



The cannabinoid receptor-2 is involved in allergic inflammation

Takayuki Mimura ^{a,*}, Yoshifumi Ueda ^b, Yoshihiro Watanabe ^a, Takayuki Sugiura ^c

^a Pharmaceutical Frontier Research Laboratories, Central Pharmaceutical Research Institute, Japan Tobacco Inc., 1-13-2 Fukuura, Kanazawa-ku, Yokohama, Kanagawa 236-0004, Japan

^b Biological/Pharmacological Research Laboratories, Central Pharmaceutical Research Institute, Japan Tobacco Inc., 1-1 Murasaki-cho, Takatsuki, Osaka 569-1125, Japan

^c Faculty of Pharmaceutical Sciences, Teikyo University, 1091-1 Suzarashi, Midori-ku, Sagami-hara, Kanagawa 252-5195, Japan

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ABSTRACT

Aim: To investigate the role of cannabinoid receptor-2 (CB2) in allergic inflammation in CB2 knockout (CB2-KO) mice.

Main methods: The swelling reaction of the pinna to various stimuli was compared between CB2-KO and wild-type (WT) mice in terms of edema and acanthosis.

Key findings: Ear swelling induced by repeated application of 2,4-dinitrofluorobenzene in CB2-KO mice was significantly decreased compared with that in WT mice. In an ovalbumin model, pinna edema was significantly suppressed in CB2-KO mice in comparison with that in WT mice. The contribution of CB2 to edema was investigated in a more extreme dermatitis model using oxazolone. Delayed-type hypersensitivity reactions in this model were also suppressed in CB2-KO mice. In each of these three different allergic dermatitis models, there was a significant decrease in edema and acanthosis in CB2-KO mice compared with WT mice. **Significance:** These results clearly demonstrate that CB2 and its endogenous ligands participate not only in the acute, edematous phase of allergic dermatitis, but also in the chronic irreversible acanthosis reaction.

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Introduction

The major psychoactive constituent of marijuana is Δ^9 -tetrahydrocannabinol (Δ^9 -THC) (Gaoni and Mechoulam, 1964). Δ^9 -THC exhibits a variety of pharmacological activities in vitro and in vivo, where it has been reported to induce an altered perception, euphoria, hallucination and enhanced appetite in humans, while reducing spontaneous motor activity and inducing immobility, analgesia and impairment of short-term memory in experimental animals (Chaperon and Thiébot, 1999; Dewey, 1986; Di Marzo et al., 1998; Klein et al., 1998). Two types of cannabinoid receptors (CB1 and CB2) have been identified (Matsuda et al., 1990; Munro et al., 1993) and these belong to the G-protein-coupled seven-transmembrane (TM) receptor family (Howlett, 1998). CB1 is found predominantly in the brain, with the highest densities in the hippocampus, cerebellum, and striatum (Ameri, 1999). Locations outside the central nervous system have also been indicated, including the testis, uterus, ovary, adrenal gland, heart and lung (Gérard et al., 1991; Wenger et al., 2001; Das et al., 1995; Galiègue et al., 1995). In contrast, CB2 has been reported as being expressed predominantly in various lymphoid organs, such as the spleen, thymus, tonsils, lymph nodes and Peyer's patches (Munro et al., 1993; Lynn and Herkenham, 1994), and a contribution of CB2 to immune cell function has been reported (Miller and Stella, 2008).

Human and mouse CB1 share 96% homology (Chakrabarti et al., 1995), while human and mouse CB2 share 82% homology (Shire et al., 1996a). Overall, there is 66% homology between the mouse CB1 and CB2 and 78% at the TM level (Shire et al., 1996b). Human CB1 and CB2 share 44% overall homology and 68% at the TM level (Munro et al., 1993). Although the homology between CB1 and CB2 is not particularly high, two endogenous ligands (2-arachidonoyl glycerol (2-AG) and anandamide) bind to and stimulate function via both CB1 and CB2 (Maccarrone and Finazzi-Agró, 2003; Oka et al., 2005; Gonsiorek et al., 2000). To clarify the pathological role(s) of CB2 in various diseases, the use of CB2 knockout (CB2-KO) mice as well as CB2-specific synthetic antagonists/inverse agonists has been employed.

Previous reports using CB2-KO mice revealed that CB2 and its endogenous ligands are involved in pain, acute inflammation, sepsis and alcoholic hepatic injury (Yamamoto et al., 2008; Tschöp et al., 2009; Trebicka et al., 2011). In the case of chronic allergic diseases, however, there are conflicting reports of cannabinoid involvement in inflammation. It has been reported that CB2-KO mice experienced pronounced chronic inflammation that was exacerbated by CB2-specific antagonists and alleviated by CB2 agonists (Karsak et al., 2007). In contrast, a CB2-specific antagonist/inverse agonist was found to alleviate chronic inflammation in WT mice (Ueda et al., 2005).

To clarify this inconsistency in the roles of CB2 in chronic allergic inflammation, we investigated several different allergic dermatitis models using CB2-KO mice.

* Corresponding author. Tel.: +81 45 786 7690; fax: +81 45 786 7692.

E-mail address: takayuki.mimura@jt.com (T. Mimura).

Materials and methods

Animals

Female CB2-KO mice on the C57BL/6 background and their age-matched WT littermates were provided by Prof. A. Zimmer, (Bonn University, Bonn, Germany), and breeding and genotyping were performed as previously described (Buckley et al., 2000). They were housed in plastic cages in a room illuminated on a 12-h 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.

Reagents

2,4-Dinitrofluorobenzene (DNFB), oxazolone (OXA) and acetone 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). DNFB and OXA were dissolved in acetone/olive oil (3:1, v/v), and were prepared at final concentrations of 1% and 3%, respectively.

Repeated DNFB-induced ear allergic dermatitis model

WT and CB2-KO mice were sensitized by repeated topical application of DNFB as previously described (Tomimori et al., 2002). Briefly, 20 μL of 1% DNFB was applied to each side of the right and left ears of mice five times at 7-day intervals. Ear thickness was measured in a blinded manner using a thickness gage (Mitutoyo Corporation, Kawasaki, Japan) immediately prior to and 24 h after DNFB challenge. Results are expressed as the increase in ear thickness relative to the value measured immediately before exposure to DNFB.

OVA-induced ear allergic dermatitis model

Immunization was carried out by intraperitoneal injection of 0.5 mL of saline containing 1 μg of OVA and 1 mg of alum in WT and CB2-KO mice on Days 0 and 7. On Day 14, ear thickness was measured in a blinded manner with a thickness gage. Allergic reaction was induced by intradermal injection of 20 μL of saline containing 1 μg of OVA in the left ear; saline was used in the right ear as a control. Ear thickness was measured as over 24 h after intradermal injection.

OXA-induced ear dermatitis model

WT and CB2-KO mice were sensitized by a single epicutaneous application of 100 μL /site of 3% OXA to the shaved abdomen. Seven days later, 20 μL /site of vehicle (acetone:olive oil, 3:1, v/v) was applied to each side of the right ear, and 20 μL /site of 1% OXA solution was applied to each side of the left ear. The increase in ear thickness was measured in a blinded manner with a thickness gage as above.

Repeated OXA-induced ear allergic dermatitis model

To establish a chronic and severe allergic model, repeated application of OXA was conducted. Briefly, mice were sensitized with 3% OXA solution to the shaved abdomen. Seven days later, 10 μL /site of vehicle (acetone:olive oil, 3:1, v/v) was applied to each side of the right ear, and 10 μL /site of 1% OXA solution was applied to each side of the left ear 20 times at 2-day intervals. Ear thickness was measured in a blinded manner with a thickness gage just before OXA challenge.

Statistical analysis

Data are represented as mean $\pm$ S.D. Variation between data from two groups was revealed by the F-test, with post hoc Student's *t*-test or Aspin-Welch test if this analysis revealed significant or non-significant differences in the variance, respectively. Variation in the multiple-group data was revealed by Bartlett's test, with post hoc Dunnett's test or Steel test if the variance was significantly or not significantly different, respectively. A *p*-value of less than 0.05 was considered significant.

Results

Repeated DNFB-induced ear allergic dermatitis model

In this model of pinna swelling, we studied the involvement of CB2 in edema and acanthosis following the repeated application of the hapten antigen, DNFB. Fig. 1 shows the increase in ear thickness measured following each DNFB application.

In WT mice, acanthosis increased following each application, while edema was reinforced whenever DNFB application was repeated. In contrast, the CB2-KO mice exhibited a significant suppression in edema and acanthosis after the third application of DNFB compared with WT mice. These marked differences between WT mice and CB2-KO mice after antigen challenges were sustained until the fifth challenge. Importantly, the acanthosis induced by several antigen challenges did not worsen in CB2-KO mice.

OVA-induced ear allergic dermatitis model

This model was used to study the role of CB2 in edema. The swelling reaction was induced in both WT and CB2-KO mice 24 h after saline or OVA injection. No swelling was observed in either mouse after saline injection, but there was significant swelling seen in both mice following OVA injection (Fig. 2). However, CB2-KO mice showed more than 50% reduction in the degree of edema compared with WT mice, suggesting that CB2 has a role in the swelling reaction induced by sensitized antigen.

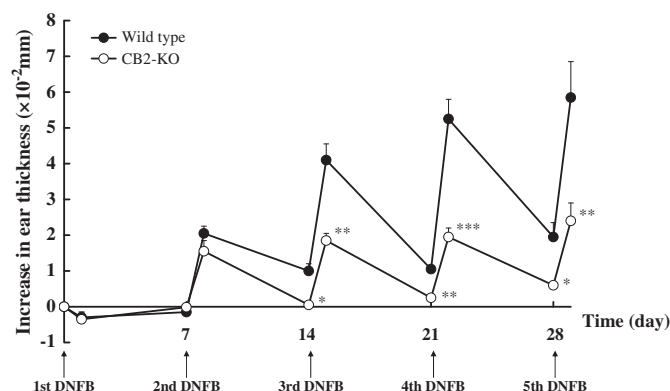


Fig. 1. Time-course study of 2,4-dinitrofluorobenzene (DNFB)-induced ear swelling reactions in WT and CB2-KO mice. Twenty microliters of 1% DNFB in acetone/olive oil (3:1, v/v) was applied to each side of the right and left ears at 7-day intervals. Ear thickness was measured in a blinded manner with a thickness gage immediately before and 24 h after DNFB challenge. Data presented here are expressed as the increase in ear thickness relative to its initial value. Data were normalized by subtracting initial ear thickness from all values, such that ear thickness before the first application was 0. Data are expressed as mean $\pm$ S.D. *, ** and *** denote statistically significant differences ($p < 0.05$, $p < 0.01$ and $p < 0.001$, respectively) compared with wild-type mice, as determined by Student's *t*-test.

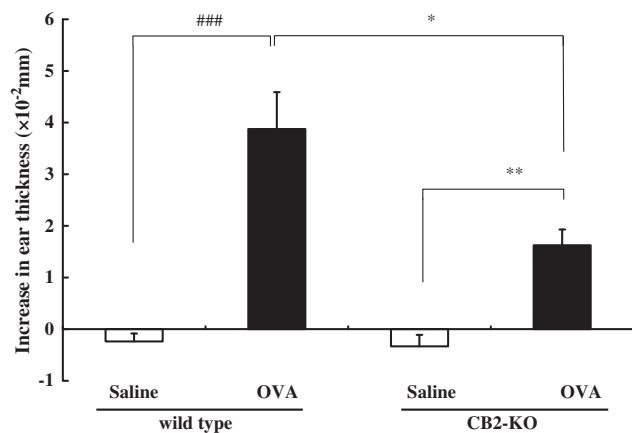


Fig. 2. Ovalbumin (OVA)-induced ear swelling in WT mice and CB2-KO mice. Primary immunization was carried out by intraperitoneal injection of 0.5 mL of saline containing 1 µg of OVA and 1 mg of alum in WT and CB2-KO mice on Days 0 and 7. On Day 14, ear thickness was measured in a blinded manner with a thickness gage. Allergic reaction was induced by intradermal injection of 20 µL of saline containing 1 µg of OVA in the left ear; saline was injected in the right ear as a control. Ear thickness was measured in a blinded manner with a thickness gage 24 h after intradermal injection. Data are expressed as mean ± S.D. ###, $p < 0.001$ vs. saline (Aspin–Welch test). *, $p < 0.05$ vs. wild type mice (Student's *t*-test). **, $p < 0.01$ vs. saline (Student's *t*-test).

OXA-induced ear dermatitis model

In this model, we used OXA, a chemical well known to induce a complex severe dermatitis and that has been previously employed in a study examining the role of CB2 in swelling reactions (Oka et al., 2006). An intense swelling reaction of the pinna was observed in WT mice that peaked at 24 h after OXA challenge and was over 5-fold greater than that induced in the DNFB and OVA challenge models. In this severe dermatitis model, the CB2-KO mice also developed swelling; this edema was significantly less than in WT mice at 6–24 h after antigen application, although it reached parity after 48 h (Fig. 3). These results suggest that CB2 is involved in the acute (<24 h) inflammatory swelling induced by OXA.

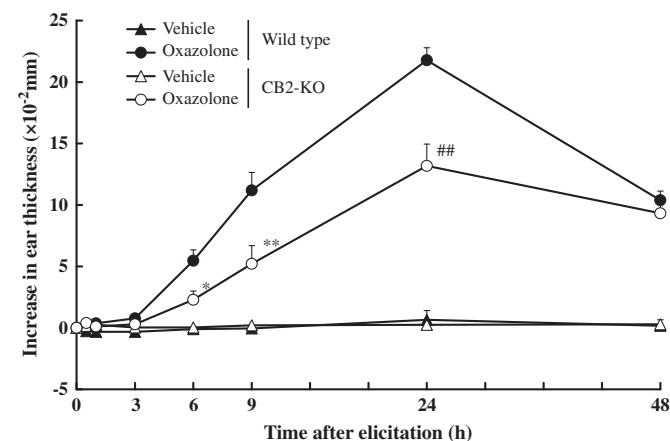


Fig. 3. Time-course study of oxazolone (OXA)-induced ear swelling in WT and CB2-KO mice. WT and CB2-KO mice were sensitized by a single epicutaneous application of 100 µL/site of 3% OXA in acetone/olive oil (3:1, v/v) to the shaved abdomen. Seven days later, 20 µL/site of vehicle (acetone/olive oil = 3:1, v/v) was applied to each side of the right ear, and 20 µL/site of 1% OXA solution was applied to each side of the left ear. Ear thickness was measured in a blinded manner with a thickness gage. Data are expressed as mean ± S.D. *, $p < 0.05$, **, $p < 0.01$ vs. OXA applied on the left ear of wild-type mice (Student's *t*-test). ##, $p < 0.001$ vs. OXA applied on the left ear of wild type mice (Aspin–Welch test).

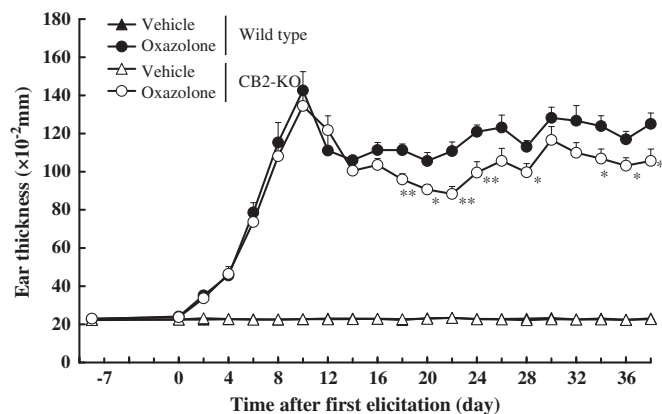


Fig. 4. Time-course study for ear swelling induced by repeated challenge with OXA in WT and CB2-KO mice. WT and CB2-KO mice were sensitized by a single epicutaneous application of 100 µL/site of 3% OXA in acetone/olive oil (3:1, v/v) to the shaved abdomen. Seven days later, 10 µL/site of vehicle (acetone/olive oil = 3:1, v/v) was applied to each side of the right ear, and 10 µL/site of 1% OXA solution was applied to each side of the left ear. This application was repeated a further 20 times at 2-day intervals. Ear thickness was measured in a blinded manner with a thickness gage just prior to OXA challenge. Data are expressed as mean ± S.D. * and ** denote statistically significant differences ($p < 0.05$ and $p < 0.01$, respectively) in left-ear thickness after OXA treatment between wild-type and CB2-KO mice (Student's *t*-test).

Repeated OXA-induced ear allergic dermatitis model

Finally, in a severe allergic dermatitis model, we examined the contribution of CB2 to the chronic inflammation. OXA was repeatedly applied to mouse ears 20 times at 2-day intervals (Fig. 4).

In WT mice, the level of acanthosis increased following each application of OXA and was associated with the rapid formation of a crust, which peaked after six repeated applications. After the crust exfoliated, repeated application of OXA did not induce any re-appearance of the crust formation, but the acanthosis continued to thicken. In the CB2-KO mice, crust formation was also observed during the initial phase and was not significantly different from the WT mice. However, in the latter phase, crust occurrence was significantly suppressed compared with WT mice.

Discussion

The aim of this study was to clarify the contribution of CB2 in allergic dermatitis. When mice were sensitized with repeated topical application of DNFB, inflammatory edema and proliferation of matrix tissues (acanthosis) were observed, events that became more severe as the number of applications increased. CB2-KO mice exhibited a significant suppression of both the acanthosis before antigen application and the edema 24 h after antigen application compared with WT mice. We have recently reported that the endogenous cannabinoid receptor ligand, 2-AG, contributes to this model and activates promyelocytic HL-60 cells to secrete various proinflammatory cytokines and chemokines (Mimura et al., 2012). It was previously reported that 2-AG induces ear edema to release nitric oxide via activation of the p42/44 MAP kinase, and that 2-AG itself has chemotactic activity toward migrating eosinophils and macrophage-like cells (Oka et al., 2004; Oka et al., 2005; Kishimoto et al., 2003). The repeated topical application model used here is characterized by ear swelling with increased eosinophil migration into the tissue (Nagai et al., 1997). Taken together, the results of this and previous reports suggest that CB2 and its endogenous ligands are involved in the edema and acanthosis that characterize allergic dermatitis.

In contrast to these findings, Karsak et al. (2007) reported that edema was slightly exacerbated in CB2-KO mice compared with WT mice after repeated application of DNFB. Moreover, CB1–CB2

double-KO mice had markedly enhanced dermal swelling following DNFB challenge in comparison with CB2-KO mice. However, this group used a low concentration of DNFB to induce a dermal reaction, which may have produced a different effect to the higher concentrations used here. Furthermore, a CB2-specific agonist enhanced ear swelling, and the monocyte chemotactic protein-2 mRNA level in the ear of CB2-KO mice was slightly lower than that in WT mice under their conditions. Therefore, we believe that at higher concentrations of DNFB, CB2 and its endogenous ligand system act to exacerbate dermal reactions. To assess further the role of CB2 in dermal allergic inflammation, we used a protein antigen, OVA, instead of a chemical hapten and examined inflammatory edema in CB2-KO mice. The extent of the edema induced by OVA was equivalent to that seen with three repeated applications of DNFB, and was significantly decreased compared with WT mice. Because the suppression of edema in CB2-KO mice was evident irrespective of the antigen used to promote edema, we conclude that CB2 has a universal role in the inflammatory process.

Furthermore, we used the chemical hapten, OXA, to induce a strong inflammatory reaction, including very severe edema and acanthosis. The edema was significantly decreased in CB2-KO mice at 6–24 h after OXA application, demonstrating that CB2 and its endogenous ligand system contribute to and enhance the inflammatory edema induced by OXA. Moreover, the magnitude of the acanthosis induced by repeated applications of OXA in the CB2-KO mice was significantly reduced compared with WT mice. It has been reported that repeated application of OXA is associated with increased immunoglobulin-E (IgE) in serum and Th2 cytokines at the site of dermatitis (Kitagaki et al., 1995; Tsukumo et al., 2010). Taniguchi et al. (2007) had reported that the pinna acanthosis was accompanied by an increase in the amount of epithelium, over-expression of the nerve growth factor (NGF) in the epithelium, and cuticle expression of the p75-positive fiber that plays a role in epithelial thickening. In addition, Sánchez et al. (2003) reported that NGF is produced following CB agonist stimulation of the PC-3 human prostate cancer cell line. It has been suggested that CB2 plays a critical role in acanthosis formation. Therefore, it is highly likely that CB2 plays significant roles in the promotion and exacerbation of edema and acanthosis. These results are in agreement with our previous reports showing that CB2 antagonists/inverse agonists suppress swelling reactions (Ueda et al., 2005, 2007).

Allergic dermatitis, especially atopic dermatitis (AD), often involves hyperproduction of IgE, which mediates histamine release from mast cells and exacerbates the itch–scratch cycle, an action that can worsen AD (Werfel, 2009). Langerhans cells expressing the high-affinity IgE receptor, FcεRI, are present in greater numbers in the skin of patients with AD than in those without the disorder (Beltrani, 2003). IgE is therefore clearly an important factor in the etiology of AD. Furthermore, Agudelo et al. (2008) reported CB2-mediated immunoglobulin class switching from IgM to IgE in cultures of B lymphocytes, which may be part of the mechanism by which these receptors exacerbate inflammatory reactions. Indeed, we also have evidence that IgE production following repeated application of OXA in the CB2-KO mice was much lower than that in WT mice (data not shown), suggesting that elimination of CB2 limits the inflammatory activity of the IgE pathway.

Conclusion

Our results suggest that CB2 and its endogenous ligand system participate not only in the acute edema phase of allergic dermatitis but also in the chronic phase of acanthosis that characterizes this disease.

Conflict of interest statement

There is no conflict of interest.

Acknowledgment

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