Immunomodulatory Effects of Cannabidiol: Relevance in Multiple Sclerosis

Alessia Furgiuele[1], Alex Mabou Tagne[1], Barbara Pacchetti[2], Mikael Sodergren[2], Marco Cosentino[1] and Franca Marino[1]

(1) Center for Research in Medical Pharmacology, University of Insubria, Varese, I, and (2) Emmac Life Sciences, London, UK

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Introduction

Multiple sclerosis (MS) is an autoimmune disorder of the CNS characterized by inflammation, demyelination and neurodegeneration. Available evidence suggests the involvement of immune processes in MS pathogenesis and progression, with CD4+ T helper lymphocytes playing a key role [1]. Cannabidiol (CBD), is the main non-psychoactive component of cannabis plants (*Cannabis sativa L.*) and together with delta-9- tetrahydrocannabinol (THC) is contained approximately in a 1:1 ratio in the oral spray Nabiximol (Sativex®). This is a standard treatment for chronic pain and spasticity in multiple sclerosis.
Emerging evidence suggests a role of CBD in modulating immune function of peripheral blood mononuclear cells (PBMC) and T lymphocytes [2]. However, the immunomodulating potential of CBD received so far little attention.

Aim

To evaluate the effects of CBD on human PBMC functional responses, including cell proliferation and cytokine production.

Materials and Methods

Test Substance - Pure CBD (code n. 2 000 01 P) was kindly provided by EMMAC Life Sciences (https://www.emmac.com/).
Isolation of human PBMC - Human PBMC were isolated from buffy coats of healthy donors by Ficoll-Paque Plus density-gradient centrifugation, as previously described [3].
Cytokine mRNA expression assay - mRNA expression was assessed in freshly isolated PBMCs resuspended in RPMI/10% FBS, stimulated with anti-CD3/anti-CD28 Abs (0.1 µg/ml) and cultured for 48h at 37°C under a 95/5% O_2/CO_2 atmosphere alone or in the presence of CBD 1 µM. Following incubation, cells were harvested, and mRNA was extracted and quantified by real-time PCR using assay-on-demand kits [4]. Gene expression level in a given sample was presented as $2^{-\Delta Ct}$ where ΔCt = [Ct (sample) - Ct (housekeeping gene)]. Relative expression was determined by normalization to 18S cDNA.
Enzyme-linked immunosorbent assays (ELISA): PBMCs production of TNF-α and IFN-γ was measured in culture supernatants using commercial ELISA kits (Thermofisher, Italy) according to manufacturer's instructions.
Flow cytometry analysis - Measurement of PBMC proliferation was performed after 120 h, by means of staining with Cell Proliferation Dye eFluor 670 (eBioscience-Prodotti Gianni, Italy, code 65-0840) and flow cytometric analysis [3]. PBMC were cultured at 37°C in a moist atmosphere of 5% CO_2 alone or in the presence of CBD 1 µM in resting conditions or added with anti-CD3/anti-CD28 Abs (0.1 µg/ml).

Results

Stimulation of PBMC with anti-CD3/anti-CD28 Abs (0.1µg/mL) increased TNF-α and IFN-γ mRNA levels by about 8- and 11-folds respectively ($P<0.01$ vs resting levels). Co-incubation of stimulated PBMC with CBD 1 µM reduced TNF-α mRNA levels down to 32.4% and IFN-γ down to 21.9% of stimulated levels **(Figure 1)**, except for one single preparation in which CBD 1 µM increased TNF-α and IFN-γ mRNA levels.
Cell supernatants for ELISA measurement of TNF-α and IFN-γ are currently stored at -80°C and will be soon processed.
Treatment of PBMC with anti-CD3/anti-CD28 Abs increased the percentage of proliferating cells on average from 1.2% to 81.2% ($P<0.01$ vs resting levels]. Co-incubation with CBD 1 µM showed no apparent effects **(Figure 2)**.

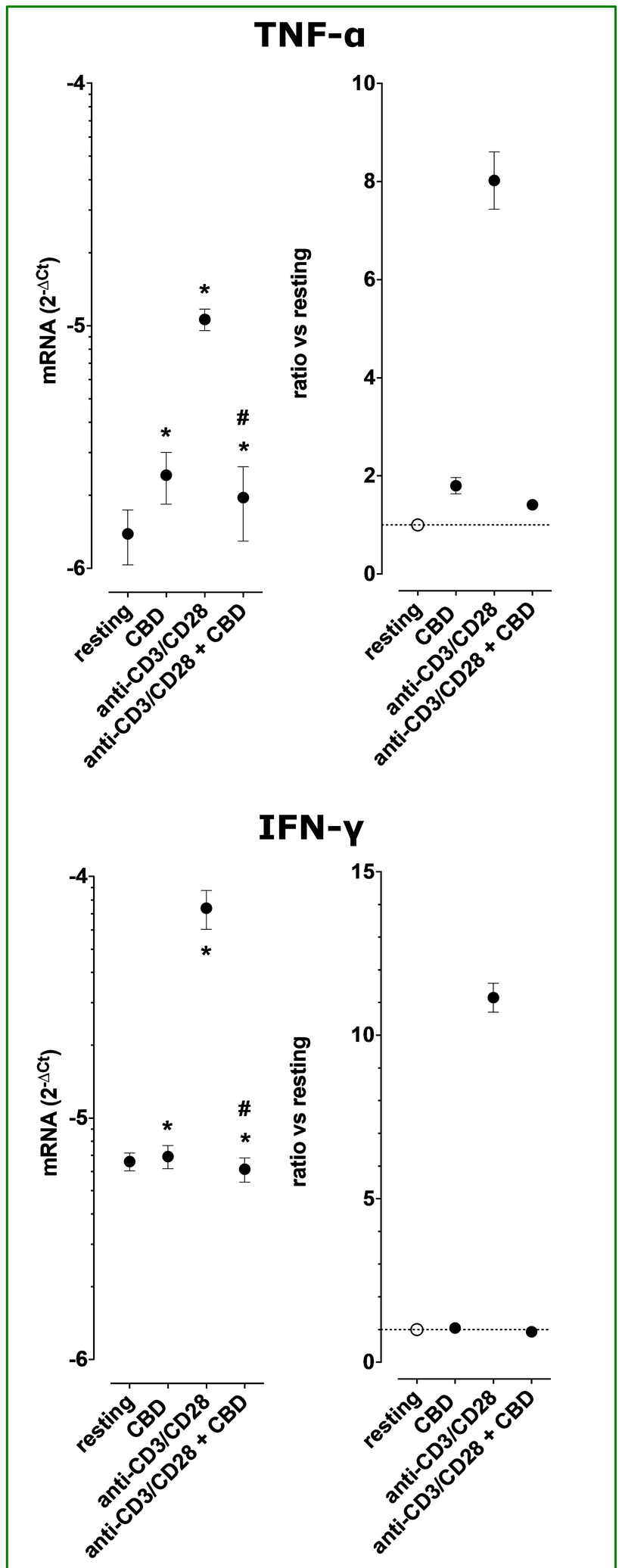

Figure 1. *Effect of CBD on TNF-α and IFN-γ mRNA levels in human PBMC. Values are means±SD of 8-9 experiments and are shown as absolute values (left) and as ratio vs resting levels (right). *= P<0.01 vs resting levels and # = P<0.01 vs anti-CD3/CD28 Abs alone by paired Student's t test.*

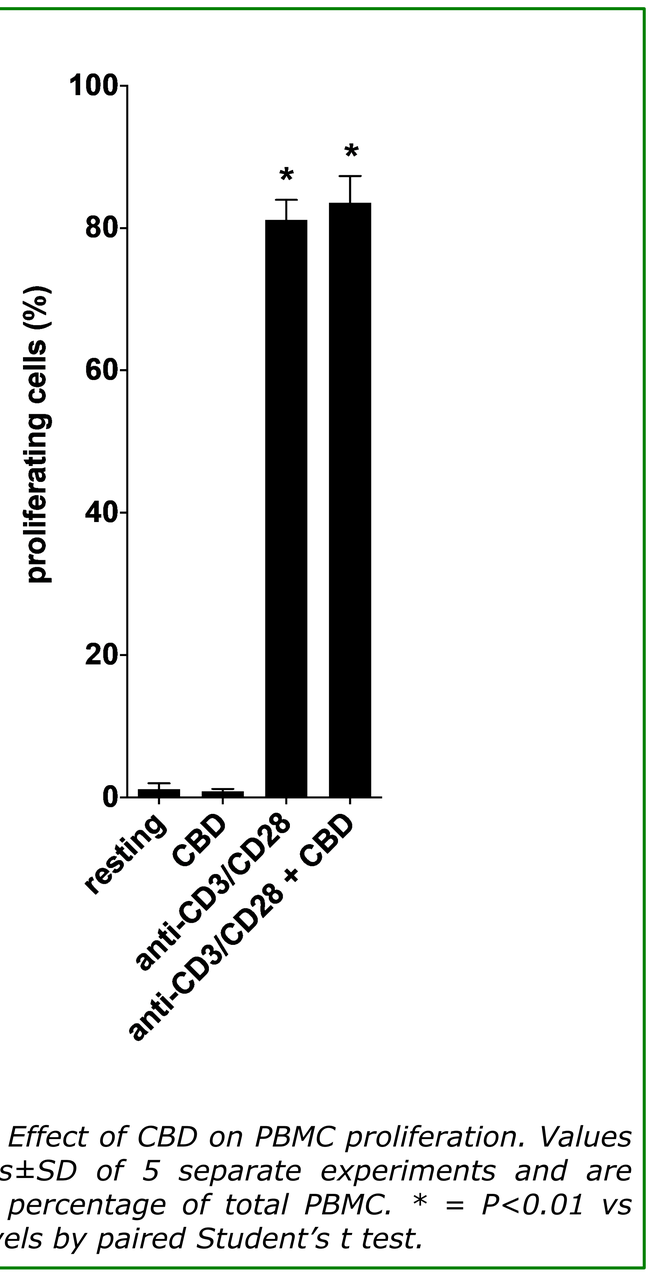

Figure 2. *Effect of CBD on PBMC proliferation. Values are means±SD of 5 separate experiments and are shown as percentage of total PBMC. * = P<0.01 vs resting levels by paired Student's t test.*

Conclusions

Preliminary experiments about the effects of CBD on functional responses of human PBMC, including cell proliferation and cytokine production, suggest that CBD may inhibit TNF-α and IFN-γ production without affecting cell proliferation.
Further research is required to unravel the effect of CBD on biologically active proteins as well as to pursue the potential immunomodulating effects of these selected therapeutic agents on CD4+T lymphocytes subsets.
In view of the role of TNF-α and IFN-γ in MS, the immunomodulatory effect of CBD on human PBMC may have significant therapeutic relevance.

References

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Correspondence to:
Alessia Furgiuele, PharmD
PhD student in Clinical and Experimental Medicine and Medical Humanities
Center of Research in Medical Pharmacology, University of Insubria
Via Monte Generoso n. 71, 21100 Varese, VA, Italy
Phone: +39 0332 217410, Fax: +39 0332 217409
E-mail: afurgiuele@uninsubria.it

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