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Cannabinoids Affect Dendritic Cell (DC) Potassium Channel Function and Modulate DC T Cell Stimulatory Capacity

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Cannabinoids affect diverse biological processes, including functions of the immune system. With respect to the immune system, anti-inflammatory and immunosuppressive effects of cannabinoids have been reported. Cannabinoids stimulate G protein-coupled cannabinoid receptors CB1 and CB2. These receptors are found primarily on neurons. However, they are also found on dendritic cells (DC), which are recognized for their critical role in initiating and maintaining immune responses. Therefore, DC are potential targets for cannabinoids. We report in this study that cannabinoids reduced the DC surface expression of MHC class II molecules as well as their capacity to stimulate T cells. In the nervous system, CB1 receptor signaling modulates K⁺ and Ca²⁺ channels. Interestingly, cannabinoid-treated DC also showed altered voltage-gated potassium (Kᵥ) channel function. We speculate that attenuation of Kᵥ channel function via CB1 receptor signaling in DC may represent one mechanism by which cannabinoids alter DC function. The Journal of Immunology, 2008, 181: 3057–3066.

Human and animal cannabinoids belong to the group of endocannabinoids that are cell membrane-derived signaling molecules formed during the metabolism of eicosanoid fatty acids (1). The endocannabinoids, such as anandamide, can activate a group of G protein-coupled cannabinoid receptors, CB1 and CB2 (2). Plant-derived cannabinoids and synthetic structural analogs of cannabinoids referred to as exogenous cannabinoids can also function to activate the specific cannabinoid receptors, normally activated by the endocannabinoids (3). The CB1 and CB2 receptors are found primarily on neurons (4). In the nervous system, CB1 receptor signaling modulates K⁺ and Ca²⁺ channels (5–9). The CB1-mediated modulation of voltage-gated potassium (Kᵥ) channel function can be regulated by both endogenous and exogenous cannabinoids (6, 9). Cannabinoids affect not only the nervous system but also the immune system, and anti-inflammatory effects of cannabinoids have been observed in cannabinoid-based drug therapies (10). The outcome is attenuation of the symptoms and progression of neuroinflammatory disorders and inflammatory bowel diseases. These anti-inflammatory effects are mediated, at least partially, through the binding of cannabinoid receptors and correlate with decreased T cell responses and reduced production of inflammatory mediators (10–12). Although, a wealth of information indicates that cannabinoids have immune suppressive and anti-inflammatory activities, the exact mechanisms of immune modulation remain unknown. Because CB1 receptors and Kᵥ channels are present on lymphocytes, macrophages, and dendritic cells (DC) (13–15), the anti-inflammatory effects of cannabinoids might involve regulation of Kᵥ channels via CB1 receptor signaling in immune cells, including DC.

DC are ubiquitous sentinels of the immune system and participate in regulating both innate and adaptive immunity (16). By possessing a range of cellular receptors, DC respond to microbial and inflammatory stimuli and undergo a process of cellular activation termed maturation (17). Upon maturation, DC enhance their Ag-presenting capacity by changing their surface phenotype involving redistribution of MHC class II (MHC-II) from intracellular compartments to the plasma membrane (18–22). Maturation is also associated with increased cell surface levels of costimulatory molecules and enhanced production of soluble inflammatory mediators (23–26). Consequently, mature DC possess important properties for activating and directing functional differentiation of Ag-specific T cells (27). Regulation of the immunostimulatory capacity of DC is therefore a key step for determining the nature and effectiveness of T cell-mediated immune responses. In addition, various mechanisms may act at distinct levels in fine-tuning DC function to prevent excessive immune responses and the onset of pathophysiological conditions. Targeting such mechanisms may serve as a means for therapeutic modulation of DC function in chronic inflammatory diseases associated with, for example, autoimmunity and transplantation (28).

Attenuation of Kᵥ channel function offers a promising means to downmodulate immune responses (14, 15, 29). As previously reported, DC express functional ion channels (30–32), including Kᵥ channels (13). However, because knowledge about possible means of Kᵥ channel modulation in DC is limited, we initially set out to study whether CB1 receptor activation impacts that process. Our data revealed that Kᵥ channel function in murine DC does undergo changes in response to CB1 receptor signaling. Subsequent studies showed that DC responded to both endogenous and exogenous cannabinoids, and that this...
response involves attenuated $K_v$ channel-mediated outward currents. Interestingly, this cannabinoind-induced attenuation of $K_v$ channel function in DC correlated with a reduced surface expression of MHC-II on DC and rendered DC less potent activators of T cells.

### Materials and Methods

#### Animals

C57BL/6 mice were bred at the Department of Neuroscience, Karolinska Institutet (Stockholm, Sweden). They were used at 5–8 wk of age and were housed under conventional conditions with free access to food and water. All animal care and experimental procedures were approved by the Animal Ethics Committee of Stockholm, Sweden.

#### Chemicals and drugs

Tetraethyl-ammonium (TEA), pertussis toxin (PTX), HEPES, EGTA, and barium chloride were all from Sigma-Aldrich. The endogenous cannabinoid, anandamide (also known as arachidonylethanolamide), the CB1 receptor agonist ACPA (arachidonylecyclopropanamide), a structural analog to anandamide, and the CB1 receptor antagonist AM251 were all from Tocris. Stock solutions of anandamide and ACPA were prepared in Tocrisolve 100 (Tocris) and stored at −20°C. Aliquots prepared in sterile water were diluted to their final concentration with extracellular solution (see below). A stock solution of AM251 was prepared in DMSO (Sigma-Aldrich) and diluted to its final concentration with extracellular solution. In all control experiments, this “vehicle” contained the appropriate concentrations of stock solution diluents, i.e., DMSO or Tocrisolve.

### DC generation and isolation

DC were generated from bone marrow cells cultured as previously described (33). Bone marrow cells were obtained from the femurs and tibias of normal C57BL/6 mice and seeded at $3 \times 10^5$ cells/ml in DMEM (Invitrogen) with glutamax I (Invitrogen), 10% FCS (Invitrogen), 100 U/ml penicillin, and 100 g/ml streptomycin (Invitrogen), supplemented with recombinant murine GM-CSF (10 ng/ml; PeproTech) in the absence of murine IL-4 (10 ng/ml; PeproTech). At day 3 of culture, the medium was gently removed, and fresh medium supplemented with the growth factors was added. After 6 days of culture, floating and lightly adherent cells were collected and seeded at $3 \times 10^5$ cells/ml in new tissue culture plates. On the following day, floating and adherent cells were collected (34), and DC were purified using CD11c microbeads (Miltenyi Biotech). The majority of purified cells had a typical DC morphology and more than 95% of them expressed CD11c. Purified DC were seeded in tissue culture plates at 1–5 $\times 10^5$ cells/ml in medium containing GM-CSF. For the electrophysiologic recording assay, DC were cultured for 1–4 days postpuration. DC were also isolated from spleens of C57BL/6 mice. Spleens were digested in RPMI 1640 supplemented with 0.5 mg/ml collagenase and 0.5 mg/ml DNase (Sigma-Aldrich) for 10 min at 37°C. Then DC were enriched by magnetic cell sorting using CD11c microbeads and a MidiMACS separation column (both from Miltenyi Biotec) following the manufacturer’s protocol. In assays performed to study the effect of $K_v$ channel blocking on DC function, expression of MHC-II and costimulatory molecules, DC were used directly or cultured in the absence or presence of LPS for 16 h before adding the $K_v$ channel blocking reagent TEA and the cannabinoinds ACPA and anandamide for 30 min.

### Electrophysiology

The DC culture medium was replaced with an extracellular solution composed NaCl 140 mM, KCl 5 mM, CaCl$_2$ 1.8 mM, MgCl$_2$ 1 mM, sucrose 10 mM (all from KEBO Laboratory) and HEPES 10 mM. The pH was adjusted to 7.4 with sodium hydroxide and osmolality to 305 mosM with sucrose. In some experiments, calcium chloride was replaced with barium chloride BaCl$_2$. Whole cell patch-clamp recordings (35) were then made from DC using capillary glass tubing (1.5 mm in diameter, GC150–10; Harvard Apparatus) pulled to form tips having a resistance of ~5 MΩ. The pipettes contained an intracellular solution composed of KCl 140 mM, NaCl 4 mM, HEPES 10 mM, EGTA 5 mM, CaCl$_2$ 0.5 mM, MgCl$_2$ 1 mM. The pH was adjusted to 7.4 with potassium hydroxide and osmolality to 300 mosM with sucrose. Control and test solutions were exchanged using a gravity-fed perfusion system with electronically controlled valves (ValveLink 8; AutoMate Scientific). Cells chosen for recording were 20–30 μM in diameter and typically had minimal ruffling or processes; this provided the best condition for obtaining a seal with the patch pipette. An Axopatch 200B (Axon Instruments) was used for recordings. For voltage clamp recordings, series resistance was compensated by 75–85%. Computer software (pClamp8; Axon Instruments) together with a digital interface (Digidata 1320A; Axon Instruments) were used to control the amplifier and data acquisition. All experiments were done at room temperature with voltage-clamp holding potential ($V_{\text{hold}}$) of −70 mV. Voltage steps were then increased by 5-mV increments from voltage-clamp holding potential +10 or +25 mV.

### Immunofluorescence and confocal analysis

For immunofluorescence labeling of the CB1 receptor, DC were grown on tissue culture plates. On the following day, floating and lightly adherent cells were collected and seeded at $3 \times 10^5$ cells/ml in DMEM (In Vitrogen) with glutamax I (In Vitrogen), 10% FCS (In Vitrogen), 100 U/ml penicillin, and 100 g/ml streptomycin (In Vitrogen), supplemented with recombinant murine GM-CSF (10 ng/ml; PeproTech). At day 3 of culture, the medium was gently removed, and fresh medium supplemented with the growth factors was added. After 6 days of culture, floating and lightly adherent cells were collected and seeded at $3 \times 10^5$ cells/ml in new tissue culture plates. On the following day, floating and adherent cells were collected (34), and DC were purified using CD11c microbeads (Miltenyi Biotech). The majority of purified cells had a typical DC morphology and more than 95% of them expressed CD11c. Purified DC were seeded in tissue culture plates at 1–5 $\times 10^5$ cells/ml in medium containing GM-CSF. For the electrophysiologic recording assay, DC were cultured for 1–4 days postpuration. DC were also isolated from spleens of C57BL/6 mice. Spleens were digested in RPMI 1640 supplemented with 0.5 mg/ml collagenase and 0.5 mg/ml DNase (Sigma-Aldrich) for 10 min at 37°C. Then DC were enriched by magnetic cell sorting using CD11c microbeads and a MidiMACS separation column (both from Miltenyi Biotech) following the manufacturer’s protocol. In assays performed to study the effect of $K_v$ channel blocking on DC function, expression of MHC-II and costimulatory molecules, DC were used directly or cultured in the absence or presence of LPS for 16 h before adding the $K_v$ channel blocking reagent TEA and the cannabinoinds ACPA and anandamide for 30 min.

#### FIGURE 1.

Outward ion channels are the predominant voltage-activated channels in murine bone marrow-derived DC. To identify the presence of ion channels, DC were subjected to whole cell patch-clamp analysis. A, Increased voltage steps were applied to DC and the magnitude of outward currents were monitored in control DC (left), in DC for which TEA replaced sodium chloride (middle), or in DC for which TEA replaced sodium chloride followed by extensive washing with control solution (right). B, Increased voltage steps were applied to DC, and the presence of inward as well as outward currents were monitored in control DC (left), in DC for which Ba$^{2+}$ replaced Ca$^{2+}$ (middle), or in cells in which Ba$^{2+}$ replaced Ca$^{2+}$ followed by extensive washing with control solution (right). Voltage-clamp holding potential was −70 mV with steps to +25 mV ($n = 166$ cells).

#### FIGURE 2.

DC express the CB1 receptor. A, DC were stained for the CB1 receptor by using anti-N terminus CB1 receptor Ab and analyzed by immunofluorescence confocal microscopy. B, Lysates of DC and brain tissue were analyzed by Western immunoblotting using the anti-N terminus CB1 receptor Ab. The 87-kDa immunoreactive band corresponds to the CB1 receptor N terminus in mouse brain (left lane) and in DC from duplicate samples (middle and right lanes).
diluted in 1.5% normal goat serum and 0.1% saponin in PBS for 45 min at room temperature. Samples were then incubated with purified Ab mAb (clone 2G9; BD Pharmingen). After washing cells four times in 1.5% of initial current. Holding voltage was −70 mV, with voltage step to +10 mV. Value for number of repeated measures (n) is shown within parentheses or as inset in box, and error bars represent SEM. *p < 0.01, ACPA treatment compared with vehicle control.

For immunofluorescence labeling of MHC-II, DC were spun onto glass slides by cytopsin. Cells were fixed in 4% paraformaldehyde in PBS for 30 min at room temperature. After fixation, samples were blocked and permeabilized in 1.5% normal goat serum and 0.1% saponin in PBS for 45 min at room temperature. As primary reagent, we used rat anti-mouse MHC-II mAb (clone 2G9; BD Pharmingen). After washing cells four times in 1.5% normal goat serum and 0.1% saponin in PBS, specific staining was detected by Alexa Fluor 488 conjugated goat anti-rabbit IgG Ab (Molecular Probes). Confocal microscopy was performed with a Zeiss LSM 510 Meta Axioplan 2 System. Projections were made from five 0.8 –1.0 µm thick optical sections.

Detection of CB1 receptor by Western immunoblotting and PCR
Control or LPS-matured DC were lysed in 250 µl of boiling lysis buffer (28 mM Tris-HCl, 22 mM Tris base, 200 mM DTT, 0.3% SDS; all from Sigma-Aldrich), precipitated in 80% methanol, and the pellets were diluted and boiled in SDS sample buffer. Electrophoresis was performed using

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(9), diluted in PBS containing 2% BSA overnight at 4°C. Specific staining was detected by incubating with Cy3-conjugated donkey anti-rabbit IgG Ab (Jackson ImmunoResearch Laboratories), diluted to 3.75 µg/ml in PBS containing 2% BSA for 2 h at room temperature. The cells were rinsed in PBS between treatments and mounted in glycerol with 2.5% diazabicycloclooctane (Sigma-Aldrich). Confocal microscopy was performed with a Zeiss LSM 510 Meta Axioplan 2 System. Projections were made from five 0.8 –1.0 µm thick optical sections.

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**FIGURE 3.** Activation of the CB1 receptor attenuates Kv channel function in DC. To measure the magnitude of outward currents in response to CB1 receptor activation, DC were subjected to whole cell patch-clamp analysis. A. Increased voltage steps were applied to DC, and the magnitude of outward currents was measured in control DC and DC treated with ACPA, a specific CB1 receptor agonist. The response was measured 10 min after addition of ACPA. B. Effects of ACPA on outward currents in DC compared with control DC over the test voltage step range. The attenuation by ACPA is observed starting at −35 mV and continues throughout the subsequent positive steps. C. The outward current response to a +10 mV voltage step was measured for 30 min in DC treated with ACPA or vehicle. The outward currents were normalized to initial values of individual DC. Data are presented as a percentage of initial current. D. The mean outward current response to a +10 mV voltage step was measured after 3–10 min in DC treated with vehicle or ACPA, as indicated. The response was normalized to initial values and presented as a percentage of initial current. Significant differences were analyzed by comparing responses to ACPA treatment with vehicle control. E. Increased voltage steps were applied to DC, and the magnitude of outward currents were monitored in DC treated with ACPA in combination with the CB1 receptor antagonist, AM251, or DC treated with AM251 only. F. Increased voltage steps were applied to DC and the magnitude of outward current was monitored in DC treated with ACPA together with PTX, an inhibitor of certain G proteins, or DC treated with PTX only. G. The outward current response to a +10 mV voltage step was measured for 10 min in DC treated with vehicle, ACPA, ACPA combined with AM251, or ACPA combined with PTX. The outward currents were normalized to initial values of individual DC. Data are presented as percentage of initial current. Holding voltage was −70 mV, with voltage step to +10 mV. Value for number of repeated measures (n) is shown within parentheses or as inset in box, and error bars represent SEM. *p < 0.01, ACPA treatment compared with vehicle control.
FIGURE 4. The endogenous cannabinoid, anandamide, attenuates K\(_\text{v}\) channel function in DC. To measure the magnitude of outward currents in response to anandamide, DC were subject to whole cell patch-clamp analysis. A, Increased voltage steps were applied to DC and outward currents were monitored in control and DC treated with anandamide. The response was measured 10 min after the addition of anandamide. B, Effect of anandamide on outward currents in DC compared with control DC over the voltage step range. C, The outward current response to a +10 mV voltage step was measured for 30 min in DC treated with anandamide or vehicle. The outward currents were normalized to initial values of individual DC. Data are presented as a percentage of initial current. D, The mean outward current response to a +10 mV voltage step was measured after 3–10 min in DC treated with vehicle or anandamide as indicated. The response was normalized to initial values and presented as a percentage of initial current. Significant differences were analyzed by comparing responses to anandamide treatment with vehicle control. Holding voltage was –70 mV, with voltage step to +10 mV. Values for number of repeated measures (n) is shown within parentheses or as inset in box, and error bars represent SEM. *p < 0.01.
FIGURE 5. Anandamide-induced attenuation of K_v channel function in DC involves CB1 receptor signaling. To study the mechanism of anandamide-induced attenuation of outward currents, DC were subject to whole cell patch-clamp analysis. A, Increased voltage steps were applied to DC and the magnitude of outward currents were monitored in DC treated with anandamide in combination with AM251, or DC treated with AM251 only. B, Increased voltage steps were applied to DC, and the magnitude of outward currents was monitored in DC treated with anandamide together with PTX, or DC treated with PTX only. C, The outward current response to a +10 mV voltage step was measured for 10 min in DC treated with vehicle, anandamide, anandamide together with AM251, or anandamide combined with PTX. The outward currents were normalized to initial values of individual DC, and data are presented as a percentage of initial current. D, The mean outward current response to a +10 mV voltage step was measured after 3–10 min in DC treated with anandamide together with AM251, AM251 only, or anandamide together with PTX as indicated. The response was normalized to initial values and presented as a percentage of initial current. Holding voltage was −70 mV, with voltage step to +10 mV. Value for number of repeated measures (n) is shown within parentheses or as inset in box, and error bars represent SEM.

Results

K_v channels are the predominant voltage-dependent ion channels in bone marrow-derived murine DC

CD11c^+ DC were expanded from bone marrow with GM-CSF and initially tested with whole cell patch-clamp analysis for the presence of ion channels of different types to establish protocols for investigating their regulation and involvement in DC function. In response to voltage steps increased by 5 mV increments from V_hold −70 mV to +25 mV, mainly outward currents were observed (Fig. 1A). Outward currents were highly attenuated by replacing sodium chloride in the extracellular solution with TEA (Fig. 1A), which blocks K_v channels. At a concentration of 145 mM TEA, outward currents were reduced by 100% (Fig. 1A), and when used at 15 mM, outward currents were reduced by 50% (data not shown). The blocking effect of TEA was reversible as extensive washing with the control solution restored the outward currents (Fig. 1A). Furthermore, because the intracellular solution contains a 28-fold higher K^+ concentration than the extracellular solution, but approximately the same Cl^− concentration, the only driving force for outward current would be K^+. In 87% of the cells (n = 166), the predominant voltage-activated currents were outward and, therefore, specific for K^+. The remaining cells either did not respond to voltage steps (6%) or had an inward Ca^{2+} current followed by an outward Ca^{2+} -dependent current, which must also have been K^+ (7%) (Fig. 1B). Thus, K^+ channels were the predominant voltage-dependent ion channels in our bone marrow-derived murine DC.

DC express the CB1 receptor

Next, we wished to determine whether DC express the CB1 receptor, a candidate target for regulating the activity of K_v channels. To detect CB1 receptor expression on DC, we used confocal laser microscopy and observed immunopositive punctuate staining by a specific Ab against the N terminus (Fig. 2A), but not against the C terminus (data not shown), of the CB1 receptor. In Western blots using the same Ab, an 87-kDa immunoreactive band corresponding to CB1 receptor protein was present and aligned with the control CB1 receptor protein from mouse brains (Fig. 2B). Also, expression of the CB1 receptor, which was detected by using PCR, underwent no change after LPS stimulation at neither a protein nor an mRNA level (data not shown). These observations prompted us to perform a more detailed analysis of K_v channel activity in DC in response to CB1 receptor activation.

CB1 receptor signaling attenuates voltage-activated outward K^+ currents in DC

To test the impact of CB1 receptor activation on the K_v channel function in DC, we applied the CB1 receptor agonist ACPA, a structural analog to anandamide, and recorded the effects on voltage-activated responses. ACPA significantly reduced the outward currents in DC (Fig. 3A). The effect of ACPA on outward currents in DC compared with control DC was monitored over the test voltage step range (Fig. 3B). Fig. 3C shows a prolonged attenuation of outward currents in DC for up to 30 min after application of ACPA. In contrast, no such attenuation occurred in control DC.
when only vehicle was used (Fig. 3D). Statistical analysis (ANOVA) followed by the Bonferroni posthoc test for multiple comparisons revealed that ACPA, compared with vehicle, significantly attenuated \( p < 0.01 \) \( K_v \) channel function in DC.

To further test whether the influence of cannabinoids on voltage-activated outward currents was mediated by CB1 receptors in DC, we measured the effect of ACPA on voltage-activated outward \( K^+ \) currents after DC had been treated with AM251, a specific CB1 receptor antagonist. AM251 completely abolished the attenuating action of ACPA on \( K_v \) channel function (Fig. 3, E and G). As previously shown, the CB1 receptor can couple to G proteins (2), including the \( G_{i/o} \) subtypes (36); therefore, we used PTX, an inhibitor of \( G_{i/o} \) proteins, as an additional test for a G protein-mediated block of the ACPA-induced signaling in DC. Pretreatment of DC with PTX abolished the effect of ACPA on \( K_v \) channel function (Fig. 3, F and G). Together, these findings support a role for the cannabinoid signaling system in the regulation of \( K_v \) channel function in DC.

The endogenous cannabinoid, anandamide, attenuates voltage-activated outward \( K^+ \) currents in DC

When the impact of anandamide on DC was monitored over the test voltage step range, as occurred with the exogenous cannabinoid ACPA (Fig. 3B), we found that anandamide also reduced the
voltage-activated outward currents in DC (Fig. 4, A and B). As Fig. 4C shows, a prolonged attenuation of outward currents in DC occurred for up to 30 min after application of anandamide, similar to that with ACPA (Fig. 3C). In contrast, outward currents were not attenuated when vehicle only was used on control DC (Fig. 4C). Application of three different concentrations of anandamide indicated a dose-dependent reduction of outward currents in DC (Fig. 4D). Statistical analysis (ANOVA) followed by the Bonferroni posthoc test for multiple comparisons revealed that 1 and 10 μM anandamide, compared with vehicle significantly reduced \( p < 0.01 \) K\(_V\) channel function in DC.

**Attenuation of outward K\(^+\) currents by anandamide involves CB1 receptor signaling in DC**

To confirm that anandamide’s blockade of K\(_V\)-channel function was CB1 receptor-mediated in DC, we pretreated these DC with the specific CB1 receptor antagonist AM251, which partially inhibited the effect of anandamide on voltage-activated outward K\(^+\) currents (Fig. 5A). In addition, pretreatment of DC with PTX before the addition of anandamide resulted in complete inhibition of outward current attenuation (Fig. 5B). Fig. 5C shows the mean response recorded over 10 min for each of the conditions, including controls (vehicle only). Furthermore, each of the conditions was tested for significant differences using the Bonferroni posthoc test for multiple comparisons. This assessment revealed that treatment with anandamide significantly reduced outward currents \( p < 0.01 \) in DC compared with vehicle, whereas no significant reduction of outward currents was observed in DC treated with anandamide in combinations with PTX or the CB1 receptor antagonist. The mean responses recorded over 3–10 min for each of the conditions in addition to one higher concentration of anandamide (10 μM) are summarized in Fig. 5D. Taken together, our findings demonstrate that both exogenous and endogenous cannabinoids can regulate K\(_V\) channel function in DC by activation of the CB1 receptor. This led us to hypothesize that attenuation of K\(^+\) outward currents may be one mechanism by which cannabinoids modulate DC immune function. To approach this issue directly, we used first the ion channel blocker TEA (see Fig. 1A) and second, the Kv channel-modulating cannabinoids ACPA and anandamide (see Figs. 3 and 4) to attenuate K\(_V\) channels in DC.

**Blocking of K\(_V\) channels alters the immunostimulatory function of DC**

Bone marrow-derived DC were stimulated with LPS followed by blocking of K\(_V\) channels with TEA. LPS-matured DC are characterized by relatively high surface expression of MHC-II (Fig. 6, A and B). However, LPS-matured DC incubated with TEA showed a marked decrease in the surface expression of MHC-II (Fig. 6, A and B). The LPS-stimulated DC exposed to the K\(_V\) channel blocking reagent TEA exhibited a markedly immature phenotype with a
predominantly intracellular location of MHC-II (Fig. 6B). In contrast, blocking of Kv channels had no effect on cell surface expression of the costimulatory molecules CD86 and CD40. These molecules remained up-regulated on LPS-stimulated DC in response to TEA (Fig. 6C and D). This outcome suggests that blocking of Kv channels induces changes in mature DC to selectively redistribute distinct surface molecules important for T cell activation.

Bone marrow-derived DC efficiently stimulated naive T cells in MLRs, particularly after exposure to maturing agents such as LPS (Fig. 6E). In contrast, LPS-stimulated DC treated with the Kv channel blocking reagent TEA were less efficient in stimulating an MLR (Fig. 6F). At a concentration of 15 mM TEA, the T cell-stimulatory capacity of DC was reduced by ~35%, and when used at 45 mM, the degree of stimulation was reduced by almost 75% (Fig. 6F). Similar to LPS-matured bone marrow-derived DC, splenic DC stimulated naive T cells in an MLR (Fig. 6G). In addition, treatment of splenic DC with the Kv channel-blocking reagent TEA reduced the T cell stimulatory capacity of DC in an MLR (Fig. 6G). Collectively, these findings suggest that blocking Kv channels, which reduced voltage-dependent outward currents, altered the Ag-presenting and T cell-stimulatory capacity of LPS-matured DC as well as splenic DC.

Attenuation of Kv channels by cannabinoids affects immunostimulatory function of DC

Next, we tested whether the cannabinoids, which attenuated Kv channels and reduced voltage-dependent outward currents (Figs. 3 and 4), also affect the immune stimulatory characteristics of DC. Splenic DC incubated with ACPA showed a reduction of MHC-II cell surface expression, similar to that observed with TEA. Additionally, primary splenic DC treated with the cannabinoids, ACPA and anandamide, were less efficient in stimulating an MLR as compared with control splenic DC (Fig. 7B). Cannabinoids also reduced the capacity of LPS-matured bone marrow-derived DC to stimulate an MLR (Fig. 7C). Treatment of splenic DC with 10 μM ACPA or 1 μM anandamide reduced the T cell stimulatory capacity of DC to the same degree as treatment with 15 mM TEA (Fig. 7D). To exclude the possibility that treatment of DC with ACPA, anandamide, or TEA had an effect on DC survival, the proportion of dead cells in DC cultures was analyzed. Cultures of bone marrow-derived LPS-matured DC treated with ACPA, anandamide, or TEA showed no accumulation of dead cells as compared with control DC (Fig. 7E). Similar results were observed with splenic DC (data not shown). In addition, trypan blue staining of bone marrow-derived normal or LPS-matured DC, and splenic DC treated with the different reagents revealed no difference in total number of viable cells. In summary, our findings suggest that cannabinoids that attenuate Kv channel function in DC, affects DC in ways leading to impaired ability to stimulate T cells.

Discussion

Because of the widespread interest in cannabinoid-based drugs that have therapeutic potential for the treatment of many human diseases, this study was initiated to extend the limited information on the mechanisms by which cannabinoids influence immunosuppression and anti-inflammatory activities. We first showed that K+ currents are the predominant outward voltage-activated currents in murine bone marrow-derived DC. We then found that selective activation of CB1 receptors in DC, using either exogenous or endogenous cannabinoids, attenuates the voltage-activated K+ currents in a time-dependent manner. This attenuation of Kv channel function in response to the selective CB1 receptor agonist, ACPA, was completely abolished in the presence of a CB1 receptor antagonist. Similarly, uncoupling of G protein signaling by PTX abolished the ACPA-mediated attenuation of Kv channel function. Together these findings demonstrate that cannabinoid-regulated Kv channel function in DC is mediated via CB1 receptor signaling, and not by other noncannabinoid-like receptors (37–40). Importantly, we demonstrated that cannabinoids attenuated Kv channel function in DC, reduce the expression of MHC-II surface molecules, and decrease the capacity to induce T cell proliferation.

Treatment with cannabinoids has been shown to suppress both innate and adaptive immunity and their therapeutic potential is being evaluated on the basis of their anti-inflammatory activities (10). From those studies, cannabinoids evidently have several mechanisms of action for the attenuation of immune-mediated diseases. Studying DC biology reveals numerous steps that provide opportunities for pharmacological manipulation of immune responses. Indeed, substantial evidence indicates that many immune suppressive agents target DC (28). Furthermore, elucidation of the mechanisms underlying cannabinoid-mediated modulation of DC function is likely to facilitate the development of future pharmacotherapies. Our results, showing that both exogenous and endogenous cannabinoids modulate Kv channel function in DC via a CB1 receptor-mediated pathway, depict a novel mechanism by which cannabinoids regulate DC and may contribute to cannabinoid-induced immunosuppression. Given the important role of Kv channels in T cell function (15), the concept that DC are similarly regulated by Kv channel modulation is compelling, particularly in light of the recent interest in DC as targets for immunosuppressive drugs (28). Cannabinoid-mediated regulation of neurons via K+ and Ca2+ channels via synaptic transmission has also been proposed (reviewed in Ref. 41).

Our initial findings revealing the presence of voltage-dependent K+ currents in murine bone marrow-derived DC is consistent with previous findings using murine splenic DC (13) as well as human monocyte-derived DC (42). Ion currents through K+ channels determine the resting membrane potential that prevents depolarization, thus regulating Ca2+ influx and the many functions that involve Ca2+ signaling, including production and release of inflammatory mediators as well as cell proliferation and differentiation (43). Although, Kv channel function has been implicated as a regulator of DC maturation and T cell stimulatory capacity (42), the mechanism is ill defined. Cannabinoids act at two distinct types of G protein-coupled receptors, CB1 (44) and CB2 (45). CB1 receptor is highly expressed in the CNS, but is also found in some peripheral tissues, whereas CB2 receptor is found mainly outside the CNS, particularly in association with the immune system. At an intracellular level, activation of both CB1 and CB2 receptors alters cAMP levels by inhibiting stimulus-induced adenylyl cyclase (3, 46, 47). Because human DC express the cannabinoid receptors CB1 and CB2, as determined by mRNA expression (48), these receptors are potential targets for manipulation when one wishes to modulate Kv channels and thereby direct DC function. Regulation of Kv channels by cannabinoids is intriguing because DC function is inhibited by the same immunosuppressive and anti-inflammatory drugs, i.e., FK-506, rapamycin, and cyclosporine (28) that inhibit Kv channels in lymphocytes (15). Thus, drugs that regulate Kv channels can modulate DC function toward therapeutic immunosuppression. Our finding that Kv channels couple through CB1 receptor is consistent with the fact that K+ and Ca2+ channels are often modulated by activation of CB1 receptor in neurons. Therefore, our observations indicate that, by modulating functions of Kv channels, cannabinoids may modulate DC function in a less destructive way than the recently described NF-κB-dependent apoptosis in murine DC triggered by activation of cannabinoid receptors (49). Importantly, Do and coworkers (49)
reported that a simultaneous activation of CB1 and CB2 receptors or a relatively high concentration of anandamide (20 μM) was required to induce cannabinoid-mediated apoptosis in DC.

Anandamide also acts on receptor types other than cannabinoid receptors (37). For instance, Sancho and coworkers (40) showed that anandamide can inhibit NF-κB in a cannabinoid receptor-independent manner in 5.1 cells (Jurkat T lymphocyte-derived clone) and A549 cells, which do not express the anandamide-sensitive Transient Receptor Potential Vanilloid type 1 channel (39). Furthermore, anandamide inhibits voltage-gated 1.2 K⁺ channels in brain slices, N-type Ca²⁺ channels, and G protein-coupled inwardly rectifying K⁺ channels in mammalian neurons through a receptor-independent, PTX-insensitive mechanism (6, 38). Our finding that the attenuation of voltage-gated K⁺ currents by anandamide was abolished by PTX blockade of G protein signaling (Fig. 5) supports the likelihood that a G protein-coupled pathway in DC is the primary instigator of this effect on the K⁺ channels. However, activation of the CB2 receptor had no effect on the K⁺ current response (data not shown), and the CB1 receptor antagonist AM251 substantially, but not completely, blocked the anandamide-induced attenuation of voltage-gated K⁺ currents in DC (Fig. 5). Future studies, such as screening for noncannabinoid-like receptors on DC, may identify additional target receptors responsible for the PTX-sensitive non-CB1 receptor-mediated modulation of K⁺ channel function in DC stimulated with anandamide.

Voltage-gated K⁺ channels are potential targets for immunomodulation because they are present on lymphocytes, macrophages, and DC (13–15, 29, 42). In T lymphocytes, K⁺, 1.3 channels are inactivated by hypoxia (50) and inhibited by the immunosuppressors cyclosporine, rapamycin, and FK-506 (15). Inhibition of these channels reduces T cell proliferation and activation and redirects cytokytic activity and cytokine production (51). Previously the blocking of K⁺ channels in human monocyte-derived DC suppressed LPS-induced up-regulation of DC markers of maturation, i.e., the costimulatory molecules CD83, CD80, and CD86, and the proinflammatory cytokine IL-12 (42). In the current study, we observed that blocking K⁺ channels in LPS-matured DC reduced the cell surface expression of MHC-II molecules and decreased the capacity of DC to induce T cell proliferation. This finding along with the reported reduction of LPS-stimulated up-regulation of maturation markers in human blood-derived DC caused by blocking K⁺ channels (42) add to the number of targets on which K⁺ channel inhibitors, such as exogenous and endogenous cannabinoids, can exert immunosuppressive effects.

Recently, it was found that anandamide is capable of providing feedback to control activated microglia and promote neuroprotection in the CNS (52). Interestingly, peripheral neurons also express anandamide, and areas of direct communication between neurons and immune cells lie in both primary (thymus and bone marrow) and secondary (spleen, tonsils, lymph nodes and Pey-er’s patches) tissues of the immune system, as well as airway epithelium and skin (53). Functionally, neurotransmitters including catecholamines and acetylcholine as well as neuropeptides including calcitonin gene-related peptide, vasoactive intestinal peptide, somatostatin, substance P, and pre-opiomelanocortin-derived peptides have been shown to modulate immune and inflammatory responses (54). Furthermore, evidence indicates that direct innervations may control immune responses (55), and the term “neuro-immunological synapse” has been proposed for contacts between neurons and APCs (56). Langerhans cells, a subtype of DC that were originally thought to originate in the nervous system because of their close contacts with nerve fibers (57), have recently been linked closely to calcitonin gene-related peptide/substance P-containing fibers in the skin (presumably nociceptive neurons) of humans (58–60), primates (58), and rodents (58, 61) as well as in the viscera (62). These same calcitonin gene-related peptide/substance P-containing primary afferent fibers express and release the immunoregulatory endocannabinoids. Specifically, Aihluwalia et al. (63) showed that stimulation of capsaicin-sensitive primary sensory neurons induces release of anandamide. Our present finding that cannabinoids can modulate the activity of voltage-dependent K⁺ currents in DC, therefore, tallies with the hypothesis that neuro-immunological interactions may occur at the level of the nerve fiber-immune cell interface.

In conclusion, our results show that the K⁺ channel function in DC can be modulated by both exogenous and endogenous cannabinoids through a CB1 receptor in a PTX-sensitive manner. This discovery presents a mechanism by which cannabinoids can regulate DC and may contribute to cannabinoid-induced immunosuppression. Given the important role of K⁺ channels in T cell function, the concept that DC are similarly regulated by K⁺ channel modulation is interesting in the light of DC as targets for immunosuppressive drugs (28). These results also indicate a potential mechanism by which peripheral neurons may influence the function of DC through release of the endogenous cannabinoid, anandamide. The down-modulation of MHC-II on DC observed in response to blocking of K⁺ channels may explain some of the immunosuppressive effects mediated by cannabinoids. Because CB1 receptor signaling attenuates K⁺ channel function in DC, the CB1 receptor can be a potential target to regulate DC function, preventing DC-mediated inflammation and inducing beneficial immunosuppression.

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Disclosures
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