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The nonpsychoactive component of marijuana cannabidiol modulates chemotaxis and IL-10 and IL-12 production of murine macrophages both in vivo and in vitro

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Abstract

Cannabidiol is the main nonpsychoactive component of marijuana. We examined the ability of in vivo and in vitro cannabidiol to interfere with the production of interleukin (IL)-12 and IL-10 by murine macrophages and to modulate macrophage chemotaxis.

Cannabidiol added in vitro to peritoneal macrophages significantly increased IL-12 and decreased IL-10 production. The CB1 and CB2 receptor antagonists prevented this modulation. Macrophages from animals treated with cannabidiol at the dose of 30 mg kg^{-1} either orally or i.p. produced higher levels of IL-12 and lower levels of IL-10 in comparison to controls, and the CB receptor antagonists did not prevent these effects. Cannabidiol dose-dependently decreased fMLP-induced chemotaxis of macrophages, and the CB2 receptor antagonist prevented this decrease.

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1. Introduction

Marijuana contains approximately 80 constituents, termed cannabinoids. Δ^9-Tetrahydrocannabinol (THC) is the major psychoactive components, whereas cannabidiol is considered to be the main nonpsychoactive compound. A wide literature describes the immunomodulating properties of Δ^9THC and of synthetic analogues, such as CP 55940, both in vivo and in vitro on many different immune cell types (Cabral and Dove-Pettit, 1998; Massi et al., 1998, 2000; Patrini et al., 1997; Klein et al., 1998). Only recently, the attention has focussed on the immune effects of cannabidiol, since its lack of psychoactive effects and low toxicity make it a good candidate as therapeutic agent (Malfait et al., 2000; Srivastava et al., 1998). Moreover, even if it is possible that this compound will never become a single therapeutic agent, cannabidiol has a major role in the herbal preparation known as "Medical Cannabis", which is increasingly used in some countries (Straus, 2000).

Macrophages have been shown to be an important target for cannabinoid effects (Cabral and Dove-Pettit, 1998). They play a central role in both innate and adaptive immunity. They are fundamental cells of the innate immune response and their ability to be chemotactically attracted to the site of initial microbial invasion or to an inflammatory focus is crucial for the full activation of the immune response that follows (Schiffmann, 1982; Wahl, 1981). Moreover, monocytes/macrophages are the main producers of IL-12, the critical factor driving the development of T helper (Th)-1 cells (Trincheri, 1995; Mosman and Sad, 1996), linked to cellular immune responses and tissue injury. Conversely, Th2 cells, responsible of humoral responses and allergy (Mosman and

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Sad, 1996), are stimulated by the T lymphocyte cytokine IL-4 and by IL-10, produced by T lymphocytes as well as by monocytes/macrophages (Moore et al., 1993).

Therefore, on considering the importance of macrophage in orchestrating all the aspects of the immune response, we evaluated the effect of cannabidiol administered in vitro and in vivo on formyl-met-leu-phe (fMLP)-induced macrophage chemotaxis and on IL-12 and IL-10 production.

Two receptors for cannabinoids have been so far characterised and cloned, i.e. the CB1 and CB2 receptors (Galiegue et al., 1995). Although it is generally accepted that cannabinoids exert their immune effects mainly through the CB2 receptors that are present on different immune cell types (Kaminski et al., 1992; Kaminski, 1998), including macrophages (Carlisle et al., 2002), it has been suggested that cannabidiol does not bind with high affinity this receptor, and the existence of a third putative receptor has been postulated (Walter et al., 2003; Pertwee, 1999). Therefore we checked the effect of the administration of the specific CB1 or CB2 receptor antagonists (Compton et al., 1996; Rinaldi Carmona et al., 1994, 1998) in order to ascertain whether these receptors are involved in the modulation of macrophage physiology exerted by cannabidiol.

2. Materials and methods

2.1. Drugs

The chemotactic peptide fMLP (Sigma, St Louis, MO) (Prossnitz and Ye, 1997) was stored as a stock solution of 10^{-3} M in dimethyl sulfoxide (DMSO, Sigma) at −80 °C and diluted in RPMI, just prior to assay.

For the in vitro experiments, cannabidiol (GW Pharmaceuticals, UK), was stored as stock solution of 10^{-2} M in DMSO, while SR141716A and SR144528 (generous gifts of Dr. F. Barth, Sanofi Recherche Synthelabo, Montpellier, France) were prepared in DMSO at the concentration of 2×10^{-2} M. All substances were further diluted in medium, such as the final percentage of DMSO in the wells was always below 0.01%. As vehicle control, we used medium added with 0.01% DMSO.

In the vivo experiments, cannabidiol, the CB1 receptor antagonist SR141716A and the CB2 receptor antagonist SR144528 were dissolved in ethanol/cremophor/saline (1:1:18) and administered in a volume of 0.2 ml/20 g body weight. Control animals were treated with the same volume of vehicle.

2.2. Animals

Swiss male mice, 18–20 g body weight (Charles River, Calco, Italy), were used in the study. Animals were kept on a 12-h light–dark cycle with water and food ad libitum and were housed six mice to a cage.

2.3. Harvest of elicited peritoneal macrophages

In all experiments, mice were inoculated intraperitoneally (i.p.) with 2 ml of 3% Brewer's thioglycollate medium (Difco, Detroit, MI, USA) for macrophage elicitation. Peritoneal exudate cells (PEC) were harvested in cold RPMI-1640 medium (Sigma) plus 10% of foetal calf serum (FCS) (GIBCO BRL, Life Technology, Italy) 3 days after elicitation.

For the in vitro experiments PEC obtained from 10 mice were pooled, while in the in vivo experiments the macrophages obtained by the single mice (eight animals per experimental group) were used.

Viability of cells was checked by the trypan blue exclusion test, and then PEC were resuspended in RPMI plus 10% FCS at 1×10^{6}/ml and 1 ml aliquots dispensed into wells of a 24-well culture plate. Isolation and purification of macrophages were carried out by adherence to culture plates. After a period of 2 h, nonadherent cells were removed with the medium and adherent cells washed twice with warm RPMI plus 10% FCS (Limiroli et al., 2002).

2.3.1. Study of cytokines

2.3.1.1. Treatments. In the in vitro experiments, cannabidiol was added to macrophages cultures at the concentrations of 5×10^{-9}, 5×10^{-8}, 5×10^{-7}, 1×10^{-6} and 5×10^{-6} M. In the antagonist experiments, cannabidiol was used at the concentration of 5×10^{-8} and the CB1 receptor antagonist SR141716A and the CB2 receptor antagonist SR144528 were added together with cannabidiol or alone at the concentrations of 10^{-7} and 10^{-6} M. In all the in vitro experiments, all wells, including control wells, contained a percentage of DMSO minor/equal to 0.01%.

In the in vivo experiments, peritoneal macrophages were elicited with thyoglicollate, and in the third day after thyoglicollate animals were treated either p.o. or intraperitoneally with 15 and 30 mg kg^{-1} of cannabidiol and killed 1 h later for the collection of macrophages. This time point was chosen on the basis of previous experiments showing that 1 h after p.o. cannabidiol treatment the effect of the drug on other immune responses was maximal (Massi et al., 2002).

The CB1 receptor antagonist SR141716A and the CB2 receptor antagonist SR144528 were administered p.o. 1 h before oral cannabidiol (30 mg kg^{-1}) at the dose of 10 mg kg^{-1}. The doses of the antagonists were chosen on the basis of our previous work showing their ability to counteract the effect of other cannabis constituents on macrophages (Sacerdote et al., 2001a). Control animals were treated p.o. with the same volume of vehicle.

2.3.1.2. Cytokine production. Adherent macrophages, obtained either from naive animals (in vitro studies) or from treated animals (in vivo studies) were primed with 1 μg/ml of lipopolysaccaride (LPS) (Sigma) for IL-10

production or with 1 μg ml^{-1} LPS and 50 U ml^{-1} interferon (IFN-γ) (Pharmingen, San Diego, CA) for IL-12 stimulation. The different stimuli were added to the macrophage cultures in a final volume of 1 ml/well of RPMI plus 10% FCS, 1% glutamine (Sigma), 2% penicillin/streptomycin solution (Sigma), 0.1% 2-mercaptoethanol (Sigma) (complete medium). The plates were incubated at 37 °C and 5% CO_2, supernatants were collected after 24 h in culture and stored frozen at −80 °C for cytokine analysis (Limiroli et al., 2002).

2.3.1.3. Cytokine ELISA. The levels of IL-12 p70 protein were determined by enzyme-linked immunosorbent assay (ELISA) protocol as standardised by Pharmingen. The anti-IL-12 capture monoclonal antibody (mAb) (9 μg ml^{-1}) was absorbed on a polystyrene 96-well plate and the IL-12 present in the sample was bound to the antibody-coated wells. The biotinylated anti-IL-12 detecting mAb (0.25 μg ml^{-1}) was added to bind the IL-12 captured by the first antibody. After washing, avidin–peroxidase (Sigma) was added to the wells to detect the biotinylated detecting antibody and, finally, 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS, Sigma) substrate was added. A coloured product was formed in proportion to the amount of IL-12 present in the sample that was measured at optical density 405 nm. The amount of cytokine in each supernatant was extrapolated from the standard curve. The standards were recombinant cytokine curves generated in doubling dilutions from 30 to 4000 pg ml^{-1}.

IL-10 production was measured with the same ELISA protocol except for the use of anti-IL-10 capture mAb at 2 μg ml^{-1}, biotinylated anti-IL-10 detecting mAb at 0.5 μg ml^{-1} and a standard curve ranging from 15 to 2000 pg ml^{-1} (all mAbs and recombinant cytokines were from Pharmingen) (Sacerdote et al., 2000).

2.3.1.4. Chemotaxis. Peritoneal macrophages were collected as described above and pooled. Cells and chemoattractant substances were suspended in RPMI 1640+BSA 1%.

Chemotaxis was measured using a Boyden modified 48-well microchemotaxis chamber, in which the upper and the lower compartments were separated by a polycarbonate filter (Biomap, Agrate Brianza, Italy), with a pore diameter of 5 μm. Cells (2×10^5 cells/ml, 10×10^3 macrophages/well) were placed in the upper chamber, and aliquots of either medium (in order to evaluate spontaneous mobility) or of the chemoattractant fMLP (in order to evaluate chemotaxis) were added in the lower chamber. The chambers were incubated for 90 min at 37 °C, in an atmosphere of 5% CO_2 and then the migrated cells that adhered to the distal part of the filters were fixed and stained. Migrated cells were quantitated by microscopically counting random fields by a scorer that was blind to experimental conditions (Sacerdote et al., 2001a).

Cannabidiol at concentrations ranging from 10^{-5} to 10^{-9} M was added to the lower chemotaxis chamber without or together with the fixed concentration of fMLP 10^{-8} M.

In order to check the involvement of either CB1 or CB2 receptor, experiments with the CBR antagonists were performed.

CBR antagonists were added in the lower chamber at the concentrations of 10^{-7} and 10^{-6} M together with fMLP 10^{-8} M, alone or in combination with cannabidiol 10^{-6} M. In all wells, a similar amount of DMSO (minor/equal to 0.01%) was present.

In a different experiment, macrophages obtained from mice were pre-incubated in polypropylene tubes for 1 h at 37 °C, in an atmosphere of 5% CO_2, in RPMI 1640 (2×10^6 cells/ml) in the presence of medium only, cannabidiol (10^{-6} M), and cannabidiol+CBR antagonists at the concentrations of 10^{-6} M. At the end of pre-incubation, the chemotactic activity of the cells was evaluated in the Boyden chamber in the presence of 10^{-8} M fMLP.

Results are expressed as % inhibition:

$$100 - [(\text{fMLP cells} + \text{C}/\text{fMLP cells}) \times 100)];$$

where fMLP cells represent the number of cells migrated in the presence of fMLP alone, and fMLP cells+C, the number

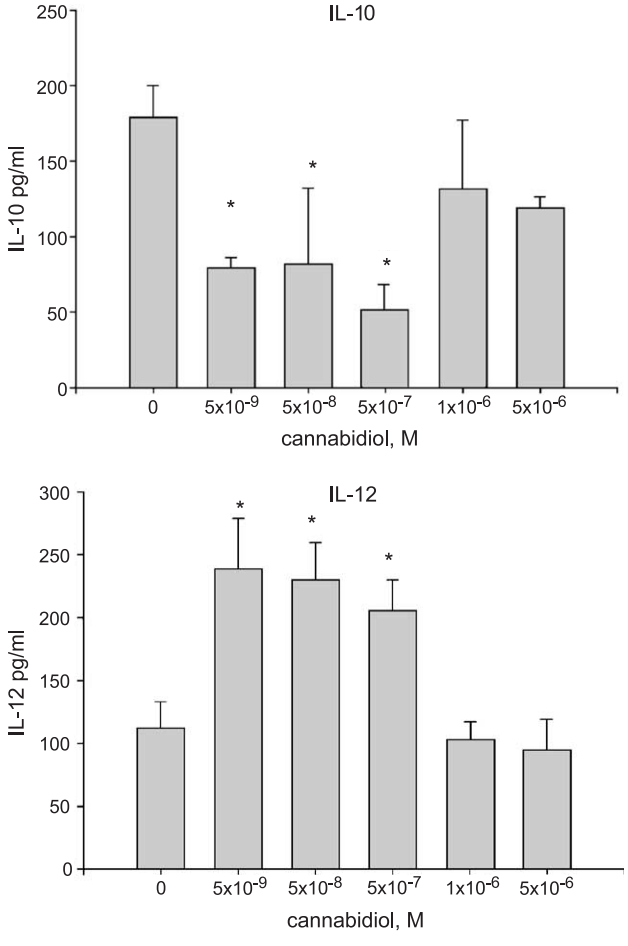

Fig. 1. Effect of the addition in vitro of cannabidiol on IL-10 (upper panel) and IL-12 (lower panel) production by peritoneal macrophages. Cannabidiol was added at the reported concentrations together with 1 μg ml^{-1} of LPS (for IL-10) and 1 μg ml^{-1} LPS+50 U ml^{-1} INF-γ (for IL-12). Media were collected 24 h later for cytokine evaluation. Values are means±S.D. of four replications. Each experiment was repeated at least three times, with similar results. *p<0.05 vs. control (0).

of cells migrated in the presence of fMLP and of the various cannabinoid compounds.

Background migration, i.e. the number of cells migrated in the presence of vehicle alone, was subtracted from all data.

2.3.2. Statistical analysis

Significant differences were assessed by one-way ANOVA, followed by Bonferroni's *t*-test for multiple comparisons.

3. Results

3.1. Effect of cannabidiol on cytokines

3.1.1. In vitro treatment

Cannabidiol was added in vitro for 24 h to macrophage cultures in the presence or absence of LPS for the stimulation of IL-10 production and of LPS+IFN-γ for IL-12. Unstimulated macrophages did not produce detectable levels of IL-12 or IL-10 (data not shown).

LPS and LPS+IFN-γ stimulation induced a significant production of IL-10 and IL-12, respectively. IL-10 levels in the culture supernatants were significantly lower than vehicle-treated cultures when cannabidiol was added at the concentrations of 5×10^{-9}, 5×10^{-8} and 5×10^{-7} M. At the higher concentrations of 1×10^{-6} and 5×10^{-6} M, the effect disappeared and no difference with control was present (Fig. 1, upper panel).

As shown in the lower panel of Fig. 1, the effect of cannabidiol on IL-12 production appeared to be the opposite. In fact, the levels of this cytokine were significantly increased at cannabidiol concentrations of 5×10^{-9}, 5×10^{-8} and 5×10^{-7} M. However, at the highest cannabidiol concentrations, the levels of IL-12 were not different in comparison to vehicle-treated culture.

In order to evaluate whether the observed effects were mediated by the activation of CB1 or CB2 receptors, the ability of the CB1 receptor antagonist SR141716A and the CB2 receptor antagonist SR144528 to prevent the cannabidiol effects on macrophage cytokines was studied. As reported in Fig. 2, when the CB1 (panel A) and CB2 (panel B) receptor antagonists were added at the concentrations of 10^{-7} M together with cannabidiol (5×10^{-8} M), the effect of cannabidiol on IL-10 was partially prevented, while the inhibition of cannabidiol effect was complete with the antagonists at the concentration of 10^{-6} M. Also, the

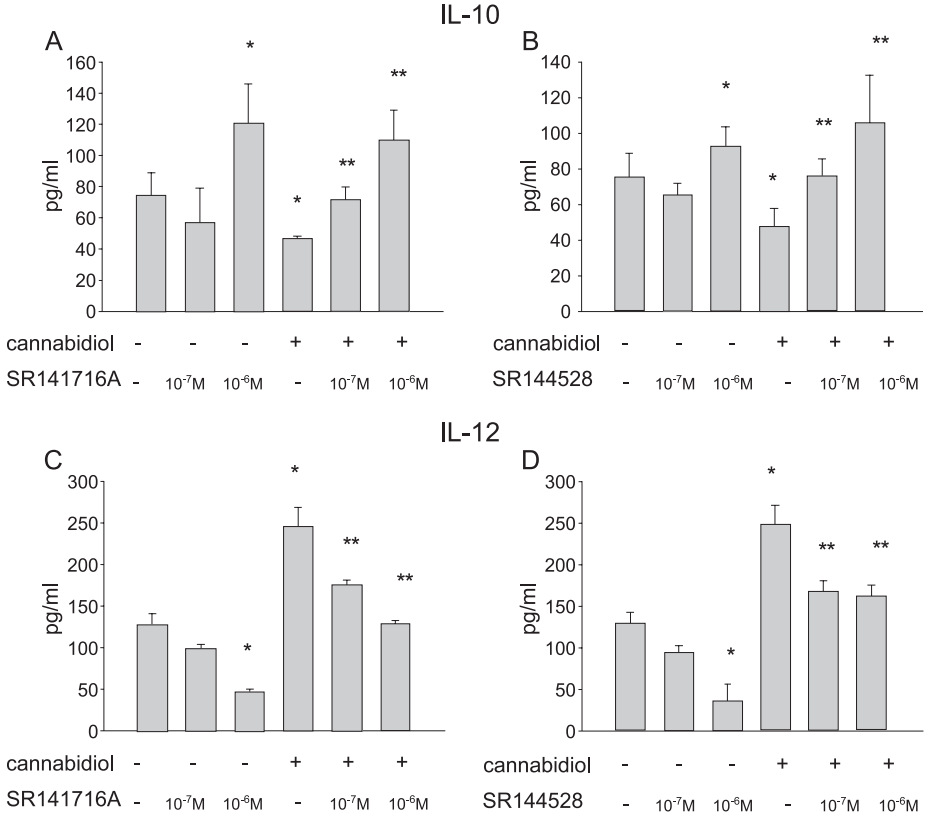

Fig. 2. Effect of cannabinoid antagonists on the modulation of macrophage IL-10 (panels A and B) and IL-12 (panels C and D) induced by cannabidiol in vitro. The CB1 receptor antagonist SR171416A (panels A and C) or the CB2 receptor antagonist SR144528 (panels B and D) were added in vitro at the concentrations of 10^{-6} and 10^{-7} M with or without cannabidiol at the optimal concentration of 5×10^{-8} M in the presence of 1 μg ml^{-1} of LPS (for IL-10) and 1 μg ml^{-1} LPS+50 U ml^{-1} INF-γ (for IL-12). Media were collected 24 h later for cytokine evaluation. Values are means±S.D. of four replications. Each experiment was repeated at least three times, with similar results. *p<0.05 vs. control (0); **p<0.05 vs. cannabidiol.

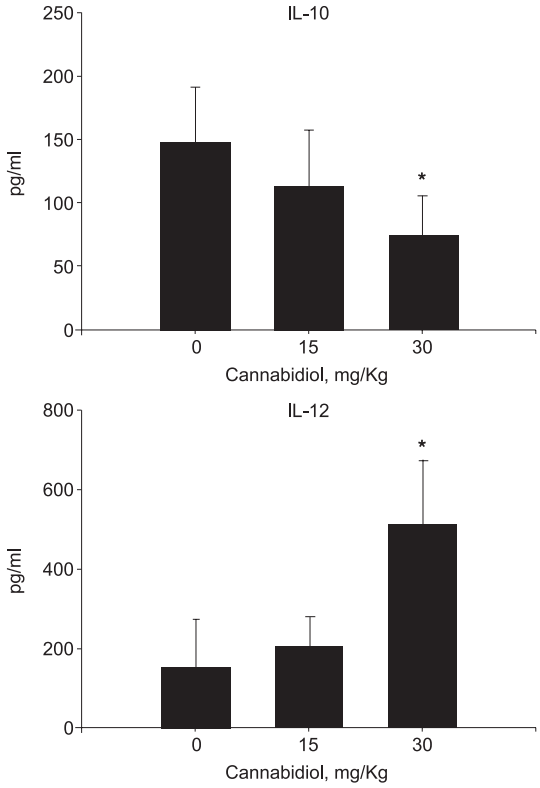

Fig. 3. Effect of the oral administration of cannabidiol on the production of IL-10 (upper panel) and IL-12 (lower panel) by peritoneal macrophages. Mouse peritoneal macrophages were collected 1 h after the administration of cannabidiol at the doses of 10 and 30 mg kg^{-1} p.o. Thereafter, cells were stimulated in vitro with 1 μg ml^{-1} of LPS (for IL-10) and 1 μg ml^{-1} LPS+50 U ml^{-1} INF-γ (for IL-12). Media were collected 24 h later for cytokine evaluation. Values are means±S.D. of eight animals. Each experiment was repeated at least three times, with similar results. *$p<0.05$ vs. vehicle-treated control (0).

cannabidiol-induced increase of IL-12 was prevented by the administrations of the CB1 (panel C) and CB2 (panel D) receptor antagonists at the concentrations of 10^{-7} and 10^{-6} M. However, in the same figure, it can be observed that at the highest dose of 10^{-6} M, both the antagonists possess an intrinsic activity, as they modulate cytokine production in a way opposite to the one induced by cannabidiol. As reported in the panels A and B of Fig. 2, in fact, SR141716A and SR144528 significantly increased IL-10 production, while they decreased that of IL-12 (panels C and D).

3.1.2. In vivo treatment

One hour after the oral administration of 15 and 30 mg kg^{-1} of cannabidiol, previously elicited peritoneal macrophages were collected and incubated for 24 h in the presence of stimuli for the production of cytokines. We chose to examine the effect on cytokines 1 h after cannabidiol treatment, since we previously observed that at this time point cannabidiol was able to modulate other immune responses (Massi et al., 2002).

Consistently with the results obtained in vitro, as reported in Fig. 3, the administration of cannabidiol in vivo decreased the production of IL-10 (upper panel) while it increased the levels of IL-12 (lower panel). The effect reached statistical significance only with 30 mg kg^{-1} of the drug.

Similar results were obtained when cannabidiol was administered by intraperitoneal route at the dose of 15 and 30 mg kg^{-1} (data not shown).

To determine whether CB1 or CB2 receptors were involved in the modulation of IL-10 and IL-12 exerted by cannabidiol, animals were pretreated with the CB1 antagonist SR141716A or the CB2 antagonist SR144528 at the dose of 10 mg kg^{-1}. As shown in Fig. 4, neither the CB1 nor the CB2 antagonists were able to prevent the IL-10 decrease (upper panel) and the IL-12 increase (lower panel) induced by 30 mg kg^{-1} of cannabidiol.

3.2. Effects of cannabidiol on chemotaxis

When added in vitro in the chemotaxis chamber, cannabidiol did not affect spontaneous mobility of peritoneal macrophages (data not shown). However, it inhibited in a concentration-dependent manner fMLP-induced chemo-

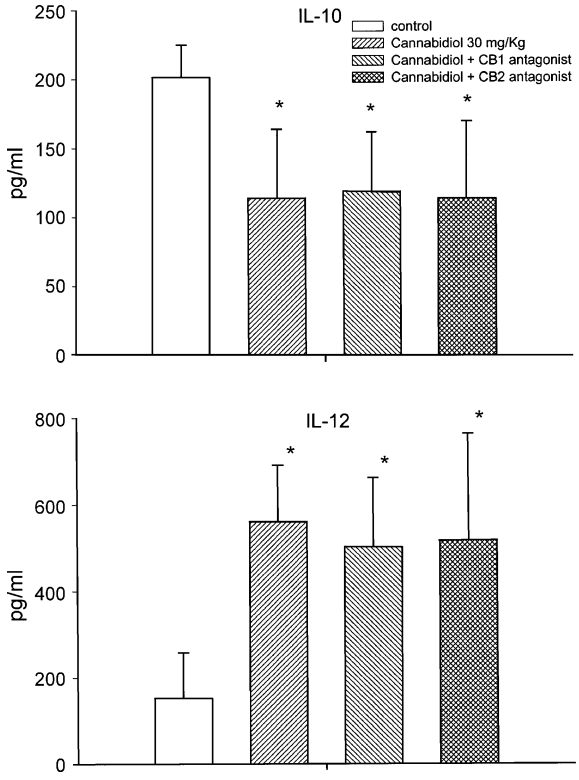

Fig. 4. Effect of pretreatment with either the CB1 receptor antagonist SR171416A or the CB2 receptor antagonist SR144528 at the dose of 10 mg kg^{-1} on the modulation of IL-10 and IL-12 production induced by cannabidiol (30 mg kg^{-1}). All drugs were administered p.o. The antagonists were administered 1 h before cannabidiol and macrophages collected 1 h after cannabidiol. Thereafter, cells were stimulated in vitro with 1 μg ml^{-1} of LPS (for IL-10) and 1 μg ml^{-1} LPS+50 U ml^{-1} INF-γ (for IL-12). Media were collected 24 h later for cytokine evaluation. Values are means±S.D. of eight animals. Each experiment was repeated at least three times, with similar results. *$p<0.05$ vs. control (0).

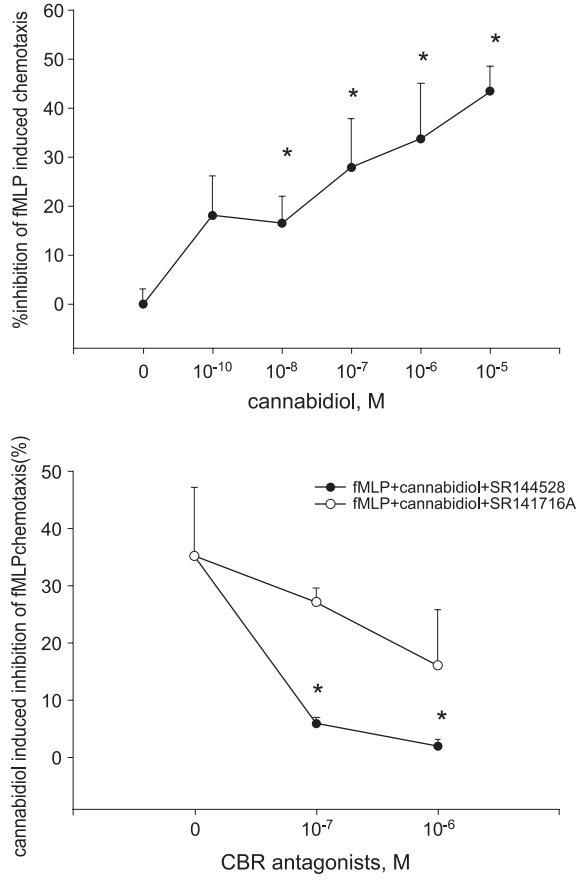

Fig. 5. Upper panel: Effect of cannabidiol added in vitro on fMLP-induced chemotaxis of murine peritoneal macrophages. fMLP was used at the concentration of 10^{-8} M. The results are the means±S.D. of five experiments. *p<0.05 vs. control (0). Lower panel: Effect of increasing concentrations of SR141716A and SR144528 on cannabidiol-induced inhibition of fMLP-induced chemotaxis of murine macrophages. The CB receptor antagonists were added in vitro at the indicated concentration in the lower chamber in combination with 10^{-6} M cannabidiol and 10^{-8} M fMLP. Values are means±S.D. of five experiments. *p<0.05 vs. cannabidiol alone (0 antagonist).

taxis. As shown in the upper panel of Fig. 5, the reduction of chemotaxis is significant starting from the cannabidiol concentration of 10^{-8} M (18% inhibition), and a maximal inhibition of 42% was reached at 10^{-5} M.

In order to test the involvement of the two cannabinoid receptors in the inhibition of macrophage migration induced by cannabidiol, two concentrations of the CBR1 and the CBR2 antagonists SR141716A and SR144528, respectively, were added in the chemotaxis chamber. The antagonists had no effect by themselves either on spontaneous migration or on fMLP chemotaxis (Table 1).

However, the CB2 receptor antagonist SR144528 was able to prevent the inhibition of chemotaxis induced by 10^{-6} M cannabidiol. As reported in the lower panel of Fig. 5, at both concentrations of the antagonists used (10^{-6} and 10^{-7} M), the cannabidiol-induced inhibition of chemotaxis was completely blocked. The addition of the CB1 receptor antagonist slightly decreased cannabidiol-induced inhibition of chemotaxis only at the highest concentration of 10^{-6} M.

Similar results were obtained when macrophages had been pre-incubated for 1 h in the presence of 10^{-6} M cannabidiol or with cannabidiol+CBR antagonists and thereafter their motility tested in the Boyden chamber in the presence or in the absence of fMLP. As shown in Table 2, in fact, cannabidiol pre-incubation significantly reduced fMLP-induced chemotaxis, and the CBR2 antagonist was able to prevent the cannabidiol inhibition.

Table 2
Spontaneous migration and fMLP (10^{-8} M)-induced chemotaxis of peritoneal macrophages after 1 h incubation with cannabidiol or cannabidiol+antagonists

Pre-incubation (1 h)	Spontaneous migration (cells/microscopic field)	fMLP chemotaxis (cells/microscopic field)
Medium	42.2±10.4[a]	109.1±22
CBD (10^{-6} M)	56.8±19.2	43.6±8.5*
CBD+SR141716A (10^{-6} M)	56.0±12.0	61.0±11.88
CBD+SR144528 (10^{-6} M)	48.0±7.7	90.6±11.5**

[a] Values are means±S.D. of three experiments.

* p<0.05 cells pre-incubated with cannabidiol vs. cells pre-incubated with medium only, and therefore assessed for fMLP chemotaxis.

** p<0.05 vs. cells pre-incubated with cannabidiol+SR144528 vs. cells pre-incubated with cannabidiol and assessed for fMLP chemotaxis.

4. Discussion

The relevance of the effects exerted by cannabidiol on the immune system is double: on one side they could constitute a toxic effect associated with recreational abuse of marijuana; on the other, they may be a desired or unsolicited feature of medicinal marijuana use. Cannabidiol, in fact, represents a relevant component of herbal preparation

Table 1
In vitro effect of the CB1 receptor antagonist SR141716A and the CB2 receptor antagonist SR144528 on spontaneous migration and fMLP-induced chemotaxis of macrophages

Concentration (M)	SR141716A		SR144528	
	Spontaneous migration (cells/microscopic field)	fMLP (10^{-8} M) chemotaxis (cells/microscopic field)	Spontaneous migration (cells/microscopic field)	fMLP (10^{-8} M) chemotaxis (cells/microscopic field)
0	20.9±4.8[a]	74.2±13.8	20.9±4.8	74.2±13.8
10^{-7}	20.5±5.2	76.0±10.2	21.4±8.2	71.9±17.8
10^{-6}	19.1±3.8	69.8±16.1	27.6±6.9	72.8±16.8

[a] Values are means±S.D. of six experiments.

known as "Medical Cannabis", which is increasingly used in many countries (Straus, 2000).

Although many studies exist on the immune modulation induced by the various natural and synthetic psychoactive cannabinoid related compounds, only a few studies have analyzed the immune properties of the nonpsychoactive compound cannabidiol. Moreover, it has been shown that each cannabinoid compound has a unique set of effects on the immune responses, and it is therefore important to characterise the effects of the single marijuana components. In the present study, we report evidence that the non-psychoactive cannabis component cannabidiol can affect different macrophage functions.

We have focussed our interest on both macrophage chemotaxis, as primary component of the innate immune response, as well as on macrophage ability to produce cytokines at the basis of regulation of acquired immune responses.

The recruitment of macrophages and monocytes to sites of inflammation, injury and infection is a crucial step in inflammation and antimicrobial immune responses. The ability to migrate towards chemoattractant substances is a first and relevant event in macrophage physiology (Schiffmann, 1982; Wenzel-Seifert et al., 1998). Cannabidiol is able to reduce the migration of murine macrophages stimulated by fMLP, indicating that it might decrease the first steps of macrophage activation. In previous work, we reported that also the synthetic cannabinoid agonist CP55-940 potently reduced macrophage migration (Sacerdote et al., 2001a). Considering the reduction of macrophage migration induced by cannabidiol, the drug can interfere with the recruitment of these cells in the early phases of inflammatory processes, delaying the onset of inflammation. Unexpected and somehow in contrast with inhibition of chemotaxis are the effects exerted by cannabidiol on IL-12 and IL-10 production. The drug, in fact, significantly stimulates the production of IL-12 while decreasing that of IL-10 both when added in vitro to cultures and when administered in vivo.

In the in vitro experiments, it must be noted that no dose–response curve seems to be present. Both for the inhibition of IL-10 and for the increase of IL-12, cannabidiol is effective at quite low concentrations, while at higher doses the effects were no longer detectable. We do not have an explanation for this response of cannabidiol, but atypical dose–response curves in vitro have been described for cannabinoid compounds as well as for cytokine modulation by other drugs of abuse such as opioid agents (Malfait et al., 2000; Chen and Buck, 2000; Chuchawankul et al., 2004; Sacerdote et al., 2001b).

Both the CB1 and the CB2 receptor antagonists added in vitro were able to prevent the modulation of chemotaxis and of cytokines induced by cannabidiol. While in the chemotaxis experiments, the CB2 antagonist was more effective that the CB1 antagonist in blocking the effect of cannabidiol, in the experiments evaluating cytokines both the antagonists similarly prevented the modulation of IL-10 and IL-12 induced by cannabidiol. The action of the antagonists is complex, since both antagonists can behave as inverse agonists in some systems (Chuchawankul et al., 2004; Croci et al., 2003; Landsman et al., 1997; Portier et al., 1999; Smith et al., 2000). Also, in our hands, the two antagonists at the quite high dosage of 10^{-6} M exert an effect on IL-10 and IL-12 production opposite to the one induced by cannabidiol. However, at lower doses, the antagonists seem to loose the inverse agonist properties, but they are still able to prevent the modulation of cytokine induced by cannabidiol. On the basis of these observations, it is difficult to state whether the ability of the antagonists to block cannabidiol effects was due to a direct interaction with cannabidiol or whether we are observing the final results of two phenomena with opposite signs. We must also consider that it has been proposed that CBD could act indirectly, enhancing the anandamide levels through an inhibition of anandamide amidase activity (Watanabe et al., 1996). Indeed, it is also known that LPS itself stimulates macrophages to produce endogenous cannabinoids (Varga et al., 1998; Matias et al., 2002; Liu et al., 2003). The possibility, therefore, that the antagonists act by interfering with the effects of endogenous cannabinoids cannot be ruled out at the moment.

It is not clear, indeed, whether cannabidiol can bind the classical CB receptors directly; it has, in fact, been proposed that cannabidiol could act through the stimulation of a novel non-CB1, non-CB2 receptor not yet identified (Walter et al., 2003; Pertwee et al., 2002; Jarai et al., 1999) that could be present in these cells. On the other hand, the lack of a dose–response curve that we observed with cannabidiol can also indicate a receptor-independent mechanism.

When cannabidiol was administered in vivo to animals, the modulation of cytokines was similar to what observed in vitro, although the treatment with either the CB1 or the CB2 receptor antagonists did not prevent the effect on cytokines. The doses of the selective antagonists administered have been shown to bind around 80% of either CB1 or CB2 receptor, and to block the effects induced by other cannabinoid compounds (Rinaldi-Carmona et al., 1995, 1998; Pozzi et al., 2003; Massi et al., 2000; Sacerdote et al., 2001a). Considering the extremely low affinity of cannabidiol for the CB receptors, the lack of effect of the antagonists cannot reasonably be ascribed to the administration of a low dosage. We do not have at the moment an explanation for this discrepancy, it can be speculated that in vivo the effects of cannabidiol and of the cannabinoid antagonists were not exerted directly on macrophages, but mediated throughout other cell systems. This aspect needs to be further evaluated.

It is well known that IL-12 has an essential role in the induction of Th1 responses and is a predominant pro-inflammatory cytokines (Trincheri, 1995). In contrast, the principal function of IL-10 appears to limit and eventually terminate the inflammatory responses (Moore et al., 1993).

Our results therefore indicate that cannabidiol can induce a pro-inflammatory phenotype of the macrophage.

In agreement with the decrease of IL-10 that we observed, a reduction of IL-10 production was reported also by Srivastava et al. (1998) in a human T cell line. As far as we know, ours is the first study evaluating the effect of cannabidiol alone on IL-12 production by macrophages.

Interestingly, a recent paper (Killestein et al., 2003), which evaluated the immune function in multiple sclerosis patients treated orally with a combination of THC and cannabidiol, showed a significant increase in plasma pro-inflammatory cytokine TNF-α in all subjects and of IL-12 in a subgroup of patients, thus suggesting a pro rather than an anti-inflammatory effect of this therapy. Since macrophage and macrophage-like cells are the main source of IL-12, on the basis of our results we can hypothesise that the enhancement of plasma IL-12 observed in cannabidiol/THC-treated patients (Killestein et al., 2003) could be due to a direct stimulation of macrophages by cannabidiol. These and our results are in partial disagreement with the report by Malfait et al. (2000), who demonstrated a therapeutic action of cannabidiol in a murine model of arthritis. They also suggested that the treatment with cannabidiol suppressed specific Th1 response to collagen, by inhibiting INF-γ production. However, our results suggest that this effect does not seem to be mediated by a modulation of macrophage cytokines, but could be a direct effect on T cells.

We must also remark that other cannabinoid compounds either natural or synthetic have been shown to modulate the T cell responses mainly in the direction of a Th1 inhibition and Th2 stimulation. These effects have been shown to be, at least in part, mediated by an action on macrophages, in this case in a manner opposite to what we now report for the effects of cannabidiol on macrophages (Klein et al., 1998, 2000, 2004; Newton et al., 1998). The description of these differential effects confirms the necessity to evaluate the immune properties of each marijuana component.

On the whole, our results and data from the literature indicate that cannabidiol can exert different effects on the macrophages. It seems to inhibit the precocious steps of the inflammatory process throughout reduction of chemotaxis and NO production (Malfait et al., 2000), whereas it can activate the following phases of macrophage activation, by increasing IL-12 and decreasing IL-10 production therefore favouring the development of a Th1 response. However, the inhibition of chemotaxis can also be interpreted as a signal of arrest, a fundamental step for the macrophage cell to fully express its antimicrobial and pro-inflammatory potential once it has reached the appropriate tissue. Indeed, it is possible that cannabidiol, by enhancing the efficacy of the macrophagic response, could lead to a faster resolution of an inflammatory/infective process.

A positive role for the increase of IL-12 induced by cannabidiol could be envisaged in the recently described antitumor properties of cannabidiol (Massi et al., 2004). We can hypothesise that the increase of IL-12 could be involved in the delay of human glioma cell growth inoculated in nude mice induced by cannabidiol.

In conclusion, it is clear that the effect exerted by cannabinoids on the immune system are complex and not univocal. In fact, on the basis of the cell and function considered, the final result could be different. Moreover, it is confirmed that all the single components of marijuana can have a unique profile as far as immunomodulation is concerned (Straus, 2000). Further studies are therefore needed for a potential therapeutic application of cannabidiol especially as anti-inflammatory drugs.

References

Cabral, G.A., Dove-Pettit, D.A., 1998. Drugs and immunity: cannabinoids and their role in decreased resistance to infectious disease. J. Neuroimmunol. 83, 116–123.

Carlisle, S.J., Marciano Cabral, F., Staab, A., Ludwick, C., Cabral, G.A., 2002. Differential expression of the CB2 receptor by rodent macrophages and macrophage-like cells in relation to cell activation. Int. Immunopharmacol. 2, 69–82.

Chen, Y., Buck, J., 2000. Cannabinoids protect cells from oxidative cell death: a receptor-independent mechanism. J. Pharmacol. Exp. Ther. 293, 807–812.

Chuchawankul, S., Shima, M., Buckley, N.E., Hartmann, C.B., McCoy, K.L., 2004. Role of cannabinoid receptors in inhibiting macrophage costimulatory activity. Int. Immunopharmacol. 4, 265–278.

Compton, D.R., Aceto, M.D., Lowe, J., Martin, B.R., 1996. In vivo characterization of a specific cannabinoid receptor antagonist (SR141716A): inhibition of Δ9-tetrahydrocannabinol-induced responses and apparent agonistic activity. J. Pharmacol. Exp. Ther. 277, 586–594.

Croci, T., Landi, M., Galzin, A.M., Marini, P., 2003. Role of cannabinoid CB1 receptors and tumor necrosis factor-alpha in the gut and systemic anti-inflammatory activity of SR 141716 (Rimonabant) in rodents. Br. J. Pharmacol. 140, 115–122.

Galiegue, S., Mary, S., Marchand, J., Dussossoy, D., Carriere, D., Carayon, P., Bouaboula, M., Shire, D., Le Fur, G., Casellas, P., 1995. Expression of central and peripheral cannabinoid receptor in human tissue and leukocyte subpopulation. Eur. J. Biochem. 232, 54–61.

Jarai, Z., Wagner, J.A., Varga, K.D., Compton, D.R., Martin, B.R., Zimmer, A.M., Bonner, T.I., Buckley, N.E., Razdan, R.K., Zimmer, A., Kunos, G., 1999. Cannabinoid-induced mesenteric vasodilation through an endothelial site distinct from CB1 or CB2 receptors. Proc. Natl. Acad. Sci. U. S. A. 96, 14136–14141.

Kaminski, N.E., 1998. Regulation of the cAMP cascade, gene expression and immune function by cannabinoid receptors. J. Neuroimmunol. 83, 124–132.

Kaminski, N.E., Abood, M.E., Kessler, F.K., Martin, B.R., Schatz, A.R., 1992. Identification of a functionally relevant cannabinoid receptor on mouse spleen cells that is involved in cannabinoid-mediated immune modulation. Mol. Pharmacol. 42, 736–742.

Killestein, J., Hoogervost, E.L.J., Reif, M., Blauw, B., Smits, M., Uitdehaag, B.M.J., Nagelkerken, L., Polman, C.H., 2003. Immunomodulatory effects of orally administered cannabinoids in multiple sclerosis. J. Neuroimmunol. 137, 140–143.

Klein, W.K., Newton, C., Friedman, H., 1998. Cannabinoid receptors and immunity. Immunol. Today 19, 373–381.

Klein, T.W., Newton, C.A., Nakachi, N., Friedman, H., 2000. delta-9-Tetrahydrocannabinol treatment suppresses immunity and early IFN-gamma, IL-12 and IL-12 receptor beta-2 responses to *Legionella pneumophila* infection. J. Immunol. 15, 6461–6466.

Klein, T.W., Newton, C.A., Larsen, K., Chou, J., Perkins, I., Lu, L., Nong, L., Friedman, H., 2004. Cannabinoid receptors and T helper cells. J. Neuroimmunol. 147, 91–94.

Landsman, R.S., Burkey, T.H., Consroe, P., Roeske, W.R., Yamamura, H.I., 1997. SR141716A is an inverse agonist of the human cannabinoid receptor. Eur. J. Pharmacol. 334, R-1–R-2.

Limiroli, E., Gaspani, L., Panerai, A.E., Sacerdote, P., 2002. Differential tolerance development in the modulation of macrophage cytokine production in mice. J. Leukoc. Biol. 72, 43–48.

Liu, J., Batkai, S., Pacher, P., Harvey White, J., Wagner, J.A., Vravatt, B.F., Gao, B., Kunos, G., 2003. Lipopolysaccaride induces anandamide synthesis in macrophages via CD14/MapK/phosphoinositide 3-kinase/NF-kappab independently of platelet activator factor. J. Biol. Chem. 278, 45034–45039.

Malfait, A.M., Gallily, R., Sumariwalla, P.F., Malik, A.S., Andreakos, N.E., Mechoulam, R., Feldmann, M., 2000. The nonpsychoactive cannabis constituent cannabidiol is an oral anti-arthritic therapeutic in murine collagen induced arthritis. Proc. Natl. Acad. Sci. U. S. A. 97, 9561–9566.

Massi, P., Sacerdote, P., Ponti, W., Fuzio, D., Manfredi, B., Vigano', D., Rubino, T., Bardotti, M., Parolaro, D., 1998. Immune function alterations in mice tolerant to tetraidrocannabinol: functional and biochemical parameters. J. Neuroimmunol. 92, 60–66.

Massi, P., Fuzio, D., Vigano', D., Sacerdote, P., Parolaro, D., 2000. Relative involvement of cannabinoid CB1 and CB2 receptors in the Δ9-tetrahydrocannabinol-induced inhibition of natural killer activity. Eur. J. Pharmacol. 387, 343–347.

Massi, P., Vaccani, A., Sacerdote, P., Parolaro, D., 2002. Effects of cannabidiol, a non psychoactive constituent of marijuna on immune system. 12th Annual Symposium on the Cannabinoids (ICRS) Pacific Grove, CA, USA, Abstract, p. 43.

Massi, P., Vaccani, A., Ceruti, S., Colombo, A., Abbracchio, M.P., Parolaro, D., 2004. Antitumor effects of cannabidiol a nonpshychoactive cannabinoid, on human glioma cell lines. J. Pharmacol. Exp. Ther. 308, 838–845.

Matias, I., Pochard, P., Orlando, P., Salzet, M., Pestel, J., Di Marzo, V., 2002. Presence and regulation of the endocannabinoid system in human dendritic cells. Eur. J. Biochem. 269, 3771–3778.

Moore, K.W., O Garra, A., De Waal Malefyt, R., Vieira, P., Mosman, T.R., 1993. Interleukin-10. Annu. Rev. Immunol. 11, 165–190.

Mosman, T.R., Sad, S., 1996. The expanding universe of T-cell subsets; Th1, Th2 and more. Immunol. Today 17, 138–146.

Newton, C., Klein, T., Friedman, H., 1998. The role of macrophages in THC-induced alteration of the cytokine network. Adv. Exp. Med. Biol. 437, 207–2014.

Patrini, G., Sacerdote, P., Fuzio, D., Manfredi, B., Parolaro, D., 1997. Regulation of immune functions in rat splenocytes after acute and chronic in vivo treatment with CP-55,940, a synthetic cannabinoid compound. J. Neuroimmunol. 80, 143–148.

Pertwee, R.G., 1999. Pharmacology of cannabinoid receptor ligands. Curr. Med. Chem. 68, 635–664.

Pertwee, R.G., Ross, R.A., Craib, S.J., Thomas, A., 2002. (-)-Cannabidiol antagonizes cannabidiol receptor agonista and noradrenaline in the mouse deferens. Eur. J. Pharmacol. 456, 99–106.

Portier, M., Rinaldi-Carmona, M., Pecceu, F., Combes, T., Poinot-Chazel, C., Calandra, B., Barth, F., Le Fur, G., Casellas, P., 1999. SR 144528, an antagonist for the peripheral cannabinoid receptor that behaves as an inverse agonist. J. Pharmacol. Exp. Ther. 288, 582–589.

Pozzi, O., Misiano, P., Clark, G.D., Visentin, L., 2003. Antagonism between the Antiinflammatory Activity of Th Cannabinoid WIN 55212-2 and SR141716A.

Prossnitz, E.R., Ye, R.D., 1997. The *N*-formyl peptide receptor: a model for the study of chemoattractant receptor structure and function. Pharmacol. Ther. 74, 73–102.

Rinaldi Carmona, M., Barth, F., Heaulme, M., Shire, D., Calandra, B., Congy, C., Martinez, S., Maruani, J., Neliat, G., Caput, D., Ferrara, P., Soubrie', P., Breliere, J.C., Le Fur, G., 1994. SR 141716A a potent and selective antagonist of the brain cannabinoid receptor. FEBS Lett. 350, 240–244.

Rinaldi-Carmona, M., Barth, F., Heaulme, M., Alonso, R., Shire, D., Congy, C., Soubrie, P., Breliee, J.C., Le Fur, G., 1995. Biochemical and pharmacological characterization of SR141716A, the first potent and selective brain cannabinoid receptor antagonist. Life Sci. 26, 1941–1947.

Rinaldi Carmona, M., Barth, F., Millan, J., Derocq, J.M., Casellas, P., Chongy, C., Oustric, D., Sarran, M., Bouaboula, M., Calandra, B., Portier, M., Shire, D., Breliere, J.C., Le Fur, G., 1998. SR 144528 the first potent and selective antagonist of the CB2 cannabinoid receptor. J. Pharmacol. Exp. Ther. 284, 644–650.

Sacerdote, P., Manfredi, B., Gaspani, L., Panerai, A.E., 2000. The opioid antagonist naloxone induces a shift from type 1 cytokine pattern to type 2 cytokine pattern in balb/cJ mice. Blood 95, 2031–2036.

Sacerdote, P., Massi, P., Panerai, A.E., Parolaro, D., 2001a. In vivo and in vitro treatment with the synthetic cannabinoid CP55,940 decreases the in vitro migration of macrophages in the rat: involvement of both CB1 and CB2 receptors. J. Neuroimmunol. 109, 155–163.

Sacerdote, P., Limiroli, E., Gaspani, L., 2001b. Experimental evidence for immunomodulatory effects of opioids. In: Machelska, H., Stein, C. (Eds.), Immune Mechanisms of Pain and Analgesia, Advances in Experimental Medicine and Biology vol. 521. Kluwer Academic, New York, pp. 106–112.

Schiffmann, E., 1982. Leukocyte chemotaxis. Annu. Rev. Physiol. 44, 553–573.

Smith, S.R., Terminelli, C., Denhart, G., 2000. Effects of cannabinoid receptor agonist and antagonist ligands on production of inflammatory cytokines and antiinflammatory inteleukin-10 in endotoxemic mice. J. Pharmacol. Exp. Ther. 293, 136–150.

Srivastava, M.D., Srivastava, B.I.S., Brouhard, B., 1998. Δ9 Tetrahydrocannabinol and cannabidiol alter cytokine production by human immune cells. Immunopharmacology 40, 179–185.

Straus, S.E., 2000. Immunoactive cannabinoids: therapeutics prospects for marijuana constituents. Proc. Natl. Acad. Sci. 97, 9363–9364.

Trincheri, G., 1995. Interleukin-12: a proinflammatory cytokine with immunoregulatory functions that bridge innate resistance and antigen specific adaptive immunity. Annu. Rev. Immunol. 13, 251–276.

Varga, K., Wagner, J.A., Bridgen, D.T., Kunos, G., 1998. Platelet- and macrophage derived cannabinoids are involved in endotoxin-induced hypotension. FASEB J. 12, 1035–1044.

Wahl, S.M., 1981. Inflammation and wound healing. In: Oppenheim, J., Rosenstreich, D., Potter, M. (Eds.), Cellular Functions in Immunity and Inflammation. Elsevier/North Holland, New York, NY, pp. 55–85.

Walter, I., Franklin, A., Witting, A., Wade, C., Xie, Y., Kunos, G., Mackie, K., Stella, N., 2003. Nonpsychotropic cannabinoid receptor regulate microglial cell migration. J. Neurosci. 23, 1398–1405.

Watanabe, K., Kayano, Y., Matsunaga, T., Yamamoto, I., Yoshimura, H., 1996. Inhibition of anandamide amidase activity in mouse brain microsomes by cannabinoids. Biol. Pharm. Bull. 19, 1109–1111.

Wenzel-Seifert, K., Hurt, C.M., Seifert, R., 1998. High constitutive activity of the human formyl peptide receptor. J. Biol. Chem. 273, 24181–24189.