



Cannabidiol arrests onset of autoimmune diabetes in NOD mice

Lola Weiss^{a,*}, Michael Zeira^a, Shoshana Reich^a, Shimon Slavin^a,
Itamar Raz^b, Raphael Mechoulam^{c, d}, Ruth Gallily^d

^a Department of Bone Marrow Transplantation and Cancer Immunotherapy, Hadassah Hebrew University Hospital, Jerusalem 91120, Israel

^b Department of Internal Medicine, Hadassah-Hebrew University Medical School, Jerusalem 91120, Israel

^c Department of Medicinal Chemistry and Natural Products, Hebrew University, Medical Faculty, Jerusalem 91120, Israel

^d Lautenberg Center for Immunology, Hebrew University Medical School, Jerusalem 91120, Israel

Received 25 March 2007; received in revised form 31 May 2007; accepted 21 June 2007

Abstract

We have previously reported that cannabidiol (CBD) lowers the incidence of diabetes in young non-obese diabetes-prone (NOD) female mice. In the present study we show that administration of CBD to 11–14 week old female NOD mice, which are either in a latent diabetes stage or with initial symptoms of diabetes, ameliorates the manifestations of the disease. Diabetes was diagnosed in only 32% of the mice in the CBD-treated group, compared to 86% and 100% in the emulsifier-treated and untreated groups, respectively. In addition, the level of the proinflammatory cytokine IL-12 produced by splenocytes was significantly reduced, whereas the level of the anti-inflammatory IL-10 was significantly elevated following CBD-treatment. Histological examination of the pancreata of CBD-treated mice revealed more intact islets than in the controls. Our data strengthen our previous assumption that CBD, known to be safe in man, can possibly be used as a therapeutic agent for treatment of type 1 diabetes.

© 2007 Elsevier Ltd. All rights reserved.

Keywords: Cannabinoids; Cannabidiol; Diabetes; NOD mice; Cytokines

1. Introduction

Type 1 diabetes mellitus (T1DM – insulin-dependent diabetes) is an autoimmune disease resulting in destruction of insulin-producing pancreatic β cells, a process which is assumed to be mediated mainly by CD4 Th1 and CD8 T lymphocytes (Atkinson and Leiter, 1999; Mandrup-Poulsen, 2003). The NOD mouse is the animal used most often for pre-clinical

evaluation of prophylactic and therapeutic treatments of diabetes. When the NOD mouse is not treated, it develops a disease with characteristics similar to autoimmune insulinitis in man (Anderson and Bluestone, 2005).

Insulinitis is an inflammation of the islands of Langerhans and is the initial lesion of insulin-dependent diabetes mellitus, during which leukocytes, lymphocytes in particular, surround and infiltrate the islets.

While numerous approaches have successfully been used to prevent diabetes in young NOD mice, the amelioration of diabetes after onset, or around onset time, is much more difficult to achieve. Importantly, some of the therapies found to be effective in this mice model, have subsequently shown efficacy in clinical trials. Raz et al. have reported immunomodulation with DiaPep277 in a phase 2 clinical trial (Raz et al., 2001) and Keymeulen et al. have successfully employed anti-CD3 antibodies (Keymeulen et al., 2005).

Abbreviations: CBD, cannabidiol; ConA, concanavalin A; FCS, fetal calf serum; NO, nitric oxide; NOD, non-obese diabetic mice; PM θ , peritoneal macrophages; SPF, special pathogen free; T1DM, type 1 diabetes mellitus; Th1, T helper 1; Th2, T helper 2; TNF- α , tumor necrosis factor- α .

* Corresponding author at: Department of Bone Marrow Transplantation and Cancer Immunotherapy, Hadassah University Hospital, POB 12000, Jerusalem 91120, Israel. Tel.: +972 2 677 6681; fax: +972 2 642 2731.

E-mail address: lolaw@hadassah.org.il (L. Weiss).

Cannabidiol (CBD) is a potent anti-inflammatory agent. It is effective in suppressing IFN- γ and TNF- α production and progression of autoimmune Th1-mediated rheumatoid arthritis by inhibition of T cell proliferation (Malfait et al., 2000). This observation led us to investigate the possible effects of CBD on additional autoimmune diseases. Recently we demonstrated that CBD lowers the incidence of diabetes in young NOD mice. It also significantly reduces the plasma levels of the proinflammatory cytokines IFN- γ and TNF- α , lowers Th1-associated cytokine production in vitro and augments Th2-associated cytokines IL-4 and IL-10 (Weiss et al., 2006). Following this observation, which shows that CBD partly prevents initiation of diabetes, we decided to explore the possibility that CBD can also prevent the development of overt or latent diabetes.

In our present report we found that CBD significantly inhibited insulinitis in 11–14 week old NOD female mice.

2. Materials and methods

2.1. Mice

Non-obese female NOD/LtJ mice, 11–14-week-old, were obtained from Harlan, Israel. Female NOD mice develop diabetes after 12–18 weeks. BALB/c female mice (11–14 weeks old) were also obtained from the same supplier. Mice were fed standard laboratory animal chow ad libitum and were kept in pathogen free (SPF) animal house facility. The study was conducted in compliance with the international laws on animal experimentation and approved by the Ethical Committee of the Hebrew University Medical School.

2.2. Cannabidiol (CBD)

CBD was extracted from Cannabis resin (hashish) and purified as previously reported (Gaoni and Mechoulam, 1971). The crystalline CBD, melting point 66–67 °C, presented a single peak on gas chromatography analysis. CBD was first dissolved in ethanol and then the same amount of Cremophor EL (Sigma) was added. This solution was then further diluted with saline so that the final solution was ethanol/cremophor/saline (1:1:18).

2.3. Experimental design

NOD female mice, 11–14 week old, with impaired glucose tolerance and 14 week old mice with normal glucose tolerance were assigned to three groups (Gross et al., 1998). One group received CBD, the second group received Cremophor/ethanol/saline (vehicle) and the third group remained untreated.

CBD was administered i.p. at a dose of 5 mg/kg per day, in 0.1 ml. The experiment was continued for 4 weeks, each mouse receiving 5 administrations per week. The mice were observed until 24 weeks of age.

The level of urine glucose was assayed once or twice a week by test strips (Medi-Test Combi 9 Macherey-Nagel, Duren) and was considered positive after the appearance of glucosuria (>1000 mg/dl) in at least two determinations, according to the manufacturer's information. A level of 1000 mg/dl glucose in the urine was found to correlate with >200 mg/dl. glucose in the blood.

2.4. Peritoneal macrophage culture

Peritoneal lavages from each mouse, containing approximately 40% macrophages (PM θ), were collected in phosphate buffer saline (PBS), centrifuged and the pellets were then resuspended and plated in 24-well plates for two hours. The wells were washed to remove non-adherent cells and fresh DMEM with 10% fetal calf serum (FCS) was added to the cells, mostly macrophages

>90%, for further over night incubation. Harvested supernatants were centrifuged to remove any cells and stored at –80 °C until assayed for cytokines.

2.5. Splenic lymphocyte culture

Spleen cells from each mouse were removed aseptically, the cells were released, washed in PBS, resuspended in RPMI complete medium supplemented with 10% FCS, 100 U/ml penicillin, 100 μ g/ml streptomycin and 2 mM L-glutamine (Beith Haemek, Israel), counted with Trypan-blue dye exclusion to distinguish the live cells (unstained) and dispensed into 24-well plates (Greiner Labs, Germany) at 2×10^6 cells/ml medium per well. Cells were stimulated with 2.5 μ g/ml Concanavalin A (ConA) (Sigma St. Louis, MI, USA) and cultured at 37 °C in a 5% CO₂ humidified incubator. Supernatants were harvested after 48 h incubation, cleared by centrifugation and stored at –80 °C until assayed for cytokines.

2.6. Cytokine assay

Levels of cytokines in the plasma and in the supernatants of splenic lymphocytes and peritoneal macrophages of the mice were assayed by the “sandwich” ELISA technique. ELISA reagents were purchased as Opt. EIA Cytokine ELISA sets from BD Biosciences (San Diego, CA, USA) and were used according to the manufacturer's protocol as previously described (Ji et al., 2003).

2.7. Histopathology

Pancreatic tissue was fixed in 4% buffered formalin, embedded in paraffin, and 5- μ m sections were stained with hematoxylin and eosin. Twenty sections were screened and scored by two independent observers and were graded as follows: intact islets; partially infiltrated islets and peri-infiltrated islets; totally infiltrated islets; destroyed islets.

2.8. Statistical analysis

The statistical significance of the results of diabetes occurrence in Fig. 1 was calculated by the Kaplan–Meier method, and statistical significant differences between the curves were analyzed by log rank. Differences between experimental and control values (Table 1) were analyzed for significance ($p \leq 0.05$) by one-tail distribution-free Mann–Whitney *U*-test.

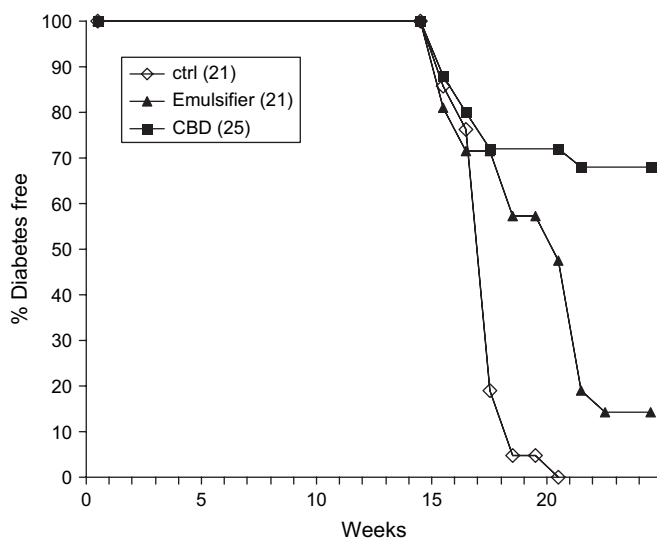


Fig. 1. Incidence of diabetes in NOD mice treated with 5 mg/kg CBD i.p., vehicle control and untreated mice. The incidence of diabetes in the CBD treated group, compared with vehicle-treated mice and untreated controls was significant $p < 0.001$ (log rank test).

Table 1
Cytokine levels in the plasma and supernatants of peritoneal macrophages and spleen cells from untreated and CBD treated mice at 24 weeks of age

In vivo treatment of NOD mice	Number of mice	In vitro spleen cell activation	Cytokine evaluated	pg/ml mean \pm SE	<i>p</i> =
CBD	5	Plasma	IL-6	2.0 \pm 2.0	0.016
Vehicle	4	Plasma	IL-6	100.7 \pm 44.1	
CBD	4	PM θ	IL-6	50.2 \pm 17.7	0.015
Vehicle	4	PM θ	IL-6	100.0 \pm 20.0	
CBD	3	PM θ	IL-10	234.7 \pm 85.3	0.05
Vehicle	3	PM θ	IL-10	1.0 \pm 1.0	
CBD	4	Sp	IL-10	97.5 \pm 57.5	0.029
Vehicle	4	Sp	IL-10	1.0 \pm 1.0	
CBD	9	Sp	TNF α	20.2 \pm 10.2	0.068
Vehicle	8	Sp	TNF α	148.0 \pm 44.0	
CBD	13	Sp	IL-12	36.9 \pm 15.3	0.018
Vehicle	9	Sp	IL-12	114.0 \pm 38.1	
CBD	5	Sp	IL-4	278 \pm 97.9	0.056
Vehicle	4	Sp	IL-4	100 \pm 21.9	

Differences between CBD and control values were analyzed for significance by one-tail distribution-free Mann–Whitney *U*-test.

3. Results

3.1. CBD partly prevents the development of overt diabetes in NOD female mice

CBD was administered i.p. to 11–14 week old female NOD mice. Treatment was continued for 4 weeks (5 days a week) at a dose of 5 mg/kg per day. Treatment was then withdrawn and the mice were observed until 24 weeks of age. A remarkable reduction of diabetes development was observed in CBD-treated mice, compared with vehicle-treated mice and untreated controls ($p < 0.001$, log rank test). At the end of the 24 weeks, only 8 of 25 (32%) of the CBD-treated mice developed glucosuria, compared with 18 of 21 (86%) vehicle-treated mice and 100% (21 mice) of the untreated controls (Fig. 1).

3.2. Effect of CBD on cytokine profile

Cytokine concentration of IL-12, TNF- α , IL-6 and IL-10 in the supernatants of NOD splenocytes and macrophages (stimulated and unstimulated in vitro) as well as IL-6 in plasma were assessed by ELISA. A significant reduction of IL-6 level was noted in the plasma of CBD-treated mice ($p = 0.016$), as well as in the levels of IL-6 and IL-12 in cell supernatants of CBD-treated NOD mice, compared with the levels in vehicle-treated mice ($p = 0.015$ and $p = 0.018$, respectively). The level of TNF- α in splenocyte supernatants was also reduced, but the reduction was not significant ($p = 0.068$). In contrast the levels of the Th2-associated cytokines, IL-10, in supernatants of macrophages and splenocytes and IL-4 secreted by splenocytes were higher, although in the case of IL-4, the enhancement was not significant ($p = 0.05$, $p = 0.029$ and $p = 0.056$ respectively) (Table 1). The changes in the cytokine profile suggest that CBD administration induces a Th1 \rightarrow Th2 shift (Crane and Forrester, 2005; Chan et al., 1991).

3.3. Histology of pancreas

The morphological structure of intact islets from CBD-treated NOD mice was similar to normal islets, in contrast to the abundant mononuclear cell invasion into islets in vehicle-treated and untreated mice. The number of intact islets in pancreata taken from untreated (6 out of 73, 8%) and vehicle treated mice (12 out of 94, 13%) decreased, compared to CBD-treated mice (108 out of 140, 77%). As most islets in the control diabetic mice were fully destroyed and disappeared, the total number of islets counted was lower than that in the CBD-treated (Table 2).

Cell infiltration into islets (leading to insulinitis) was also lower in the pancreata of CBD-treated mice when they were examined 6–8 weeks after the treatment was stopped. The morphological appearance of intact islets was similar to that of normal islets. In contrast, abundant mononuclear cell invasion into islets and destroyed islets were seen in the pancreata of vehicle and untreated controls (Fig. 2).

4. Discussion

Studying the levels of various cytokines secreted in vitro by macrophages and splenocytes derived from CBD-treated NOD mice demonstrated a decrease in production of IL-6, TNF- α and IL-12. In contrast the levels of IL-10 (by PM θ and splenocytes) and IL-4 (by splenocytes) were enhanced. However the changes in TNF- α and IL-4 levels indicated a trend only, as they did not achieve statistical significance. Numerous investigations have shown that suppression of proinflammatory cytokines TNF- α , IL-6 as well as IL-12 inhibits inflammatory damage. Moreover, the increase of IL-10 production, which suppresses proinflammatory cytokines production, is known to abrogate inflammatory manifestation.

We have previously shown that treatment of NOD mice (6–12 weeks old) with CBD, leads to augmentation of IL-4 and IL-10 production by splenocytes and decrease in IFN- γ (Weiss et al., 2006). We assumed that this phenomenon demonstrates a Th1 to Th2 cytokine shift, a mechanism that may prevent development of diabetes (Elenkov et al., 2005).

The results of the present study indicate that treatment of 11–14 weeks old female NOD mice, either in a latent diabetes stage (after 14 weeks) or with initial symptoms of diabetes (appearing up to 14 weeks), with CBD for 4 weeks, led to sustained inhibition of insulinitis. CBD treatment induced a qualitative modification of the islets infiltrated by mononuclear cells,

Table 2
Histological analysis of pancreas tissue from mice treated with CBD, or with vehicle or untreated

Treatment	No. of scored islets	Intact islets	Total ruined islets	Full infiltrated islets	Partial infiltrated islets	Percent intact islets
Control	73	6	29	28	5	8
Vehicle	94	12	30	43	7	13
CBD	140	108	–	12	15	77

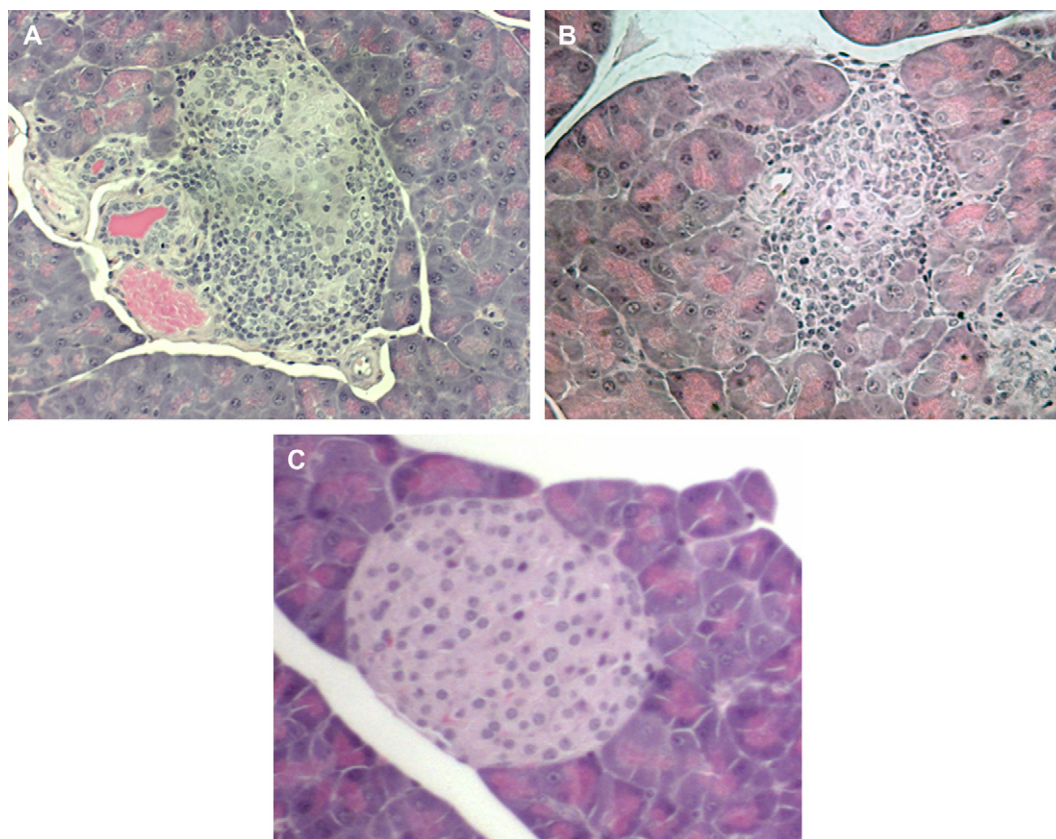


Fig. 2. Representative histology of Hematoxylin-Eosin stained pancreases from (A) untreated control mice, mag. $\times 800$, (B) vehicle treated mice, mag. $\times 800$, and (C) CBD-treated mice, mag. $\times 800$. Abundant mononuclear cell invasion into islets and pri-insulinitis in the pancreata of untreated and vehicle treated mice (A and B). The pancreas of CBD-treated non-diabetic mice showed neither cell-infiltration nor islet destruction (C).

inhibited specific destruction of the islets and eventually prevented diabetes (Fig. 2, Table 2). CBD treatment reduced diabetes from 86% in untreated mice to 32% in CBD-treated mice. The direct effect of CBD on glucose load tests (i.e. 2 g/kg i.p.) in BALB/c versus NOD mice showed no difference in treatment with saline compared to CBD. This result shows no direct effect of CBD on glucose levels in the blood. It was also demonstrated that CBD treatment inhibited IL-12 production by splenocytes. IL-12 is a cytokine that plays a major role in autoimmunity including diabetes (Rothe et al., 1996; Trembleau et al., 1995; Rabinovitch et al., 1996; Trinchieri, 1995). Macrophages and dendritic cells are known to produce IL-12, which triggers generation of IFN- γ (Verma et al., 2006).

Th1 cytokines activate cytotoxic T cells and stimulate macrophages to produce proinflammatory cytokines and nitrogen free radicals (NO). Macrophages and dendritic cells are known to produce IL-12, which triggers generation of IFN- γ (Verma et al., 2006). All these molecules contribute to islet destruction. These results indicate that, CBD suppression of macrophage IL-12 production can inhibit Th1 mediated autoimmunity.

The difference between Th1 and Th2 cells in diabetes are discussed in various studies (Rabinovitch 2003; Meierhoff et al., 2002). The aim in treatment of diabetes is to decrease cytokine levels of the Th1 related cytokines IFN- γ and IL-12 and to increase Th2 related cytokines IL-4, IL-10.

The data in our NOD study suggest that IL-6 participates in islet inflammation, and CBD, by significant suppression of IL-6 production, decreases islet destruction. Additional factors, such as proinflammatory cytokines and mediators presumably participate in the pathological events.

Furthermore, in this study, we show that immune cells from CBD-treated mice enhance *in vitro* IL-4 and IL-10 cytokine production. These cytokine alterations correlated with disease suppression. Several reports support that immunological interventions, which enhance Th2 immune responses in NOD mice are protective against the disease. For example, treatment of NOD mice with IL-4, a Th2 cytokine, prevents overt diabetes (Rapoport et al., 1993; Cameron et al., 1997; Tominaga et al., 1998) and local expression of IL-4 in pancreatic beta cells in transgenic mice inhibits insulinitis (Mueller et al., 1996; Shi et al., 2006) have demonstrated that abrogation of recurrent autoimmunity requires host IL-4. In addition, levels of IL-10, a Th2 associated cytokine, correlate with protection (Mueller et al., 1996) and IL-4 and IL-10 inhibit diabetes by suppressing Th1 cytokine production in islet grafts (Rabinovitch et al., 1995).

Histological examination of the pancreas indicated the absence of insulinitis in 77% of the islets in the CBD-treated group, compared to 13% in the pancreas taken from vehicle treated mice; thus, β cell destruction in the CBD-treated mice was markedly suppressed (Table 2, Fig. 2C).

We have previously shown that CBD treatment of young NOD mice preserved more than 85% of the islets as determined by histological analysis (Weiss et al., 2006). We also reported that CBD inhibited TNF- α production in vitro and in vivo (Malfait et al., 2000) and have noted the same trend now in a different system. We have also previously seen that nitric oxide formation is decreased in vitro (Ben-Shabat et al., 2006). As TNF- α and nitric oxide are important mediators of IL-1 β induced β -cell damage (Corbett et al., 1991) it is possible that this inhibition represents one of the protective pathways through which CBD acts.

It was recently suggested by Carrier et al. (2006) that CBD has anti-inflammatory properties due to its binding capacity to A2A adenosine receptor, which leads to inhibition of adenosine uptake and enhancement of endogenous adenosine signaling. It was further demonstrated that adenosine transport inhibitors suppress the production in vivo of TNF- α , one of the key cytokines of inflammation. Thus the observed CBD effect in diabetes may be due, at least partly, to its binding and activation of the A2A receptor. Indeed, there are several reports which indicate that A2A receptor activation is involved in causing diabetes although in some cases it may represent the result of diabetes (Grden et al., 2005; Thong and Graham, 2002; Awad et al., 2006).

It is possible that CBD acts by antagonism of cannabinoid agonists. Bermudez-Siva et al. (2006) demonstrated that activation of cannabinoid CB1 receptors induces glucose intolerance in mice and Juan-Pico et al. (2006) reported that the endocannabinoid 2-arachidonoyl glycerol (2-AG), through CB2 receptors, affects Ca²⁺ signals in β -cells and as a consequence decreases insulin secretion. As CBD was recently found to be an antagonist of CB1 and CB2 receptor agonists (Thomas et al., 2007) it is possible that via this route it affects the development of diabetes in NOD mice. Indeed the CB1 cannabinoid receptor antagonist, rimonabant, was reported to lead to lower plasma glucose and insulin levels, as well as improved insulin resistance in animals and to an improvement in glycemic control in prediabetic and type 2 diabetic patients (Gelfand and Cannon, 2006).

We have also demonstrated that in vivo CBD treatment augments IL-4 and IL-10 production, which can prevent the development of diabetes through a Th1 \rightarrow Th2 cytokine shift, as other studies have noted (Weiss et al., 2006).

An extensive list of therapeutic strategies has been shown to provide some protection from the development of overt diabetes in the NOD mouse model. A number of clinically available immunomodulatory compounds, such as cyclosporine, FK506 and Rapamycin may suppress the disease progression, but some of them are toxic or they can augment the risk of infection and tumorigenesis (Kai et al., 1993; Baeder et al., 1992).

Successful induction of remission in 17 of 25 diabetic mice by CBD treatment long after cessation of treatment, indicates that CBD exerts long lasting beneficial influence on β -cell mass and glycemia. We assume that by effectively controlling autoimmune effector T-cells or by influencing cytokine balance resulting in a Th1 \rightarrow Th2 shift, CBD caused amelioration of autoimmune insulinitis and allowed endogenous

precursor cells to slowly give rise to increased β -cell mass and thus maintain normoglycemia.

Although insulin replacement therapies provide chronic care for people with type 1 diabetes, this treatment does not restore normal glucose control and is associated with significant risk of hypoglycemia. Controlling autoimmunity may represent an alternative therapeutic pathway aimed at restoring pancreatic cell mass and function.

CBD is not psychoactive and has anti-inflammatory and anti-autoimmune properties. Based on the above presented results, on the previously documented anti-inflammatory effects of CBD (Malfait et al., 2000) and on its clinical safety (Cunha et al., 1980), it seems reasonable to consider the use of CBD for controlling type 1 diabetes at an early stage of the disease.

Acknowledgements

We acknowledge support by the US National Institute on Drug Abuse (DA-9789 to RM) and the Concern Foundation, Los Angeles (to RG).

References

- Anderson, M.S., Bluestone, J.A., 2005. The NOD mouse: a model of immune dysregulation. *Annu. Rev. Immunol.* 23, 447–485.
- Atkinson, M.A., Leiter, E.H., 1999. The NOD mouse model of type 1 diabetes: as good as it gets? *Nat. Med.* 5, 601–604.
- Awad, A.S., Huang, L., Ye, H., Duong, E.T., Bolton, W.K., Linden, J., Okusa, M.D., 2006. Adenosine A2A receptor activation attenuates inflammation and injury in diabetic nephropathy. *Am. J. Physiol. Renal Physiol.* 290, F828–F837.
- Baeder, W.L., Sredy, J., Sehgal, S.N., Chang, J.Y., Adams, L.M., 1992. Rapamycin prevents the onset of insulin-dependent diabetes mellitus (IDDM) in NOD mice. *Clin. Exp. Immunol.* 89, 174–178.
- Ben-Shabat, S., Hanus, L.O., Katzavian, G., Gallily, R., 2006. New cannabinoid derivatives: synthesis, binding to cannabinoid receptors and evaluation of their antiinflammatory activity. *J. Med. Chem.* 49, 1113–1117.
- Bermudez-Siva, F.J., Serrano, A., Diaz-Molina, F.J., Sanchez Vera, I., Juan-Pico, P., Nadal, A., Fuentes, E., Rodriguez de Fonseca, F., 2006. Activation of cannabinoid CB1 receptors induces glucose intolerance in rats. *Eur. J. Pharmacol.* 531, 282–284.
- Cameron, M.J., Arreaza, G.A., Zucker, P., Chensue, S.W., Strieter, R.M., Chakrabarti, S., Delovitch, T.L., 1997. IL-4 prevents insulinitis and insulin-dependent diabetes mellitus in nonobese diabetic mice by potentiation of regulatory T helper-2 cell function. *J. Immunol.* 159, 4686–4692.
- Carrier, E.J., Auchampach, J.A., Hillard, C.J., 2006. Inhibition of an equilibrative nucleoside transporter by cannabidiol: a mechanism of cannabinoid immunosuppression. *Proc. Natl. Acad. Sci. U.S.A.* 103, 7895–7900.
- Chan, S.H., Perussia, B., Gupta, J.W., Kobayashi, M., Pospisil, M., Young, H.A., Wolf, S.F., Young, D., Clark, S.C., Trinchieri, G., 1991. Induction of interferon gamma production by natural killer cell stimulatory factor: characterization of the responder cells and synergy with other inducers. *J. Exp. Med.* 173, 869–879.
- Corbett, J.A., Lancaster, J.R., Sweetland, M.A., 1991. Interleukin-1 beta-induced formation of EPR-detectable iron-nitrosyl complexes in islets of Langerhans. Role of nitric oxide in interleukin-1 beta-induced inhibition of insulin secretion. *J. Biol. Chem.* 266, 21351–21354.
- Crane, I.J., Forrester, J.V., 2005. Th1 and Th2 lymphocytes in autoimmune disease. *Crit. Rev. Immunol.* 25, 75–102.
- Cunha, J.M., Carlini, E.A., Pereira, A.E., Ramos, O.L., Pimentel, C., Gagliardi, R., Sanvito, W.L., Lander, N., Mechoulam, R., 1980. Chronic administration of cannabidiol to healthy volunteers and epileptic patients. *Pharmacology* 21, 175–185.

- Elenkov, I.J., Iezzoni, D.G., Daly, A., Harris, A.G., Chrousos, G.P., 2005. Cytokine dysregulation, inflammation and well-being. *Neuroimmunomodulation* 12, 255–269.
- Gaoni, Y., Mechoulam, R., 1971. The isolation and structure of Δ^1 -THC and other neutral cannabinoids from hashish. *J. Am. Chem. Soc.* 93, 217–224.
- Gelfand, E.V., Cannon, C.P., 2006. Rimonabant: a cannabinoid receptor type 1 blocker for management of multiple cardiometabolic risk factors. *J. Am. Coll. Cardiol.* 47, 1919–1926.
- Grden, M., Podgorska, M., Szutowicz, A., Pawelczyk, T., 2005. Altered expression of adenosine receptors in heart of diabetic rat. *J. Physiol. Pharmacol.* 56, 587–597.
- Gross, D.J., Weiss, L., Reibstein, I., Van den Brand, J., Okamoto, H., Clark, A., 1998. Amelioration of diabetes in nonobese diabetic mice with advanced disease by linomide-induced immunoregulation combined with Reg protein treatment. *Endocrinology* 139 (5), 2369–2374.
- Ji, Y.H., Weiss, L., Zeira, M., Abdul-Hai, A., Har-Noy, M., Slavin, S., 2003. Allogeneic cell-mediated immunotherapy of leukemia with immune donor lymphocytes to upregulate antitumor effects and downregulate antihost responses. *Bone Marrow Transplant.* 32, 495–504.
- Juan-Pico, P., Fuentes, E., Bermudes-Silva, F.J., Javier Diaz-Molina, F., Ripoll, C., Rodriguez de Fonseca, F., Nadal, A., 2006. Cannabinoid receptors regulate Ca^{2+} signals and insulin secretion in pancreatic β -cells. *Cell Calcium* 39, 155–162.
- Kai, N., Motojima, K., Tsunoda, T., Kanematsu, T., 1993. Prevention of insulinitis and diabetes in nonobese diabetic mice by administration of FK506. *Transplantation* 55, 936–940.
- Keymeulen, B., Vandemeulebroucke, E., Ziegler, A.G., Mathieu, C., Kaufman, L., Hale, G., Gorus, F., Goldman, M., Walter, M., Candon, S., Schandene, L., Crenier, L., De Block, C., Seigneurin, J.M., De Pauw, P., Pierard, D., Weets, I., Rebello, P., Bird, P., Berrie, E., Frewin, M., Waldmann, H., Bach, J.F., Pipeleers, D., Chatenoud, L., 2005. Insulin needs after CD3 antibody therapy in new-onset type 1 diabetes. *N. Engl. J. Med.* 352, 2598–2608.
- Malfait, A.M., Gallily, R., Sumariwalla, P.F., Malik, A.S., Andreakos, E., Mechoulam, R., Feldmann, M., 2000. The non-psychoactive cannabis-constituent cannabidiol is an oral anti-arthritis therapeutic in murine collagen-induced arthritis. *Proc. Natl. Acad. Sci. U.S.A.* 97, 9561–9566.
- Mandrup-Poulsen, T., 2003. Beta cell death and protection. *Ann. NY Acad. Sci.* 1005, 32–42.
- Meierhoff, G., Ott, P.A., Lehmann, P.V., Schloot, N.C., 2002. Cytokine detection by ELISPOT: relevance for immunological studies in type 1 diabetes. *Diabetes Metab. Res. Rev.* 18, 367–380.
- Mueller, R., Krahl, T., Sarvetnick, N., 1996. Pancreatic expression of interleukin-4 abrogates insulinitis and autoimmune diabetes in nonobese diabetic (NOD) mice. *J. Exp. Med.* 184, 1093–1099.
- Rabinovitch, A., 2003. Immunoregulation by cytokines in autoimmune diabetes. *Adv. Exp. Med. Biol.* 520, 159–193.
- Rabinovitch, A., Suarez-Pinzon, W.L., Sorensen, O., Bleackley, R.C., Power, R.F., Rajotte, R.V., 1995. Combined therapy with interleukin-4 and interleukin-10 inhibits autoimmune diabetes recurrence in syngeneic islet-transplanted nonobese diabetic mice. Analysis of cytokine mRNA expression in the graft. *Transplantation* 60, 368–374.
- Rabinovitch, A., Suarez-Pinzon, W.L., Sorensen, O., 1996. Interleukin 12 mRNA expression in islets correlates with beta-cell destruction in NOD mice. *J. Autoimmun.* 9, 645–651.
- Rapoport, M.J., Jaramillo, A., Zipris, D., Lazarus, A.H., Serreze, D.V., Leiter, E.H., Cyopick, P., Danska, J.S., Delovitch, T.L., 1993. Interleukin 4 reverses T-cell proliferative unresponsiveness and prevents the onset of diabetes in nonobese diabetic mice. *J. Exp. Med.* 178, 87–99.
- Raz, I., Elias, D., Avron, A., Tamir, M., Metzger, M., Cohen, I.R., 2001. Beta-cell function in new-onset type 1 diabetes and immunomodulation with a heat-shock protein peptide (DiaPep277): a randomised, double-blind, phase II trial. *Lancet* 358, 1749–1753.
- Rothe, H., Burkart, V., Faust, A., Kolb, H., 1996. Interleukin-12 gene expression is associated with rapid development of diabetes mellitus in non-obese diabetic mice. *Diabetologia* 39, 119–122.
- Shi, Q., Wang, D., Hadley, G.A., Farber, D.L., Bartlett, S.T., 2006. Abrogation of recurrent autoimmunity in the NOD mouse: a critical role for host interleukin 4. *Surgery* 140, 281–288.
- Thomas, A., Baillie, G.L., Phillips, A.M., Razdan, R.K., Ross, R.A., Pertwee, R.G., 2007. Cannabidiol displays unexpectedly high potency as an antagonist of CB(1) and CB(2) receptor agonists in vitro. *Br. J. Pharmacol.* 150, 613–623.
- Thong, F.S., Graham, T.E., 2002. The putative roles of adenosine in insulin- and exercise-mediated regulation of glucose transport and glycogen metabolism in skeletal muscle. *Can. J. Appl. Physiol.* 27, 152–178.
- Tominaga, Y., Nagata, M., Yasuda, H., Okamoto, N., Arisawa, K., Moriyama, H., Miki, M., Yokono, K., Kasuga, M., 1998. Administration of IL-4 prevents autoimmune diabetes but enhances pancreatic insulinitis in NOD mice. *Clin. Immunol. Immunopathol.* 86, 209–218.
- Trembleau, S., Penna, G., Bosi, E., Mortara, A., Gately, M.K., Adorini, L., 1995. Interleukin 12 administration induces T helper type 1 cells and accelerates autoimmune diabetes in NOD mice. *J. Exp. Med.* 81, 817–821.
- Trinchieri, 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.
- Verma, N.D., Boyd, R., Robinson, C., Plain, K.M., Tran, G.T., Hall, B.M., 2006. Interleukin-12p70 prolongs allograft survival by induction of interferon gamma and nitric oxide production. *Transplantation* 82, 1324–1333.
- Weiss, L., Zeira, M., Reich, S., Har-Noy, M., Mechoulam, R., Slavin, S., Gallily, R., 2006. Cannabidiol lowers incidence of diabetes in non-obese diabetic mice. *Autoimmunity* 39, 143–151.