The nonpsychoactive cannabis constituent cannabidiol is an oral anti-arthritic therapeutic in murine collagen-induced arthritis


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The therapeutic potential of cannabidiol (CBD), the major nonpsychoactive component of cannabis, was explored in murine collag-
en-induced arthritis (CIA). CIA was elicited by immunizing DBA/1 mice with type II collagen (CII) in complete Freund’s adjuvant. The CIA used was either bovine or murine, resulting in classical acute CIA or in chronic relapsing CIA, respectively. CBD was administered after onset of clinical symptoms, and in both models of arthritis the treatment effectively blocked progression of arthritis. CBD was equally effective when administered i.p. or orally. The dose dependency showed a bell-shaped curve, with the optimal effect at 5 mg/kg per day i.p. or 25 mg/kg per day orally. Clinical improvement was associated with protection of the joints against severe damage. Ex vivo, draining lymph node cells from CBD-treated mice showed a diminished CII-specific proliferation and IFN-γ production, as well as a decreased release of tumor necrosis factor by knee synovial cells. In vitro effects of CBD included a dose-dependent suppression of lymphocyte proliferation, both mitogen-stimulated and antigen-specific, and the blockade of the Zymosan-triggered reactive oxygen burst by peritoneal granulocytes. It also was found that CBD administration was capable of blocking the lipopolysaccharide-induced rise in serum tumor necrosis factor in C57BL mice. Taken together, these data show that CBD, through its combined immunosuppressive and anti-inflammatory actions, has a potent anti-arthritic effect in CIA.

Cannabidiol (CBD) is one of the major components of Cannabis sativa, marijuana (1). Marijuana contains approximately 80 constituents, termed cannabinoids (2, 3). CBD is not psychoactive, unlike the other major component of cannabis, Δ9-tetrahydrocannabinol (Δ9THC) (4, 5). A vast literature documents the immune modulating effects of cannabinoids, in vivo and in vitro, mainly of Δ9THC and synthetic analogues such as CP55,940 (reviewed in ref. 6). A nonexhaustive list of in vitro effects includes inhibition of the proliferative responses of T lymphocytes (7), inhibition of cytotoxic T cell activity (8), suppression of macrophage function and antigen presentation (9, 10), and inhibition of NO production by macrophages (11). Reports on the in vitro effects of CBD on immune cells are scarce and include the modulation of tumor necrosis factor (TNF), IL-1, and IFN-γ by human peripheral blood mononuclear cells (12, 13) and the suppression of chemokine production by a human B cell line (14). These potentially anti-inflammatory properties of CBD, together with the lack of psychotropic effect and low toxicity (15), prompted us to test the potential of CBD as a therapeutic agent in collagen-induced arthritis (CIA).

CIA, a murine model for rheumatoid arthritis (RA), is elicited by immunizing DBA/1 mice with type II collagen (CII) in complete Freund’s adjuvant (16). The immune response to CII involves both humoral and cellular mechanisms (17, 18), and the cellular response is THelper 1-mediated (19). CIA is characterized by rapid onset of clinical joint inflammation, resulting in destruction of joint tissues and cartilage/bone erosions. Suppression of the inflammatory process by blocking TNF with mAbs has proven an effective treatment of CIA (20, 21), and these findings led to the successful use of TNF blockade in multiple phase I, II, and III clinical trials with RA patients (reviewed in ref. 22), thus validating the predictive value of CIA as a model for RA. In the present study, we report that CBD has a beneficial therapeutic action on established CIA, and we explore its mode of action.

Materials and Methods

Purification of CBD. CBD was purified from hashish as reported (23). Its purity was established on the basis of melting point (66–67°), optical rotation (αD = 125°), and single peak on gas chromatography (23).

Induction and Monitoring of Heterologous CIA. Bovine CII was purified from hyaline cartilage (21). Male DBA/1 mice (8–12 weeks old) were immunized with 100 μg of CII emulsified in complete Freund’s adjuvant (Difco) by intradermal injection at the base of the tail. From day 15 after immunization onward, mice were examined daily for onset of clinical arthritis. Assessment of arthritis included monitoring of clinical scores where 0 = normal; 1 = slight swelling and erythema; 2 = pronounced edema; 3 = joint rigidity. Each limb was graded, resulting in a maximal clinical score of 12 per animal. The arthritis was monitored over 10 days, after which the mice were killed (21).

Induction and Monitoring of Homologous CIA. Mouse CII was purified from sternal cartilage from female DBA/1 mice, as described for bovine CII. For the chronic experiments, 6-week-old mice were immunized with mouse CII (100 μg) in complete Freund’s adjuvant. The animals were boosted 15 days later with 100 μg CII i.p. From day 30 after immunization onward, 80% of the mice developed a chronic relapsing arthritis, which was monitored for 5 weeks as described above.

Administration of CBD. CBD treatment commenced at the first clinical signs of arthritis and was administered i.p. daily until day 10 of arthritis. The CBD concentrations used were 20 mg/kg (n = 12), 10 mg/kg (n = 17), 5 mg/kg (n = 15), and 2.5 mg/kg (n = 9). CBD was dissolved in ethanol/cremophor (Sigma) (1:1, vol/vol) and further diluted in saline, so that the final solution was ethanol/cremophor/saline (1:1:18). Mice injected with ve-
hicle alone (ethanol/cremophor in saline) served as controls (n = 23).

For the oral treatment protocol, CBD was dissolved in olive oil and administered by oral gavage, daily, from the onset of arthritis for 10 days. The doses used were 10 mg/kg, 25 mg/kg, and 50 mg/kg (n = 6 per group). Control mice were fed olive oil (n = 6).

For the chronic experiments, mice were treated from the first symptoms of arthritis for 5 weeks. For the i.p. route, CBD was injected daily at 10 mg/kg (n = 7) or 5 mg/kg (n = 7). Again, mice injected with vehicle alone served as controls (n = 7). For the oral route, the treatment was administered daily (Monday to Friday) at a dose of 25 mg/kg (n = 6) and control mice were fed olive oil (n = 6).

**Histological Analysis.** At the end of each experiment, hind paws were removed postmortem, fixed in formalin, and decalcified in 5% EDTA. The paws were embedded in paraffin, sectioned, and stained with hematoxylin and eosin. Arthritic changes in the foot joints were scored as mild (mild synovial hyperplasia), moderate (pannus formation and erosions limited to the cartilage-pannus junction), or severe (extended bone and cartilage erosions with loss of joint architecture). All assessments were performed by an observer blinded to the treatment received.

**Lipopolysaccharide (LPS) Induction of Serum TNF in Mice.** Female C57BL/6 mice were injected i.p. with a sublethal dose of LPS (100 μg, *Escherichia coli* O55:B5, Difco). CBD was injected simultaneously, either i.p. or s.c., at a dose of 10 mg/kg. Ninety minutes later, the mice were bled and serum TNF levels were determined by bioassay (24).

**Reactive Oxygen Intermediate Production by Mouse Granulocytes.** C57BL/6 mice were injected i.p. with 1.5 ml thioglycollate (Difco), and 18 h later the cells were harvested by sterile lavage with PBS. The cells were washed and resuspended in Hank's balanced salt solution without phenol red, and 0.5 ml of the cell suspension was added into luminometer tubes. CBD (dissolved in ethanol) was added at this point at a final concentration of 6 μg/ml. Finally, 10 μl Luminol (Sigma) and 30 μl Zymosan (Sigma) were added, and the chemiluminescence was measured immediately in a luminometer (Biolumate LB 95, Berhold, Wildbad, Germany).

**Preparation of CBD for in Vitro Experiments.** CBD was dissolved in ethanol at a stock concentration of 10 mg/ml and stored at 4°C for up to 2 months. CBD stock was further diluted in warm medium immediately before use. All tissue culture media, vehicle control, and CBD preparations were shown to contain less than 0.1 unit/ml endotoxin, as assessed by the chromogenic Limulus Amebocyte Lysate assay (BioWhittaker). At the end of all *in vitro* experiments described below, viability of the cells was assessed with a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide test (25).

**Splenic Lymphocyte Culture.** Pooled spleens from 10-week-old DBA/1 mice were pushed through a sieve and a single cell suspension was prepared. The cells were washed, layered over a Lymphoprep-M density gradient (Cedarlane Laboratories), and spun at 2,000 rpm for 45 min. Theuffy coat containing lymphocytes was washed three times and then plated at 2 × 10^6 cells/100 μl well in complete medium comprising DMEM supplemented with 10% heat-inactivated FCS, 1% glutamine, 100 units/ml penicillin, 100 mg/ml streptomycin, and 2 × 10^-5 M 2-mercaptoethanol. Cells were stimulated with 5 μg/ml Con A in the presence of 0–10 μg/ml CBD. After 72 h, cells were pulsed with 0.5 μCi/well ^3H^thymidine (Amersham Pharmacia) overnight, harvested, and assessed for incorporation of radioactivity.

**Draining Lymph Node Cell (LNC) Culture.** Mice (controls or CBD-treated) were killed at day 3 after disease onset, and inguinal LNCs were cultured as described (26). Cells were cultured with or without bovine CII (50 μg/ml) in Tris-buffered saline, pH 7. Supernatants were collected after 72 h and stored at −20°C until cytokine measurement. Alternatively, after 72 h, cells were pulsed with ^3H^thymidine overnight and assessed for incorporation of radioactivity.

**Culture of Murine Synovial Cells.** Mice (control mice or CBD-treated) were killed at day 10 of arthritis and the knee joints were removed. Synovial cell cultures were performed as described (27). Briefly, synovial membranes were excised under a dissecting microscope and digested with 1 mg/ml collagenase A and 0.15 mg/ml DNase type IV in the presence of 33 μg/ml polymyxin B. The cells then were washed extensively and cultured in 96-well plates at a density of 2 × 10^5 cells/ml (100
Oral Administration of CBD Has an Equally Potent Therapeutic Effect on Established Arthritis. Daily oral gavage of CBD immediately after onset of arthritis resulted in suppression of acute CIA (Fig. 1A). The optimal dose was 25 mg/kg, although the higher dose of 50 mg/kg worked almost as well. The 25 mg/kg dose was used in a chronic experiment in homologous CIA and was shown to effectively suppress progression of disease over a study period of 4 weeks (Fig. 2B). The area under the curve was reduced from 72.3 in the controls to 49.7 in the treated animals (P < 0.05).

Effect of CBD on Joint Damage. Joints in the hind paws of control mice and mice treated with CBD were assessed for hyperplasia and destruction. Table 1 shows the results in acute CIA. First, in the i.p. experiments none of the control mice had normal feet, whereas 34% of the feet in mice treated with 5 mg/kg CBD were completely protected. Sixty percent of all of the feet in the i.p. experiments had normal feet, whereas 22% of the feet in mice treated with 5 mg/kg CBD was lower for 40% (20% in each category). For the oral treatment protocol, it was found that 25 mg/kg was the optimal dose. Although no normal feet were found in this treatment group, only 25% were severely affected, as compared with 75% in the controls and 50% in the 50 mg/kg group (Table 1). Thus, the histological findings confirm that CBD at an i.p. dose of 5 mg/kg was an optimal therapeutic effect on acute CIA. Those optimal doses, when tested in the chronic model, gave a good protection against histological

<table>
<thead>
<tr>
<th>Table 1. Histology of the hind feet in acute bovine CIA</th>
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<td>Intrapertoneal, n = 20/group</td>
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<tr>
<td>Arthritic changes</td>
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<td>Normal</td>
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<td>Mild</td>
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Histological findings at the end of the experiments in classical CIA. The results are shown as the percentage of all feet studied that were given a specified score. *, P = 0.0083; **, P = 0.0373 (Fisher’s exact test).

Table 2. Histology of the hind feet in chronic homologous CIA

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<td>Intrapertoneal, n = 12/group</td>
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<tr>
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Histological findings at the end of the experiments in chronic homologous CIA. The results are shown as the percentage of all feet studied that were given a specified score. *, P = 0.0373; **, P = 0.0312 (Fisher’s exact test).
CBD Blocks Mitogen-Induced Lymphocyte Proliferation As Well As Antigen-Specific LNC Proliferation. Purified lymphocytes from spleens of 10-week-old DBA/1 mice were stimulated with Con A, in the presence of increasing concentrations of CBD (0–10 μg/ml). Fig. 4A shows a dose-dependent suppression of Con A-induced proliferation. This finding was reproduced with lymphocytes from C57BL mice, indicating that the effect is not strain-dependent. Likewise, draining LNCs from control arthritic DBA/1 mice taken at day 3 of arthritis were stimulated in vitro with 50 μg/ml bovine CH in the presence of increasing CBD concentrations (Fig. 4B), and again, a dose-dependent suppression of cell proliferation by CBD was seen.

Systemic Administration of CBD Blocks LPS-Induced Serum TNF. High levels of serum TNF in C57BL/6 mice were measured by bioassay 90 min after i.p. injection of LPS. Simultaneous injection of CBD, either i.p. or s.c., effectively abrogated the rise in serum TNF (Table 5).

Discussion

Based on the reported analgesic and anti-inflammatory properties of cannabinoids, it was considered that these compounds might have anti-arthritic potency. The aim of the present study was to assess the therapeutic efficacy of CBD, a nonpsychoactive component of marijuana, in murine CIA as a model for RA. In the initial experiments, CBD was administered i.p. after the onset of clinical arthritis. It was found that CBD exerted a dose-dependent suppressive action, both on the clinical arthritis and joint damage (Fig. 1; Table 1). The dose dependency showed a bell-shaped curve, with the 5 mg/kg dose exerting an optimal therapeutic effect, whereas both the lowest dose (2.5 mg/kg) and the highest dose (20 mg/kg) were inactive. Interestingly, the therapeutic action also was observed when CBD was administered orally and 25 mg/kg, not the highest dose tested, was most effective. The same therapeutic protocols subsequently were performed in homologous CIA, a chronic relapsing form of CIA with a disease pattern that resembles human disease better (30, 31). Again, we found an optimal amelioration of clinical disease and joint damage for CBD, 5 mg/kg i.p. or 25 mg/kg orally. The clinical anti-inflammatory effect with 5 mg/kg i.p. was not statistically significant, but histological evaluation showed a significant protection of the joints. We do not have an explana-

Table 3. In vivo treatment with CBD suppresses CI-specific T helper 1 responses

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<th>Mice</th>
<th>[3H]Thymidine incorporation, cpm</th>
<th>IFN-γ production, pg/ml</th>
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<tbody>
<tr>
<td></td>
<td>−CII (±2,540)</td>
<td>12,919 (±10,055)</td>
</tr>
<tr>
<td></td>
<td>+CII (±44,321)</td>
<td>70,258 (±44,321)</td>
</tr>
<tr>
<td>Control</td>
<td>3,301</td>
<td>502 (±870)</td>
</tr>
<tr>
<td></td>
<td>6,574 (±3,779)*</td>
<td>423 (±545)**</td>
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Mice were treated with CBD, 5 mg/kg i.p., or with vehicle control from onset of arthritis. They were killed at day 3, and inguinal LNCs were cultured with or without CII. After 72 h, CI-specific cell proliferation and IFN-γ release were measured. Results are the mean +/− SD of three individual mice per treatment group, each of them tested in triplicate. This experiment is representative of three reproducible experiments. In total, 10 mice per group were tested. *, not significant; **, P = 0.0027 (Mann-Whitney U).
showed a diminished CII-specific proliferation and markedly diminished IFN-γ release (Table 3). In independent in vitro experiments, it was found that CBD suppressed the CII-specific proliferation of LNCs from arthritic mice in a dose-dependent manner, and it also suppressed Con A-induced proliferation of purified lymphocytes.

Synovial cells from mice that had been treated with an optimal dose of CBD (5 mg/kg per day i.p. for 10 days) released significantly less TNF when cultured in vitro than synovial cells from control animals (Fig. 3). This finding suggests that the therapeutic actions of CBD include the suppression of TNF-α, a proinflammatory cytokine known to be a major mediator of arthritis (22). This was corroborated by the finding that CBD, when injected i.p. or s.c at a concentration of 10 mg/kg, blocked LPS-induced serum TNF in C57BL/6 mice (Table 5). Nevertheless, we could not find suppression of TNF release by arthritic synovial cells when CBD was added in vitro (not shown), nor could we demonstrate in multiple attempts that CBD suppressed TNF release by mouse bone marrow-derived macrophages or RAW cells (data not shown). This discrepancy between in vivo and in vitro results suggests that the TNF suppression, which is observed in vivo after administration of CBD, might be mediated by an active metabolite of CBD. Another possibility is that the decreased TNF expression in vivo is an indirect consequence of a suppressed T helper 1 response.

Thus, the anti-arthritis potency of CBD seems to be the result of a combination of immunosuppression, especially of a T helper 1 response and an anti-inflammatory action by way of reducing TNF in the synovium, a combination that has proven successful when anti-IL-12 and anti-TNF were combined to treat CIA (33). Apart from these major effects, we also have demonstrated other in vitro anti-inflammatory actions of CBD that may contribute to its anti-arthritis potency, such as the inhibition of the release of reactive oxygen species by Zymosan-stimulated neutrophils (Table 4 and ref. 34). We also observed the blockade of NO production by peritoneal macrophages (not shown), as reported in the literature (11).

Cannabis has a long history as a medicinal preparation, mainly for properties such as analgesia, antiemesis, ocular hypotension, and anticonvulsion (reviewed in ref. 35). Recent research in vitro and in animal models has led to increasing evidence that cannabinoids are also important modulators of the immune system (6) and thus could have a role in the treatment of chronic inflammatory diseases, were the development of clinical trials not hampered by legal obstacles. It is therefore important to find out whether nonpsychoactive cannabinoids are suitable for treating chronic inflammatory disease. A recent report describes the effect of a nonpsychoactive synthetic derivative from tetrahydrocannabinol (THC), dimethylheptyl-THC-11-oic acid, in adjuvant arthritis in rats (36). The authors found that this compound reduced the severity of arthritis when administered from immunization onward (i.e., in a preventive protocol). The present study shows that CBD, a natural constituent of marijuana, is effective as an anti-arthritis therapeutic in established CIA. Its efficacy when given orally renders it an attractive candidate for the treatment of RA. The experiments in the chronic CIA model show that prolonged treatment with CBD does not induce tolerance, a phenomenon often observed with

### Table 4. CBD inhibits Zymosan-induced release of reactive oxygen intermediates by mouse peritoneal granulocytes

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<tr>
<th>Granulocytes</th>
<th>Chemiluminescence peak</th>
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<tbody>
<tr>
<td>Zymosan</td>
<td>300</td>
</tr>
<tr>
<td>Zymosan + CBD simultaneously</td>
<td>1,858 (55%)</td>
</tr>
<tr>
<td>Zymosan + CBD pretreatment</td>
<td>1,024 (45%)</td>
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Table 4. Thiglycollate-elicited peritoneal granulocytes from C57BL/6 mice were stimulated with Zymosan in the absence or presence of CBD, 6 μg/mL. CBD was either added simultaneously with the Zymosan or the cells were pretreated with CBD for 1 h before stimulation. Production of reactive oxygen intermediates was measured by chemiluminescence. Percentage inhibition is shown in brackets. One representative experiment of four is shown.
cannabinoids (37, 38). Moreover, clinical trials with CBD have been conducted in humans with epilepsy (39) and Huntington’s disease (40), and it was found that chronic oral administration of CBD, up to 10 mg/kg per day for 6 weeks, had no side effects. Interestingly, one paper describes that feeding 300 or 600 mg CBD to healthy human volunteers resulted in elevated plasma cortisol levels (41), yet another factor that may contribute to its anti-inflammatory/immunosuppressive actions. All of this suggests that CBD may be valuable in the treatment of other chronic inflammatory diseases as well. Indeed, preliminary studies indicate that CBD is able to delay and attenuate experimental allergic encephalomyelitis in mice (R.G. and H. Ovadia, unpublished observations) as well as inflammatory bowel disease in IL-10 knockout mice (T. Sheinid and M.F., unpublished observations).

The results presented here leave a number of questions to elucidate in the future. First, is CBD solely responsible for the anti-arithmetic effects in vivo or is there an active metabolite involved? Second, via which receptor does CBD exert its effects? Two receptors for cannabinoids have been identified, the brain receptor, CB1 (42), and the peripheral cannabinoid receptor, CB2 (43), which is present on T and B lymphocytes, natural killer cells, and macrophages (6). The affinity of CBD for the cannabinoid receptors is very low, lower than that of the other cannabinoids (43, 44). The possibility that CBD, because of its lipophilicity interferes with cell membranes, thus altering their functions, or that a metabolite acts on the CB2 receptor, cannot be ruled out. These questions should be the subject of future studies.

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