressions were examined at 1 h after the addition by DNA array analysis. Based on the data shown in figures 1–3, we deduced that CB2 has *in vivo* roles not only in inflammatory response exacerbation but also in tissue cell growth, and that CB2-expressing myeloid cells can mediate tissue cell growth. Therefore, we selected the human promyelocytic cell line HL-60 to investigate CB2-mediated gene expression and discover novel molecules that can explain the *in vivo* symptoms.

Table 1 summarizes the fold increases in various kinds of genes categorized as ‘growth factors’, ‘chemokines/cytokines’, ‘transcription factors’ and ‘others’, because the upregulated genes were mainly categorized into genes involved in cell growth, chemotaxis/inflammation and transcriptional regulation. The elevation of IL-8 and MCP-1 gene expressions was identical to the findings in previous studies by us and others [18, 19]. Interestingly, MIP-1 gene expression was much more enhanced than IL-8 gene expression, and other chemokines and TNF- α were also induced by 2-AG ether, suggesting that endogenous CB2 ligands directly activate inflammatory cells and indirectly induce the infiltration of granulocytes and macrophages as well as of lymphocytes, regardless of their CB2 expression levels. Moreover, it was shown for the first time that the growth factor-related gene CTGF was also induced by more than 5-fold, suggesting that connective tissue growth and ECM production are indirectly enhanced through CTGF secretion from inflammatory cells mediated by endogenous CB2 ligands.

Discussion

In this study, we examined the pathophysiological roles of CB2 and its endogenous ligand 2-AG using allergic models. In the mouse contact dermatitis model, the increase in 2-AG was well correlated with the increase in ear weight, with the fold-increase in the 2-AG level being higher than that of the ear weight. In addition, by comparative analyses using CB2-KO and WT mice, it was revealed that the acute and reversible tissue swelling, as well as the accumulation of irreversible tissue thickness, during the disease progression were two of the characteristic responses induced by the CB2 ligand system, and that this system enhanced both symptoms. First, the acute tissue swelling was significantly reduced in CB2-KO mice compared with WT mice, while the swelling still existed in CB2-KO mice. These findings indicate that the CB2 ligand system modulates the ear swelling, although it remains unclear whether the remaining CB1 ligand system contributes the acute tissue swelling in CB2-KO mice. Second, the accumulation of ear thickness was markedly reduced and almost cancelled in CB2-KO mice, indicating that the indirect effects via inflammatory cells expressing CB2 are largely involved in this irreversible process, because CB2 is selectively expressed on inflammatory cells but not on fibroblastoid cells. We observed that the amounts of the endogenous CB2 ligand 2-AG were increased and maintained at elevated levels during the 72-hour observation period, and it is

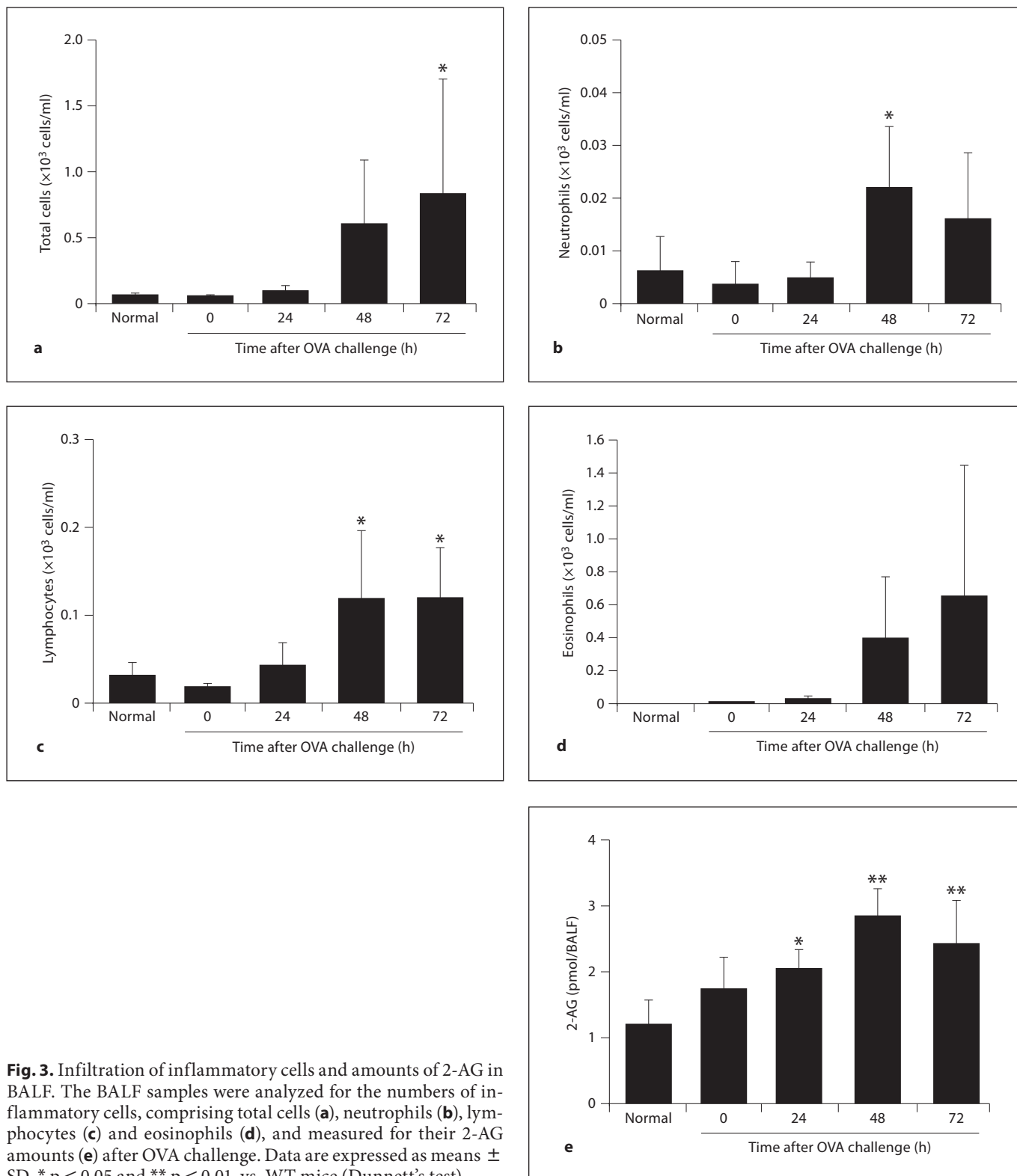


Table 1. Induction of gene expressions of both inflammatory chemokines/cytokines and growth factors in human HL-60 cells by 2-AG ether

Upregulated genes	Fold
Growth factors	
Early growth response 1 (EGR1)	8.45
Early growth response 2 (EGR2)	7.70, 6.54
Connective tissue growth factor (CTGF)	5.48
Human pilot mRNA	4.99
Early growth response 3 (EGR3)	4.76
v-fos	3.33
c-jun protooncogene/v-jun avian sarcoma virus 17 oncogene homolog (JUN)	2.67, 2.54
Transcription factor jun B (jun B)	2.49
Proinflammatory chemokines/cytokines	
MIP-1 α (CCL3)	6.69
IL-8	4.95, 4.38
GOS19-3	3.42
MIP-1 β (CCL4)	3.29
TNF- α	2.83
MCP-1 (CCL2)	2.62
Human activation (Act-2)	2.31
TNF- α -induced protein 3 (TNFAIP3)	2.30
Transcriptional factors	
Zinc finger protein (BCLIIA)	4.31
Helix-loop-helix basic phosphoprotein (GOS8)	3.87
Zinc finger protein 36	3.33, 3.29
Zinc finger protein 177 (ZNF177)	3.16
Basic transcription element-binding protein 1 (BTEB)	2.59
Others	
CD69	3.76, 2.94
Regulator of G-protein signaling 2 (RGS2)	3.16
KIAA0442	3.14
Nuclear receptor subfamily 4, group A (NR4A)	3.00
Dual specificity phosphatase 2 (DUSP2)	2.85, 2.25
Uracil-DNA glycosylase 2 (UNG2)	2.77
Prostaglandin-endoperoxidase synthase (PTGS)	2.56
Immediate early protein	2.41
Cyclin L/ania-6e	2.27
Obscurin	2.15

Data are expressed as fold values. When two probes were used, two fold values are shown.

considered that the transition from the acute inflammatory phase to the recovery and remodeling phase has already occurred on day 3. Therefore, these findings strongly suggest that the elevated 2-AG acts on inflammatory cells via CB2 to indirectly alter the tissue remodeling and recovery process from acute inflammation.

The chemical instability of 2-AG makes it difficult to study the responses mediated by the CB 2-AG system

alone. Therefore, we synthesized the ether form of 2-AG (2-AG ether) to avoid its degradation during the stimulation of human HL-60 cells, which express CB2 but not CB1. If 2-AG itself is used as a ligand for CB2, degraded arachidonic acid and its metabolites are likely to be easily generated and affect the cellular responses. Therefore, our data from the DNA array analysis using 2-AG ether are reliable for detecting molecules induced by the CB2 receptor alone. The DNA array experiments in this study provide the first data for analyzing the minimum biological responses of the direct effects via CB2.

In the DNA microarray analysis, some of the key molecules implicated in cell growth and connective tissue remodeling were identified as upregulated genes induced by 2-AG ether. CTGF is well known as a major factor that induces both growth of fibroblastoid cells and production of ECM [20], and the CTGF gene expression was enhanced by over 5-fold by 2-AG ether (table 1). It has also been reported that CTGF contributes to aberrant remodeling of inflammatory tissues, resulting in fibrotic alterations to these tissues [21]. The present data and previous findings strongly suggest that CTGF gene overexpression in inflammatory cells induced by an elevated 2-AG level contributes to the accumulation of tissue thickness. In addition, the microarray data showed that the gene expressions of EGR1/2/3, which are early growth response genes 1, 2 and 3 [22], were also upregulated by around 5-fold. It has also been reported that 2-AG induces the ERK phosphorylation cascade in the cell growth signaling pathway for fibroblastoid and synovial cells via CB1 [12, 23]. Taken together, the present results and previous findings suggest that endogenously elevated CB ligands can directly induce the growth of tissue cells capable of being responsible for irreversible accumulation of tissue thickness, although we could not clearly observe the accumulation of tissue thickness in an allergic model of CB2-KO mice. The transition from the acute phase of the inflammatory response into the chronic tissue remodeling phase is considered to be a sequential process, and it is likely that 2-AG elevation and its prolongation modulate inflammatory responses at the late phase to induce aberrant remodeling of tissues.

We previously identified IL-8 as a 2-AG-induced chemokine [18]. In this study, MIP-1 α , MIP-1 β and TNF- α were identified as upregulated genes. MIP-1 α is known as a chemokine for infiltration of monocytes, T lymphocytes, B lymphocytes and eosinophils, and TNF- α is a crucial proinflammatory cytokine for enhancing inflammation and chemotaxis [24, 25]. The DNA microarray data are also useful toward an understanding of the disease characteristics observed in the pulmonary inflam-

mation model, which is known to occur via both infiltration of inflammatory cells and compositional alterations in pulmonary epithelial and fibroblastoid cells [26]. It is suggested that the cytokines and chemokines induced by 2-AG elevated in BALF contribute to the infiltration of inflammatory cells, including CB2 non-expressing T lymphocytes, and that the overexpression of CTGF could

lead to the aberrant pulmonary remodeling and hypersensitivity.

Further studies to elucidate the qualitative alterations in the inflammatory responses induced by elevated and sustained 2-AG, especially in the late phase, are necessary to fully understand the aberrant transition from acute inflammation to tissue remodeling.

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