Dopamine suppresses Th17-cells function in multiple sclerosis.

M. Melnikov*, A. Sviridova, M. Pashenkov, A. Boyko

Department of Neurology, Neurosurgery and Medical Genetic of Pirogov Russian National Research University & Laboratory of Clinical Immunology, Institute of Immunology, Moscow

Introduction

In multiple sclerosis (MS), psychological stress may enhance the production of pro-inflammatory cytokines and promote exacerbations of the disease. Dopamine is one of the key neurotransmitters in CNS that is involved in developing psychological impairments and also has an immunomodulatory effect.[1]

Objective

This study aimed to clarify the effects of dopamine on Th17-cells function in MS.

Materials and methods

Patients

Forty patients with a documented diagnosis of MS according to the McDonald criteria (modification 2010) were examined.[2] All patients had a relapsing–remitting form of the disease. Their main demographic and clinical characteristics are shown in **Table 1**. All patients were subjected to a standard neurological examination with assessment of the EDSS score.[3] All patients were non-smoking and had no mental disorders, according to the Beck Depression Inventory and cognitive impairments, according to Montreal Cognitive Assessment. All patients were examined during clinical remission. All patients had been treated with glatiramer acetate for more than one year. At the time of blood sampling, all the patients studied had not been treated with corticosteroid or SSRIs therapy for more than six months. The control group consisted of twenty five healthy donors matched with patients by sex and age (**Table 1**). All patients signed the written informed consent to participate in this study. The study was approved by the ethics committee of the Russian National Research Medical University (protocol №192).

Flow cytometry

The percentage of circulating Th17-cells was determined by flow cytometry. Th17-cells were identified as $CD4^+CD161^+$ events and quantified as percentage of these events in relation to all $CD4^+$-T-cells.[4,5]

CD4+ T-cells cultures and stimulation

To determine functional activity of Th17- and Th1-cells, $CD4^+$ T-cells were isolated from PBMCs by magnetic cell sorting (Milteny Biotec, Germany). Then $CD4^+$ T-cells at a concentration of 8×10^4 per 200 µl per well were plated in 96-well U-bottomed culture plates in duplicate and stimulated with anti-CD3/anti-CD28-microbeads (Life Technologies, Norway) for 72 hours whereafter culture supernatants were collected and stored at –70° C.

To assess the effect of dopamine on Th17-cells function, $CD4^+$ T-cells were cultured in the presence of dopamine (Sigma, USA) at a concentration of 10^{-5} M whereafter anti-CD3/anti-CD28 microbeads were added to the cultures. To study the involvement of dopaminergic receptors in dopamine-mediated modulation of cytokine production, some samples of $CD4^+$ T-cells were pre-incubated with antagonist of D1-like (SCH23390) or D2-like (sulpiride) dopaminergic receptors (both from Sigma) a concentration of 10^{-5} M whereafter dopamine and anti-CD3/anti-CD28 microbeads were added to the cultures. In addition, $CD4^+$ T-cells were pre-incubated in the presence of antagonist of D1- or D2-like dopaminergic receptors and activated by anti-CD3/anti-CD28 microbeads.

Dopamine, DOPAC and homovanillic acid (HVA) evaluation. The levels of dopamine, DOPAC and HVA in blood plasma were determined by high-performance liquid chromatography (HPLC). Data are expressed as pmol/ml.

Cytokine evaluation

Levels of IL-17, IFN-γ, and GM-CSF in the supernatants were determined by ELISA (Invitrogen, USA). In all cases of ELISAs, the instructions of the kit manufacturers were followed. Data are expressed as pg/ml or as the percentage of cytokine production by stimulated cells in the absence of dopamine and antagonist of dopaminergic receptors.

Statistical analysis

The statistical analysis of the results was performed using Prizm 6 software.

The nonparametric Wilcoxon test was used to compare two groups. Differences were considered statistically significant if $p<0.05$.

Results

The percentages of $CD4^+$ T-cells, Th17-cells as well as IL-17, IFN-γ, and GM-CSF production by activated $CD4^+$ T-cells in MS patients and healthy subjects were comparable (**Table 2, Table 3**). The concentrations of dopamine and DOPAC were also comparable between the groups (**Table 2**). The concentration of HVA were lower in MS patients (**Table 2**).

Dopamine suppressed IL-17, IFN-γ, and GM-CSF production by $CD4^+$ T-cells in both groups (**Fig 1)** without affecting cell viability and proliferative responses (data not shown). The blockade of D2-like dopaminergic receptors with specific antagonist reduced the inhibitory effect of dopamine on IL-17 production in MS patients (**Fig 2A**) and GM-CSF production in both groups (**Fig 2E and Fig 2F**), while blockade of D1-like dopaminergic receptors increased the inhibitory effect of dopamine on GM-CSF production in both groups (**Fig 2E and Fig 2F**). Blockade of D1-dopaminergic receptors reduced IL-17 and GM-CSF production in both groups (**Fig 2A, Fig 2B, Fig 2E, Fig 2F**)

Conclusions

These data suggest an anti-inflammatory role of dopamine in MS which could be mediated by the D2-like dopaminergic receptors.

References

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Disclosure

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Table 1. Clinical and demographic characteristics of MS patients and healthy subjects. Data are medians (25^{th}; 75^{th} percentiles).

Factor	MS patients, n=40	Healthy subjects, n=25
Age, years	29 (24; 33)	30.0 (28.0; 34.5)
Men/women (% women)	13/27 (67.5)	10/15 (60)
Duration of MS, years	2 (2; 5)	NA*
EDSS score	1.5 (1.5; 2)	NA*

* NA – not applicable

Table 2. Distribution of subpopulations of blood CD4+ and Th17-cells (flow cytometry) and concentration of dopamine, DOPAC and HVA in blood plasma (HPLC) in MS patients and in healthy subjects.

Factor	MS patients, n=40	Healthy subjects, n=25
$CD4^+$ T-cells, % relative to lymphocytes	37.7 (35.5; 43.7)	39.0 (35.0; 42.0)
$CD4^+CD161^+$-Th17-cells, % relative to $CD4^+$ T-cells	20.9 (16.9; 23.5)	21.0 (19.4; 23.8)
Dopamine, pmole/ml	2.72 (1.66; 4.13)	1.93 (1.2; 2.6)
DOPAC, pmole/ml	1.79 (1.18; 2.64)	2.56 (1.2; 4.7)
HVA, pmole/ml	30.54* (14; 69.17)	71.81 (31; 110.59)

*p<0.05 compared with healthy subjects

Table 3. The secretion of cytokines by CD4+ T-cells in MS patients and in healthy subjects.

Cytokine	Stimulation	MS patients, n=40	Healthy subjects, n=25
IL-17, pg/ml	None	1 (0; 6)	3 (0; 9)
	Anti-CD3 / anti-CD28	900 (379; 1130)	857 (332; 1383)
IFN-γ, pg/ml	None	6 (3; 23)	5 (1; 28)
	Anti-CD3 / anti-CD28	4917 (2745; 8144)	4458 (2025; 5207)
GM-CSF, pg/ml	None	0 (0; 5)	5 (1; 10)
	Anti-CD3 / anti-CD28	440 (204; 778)	482 (121; 914)

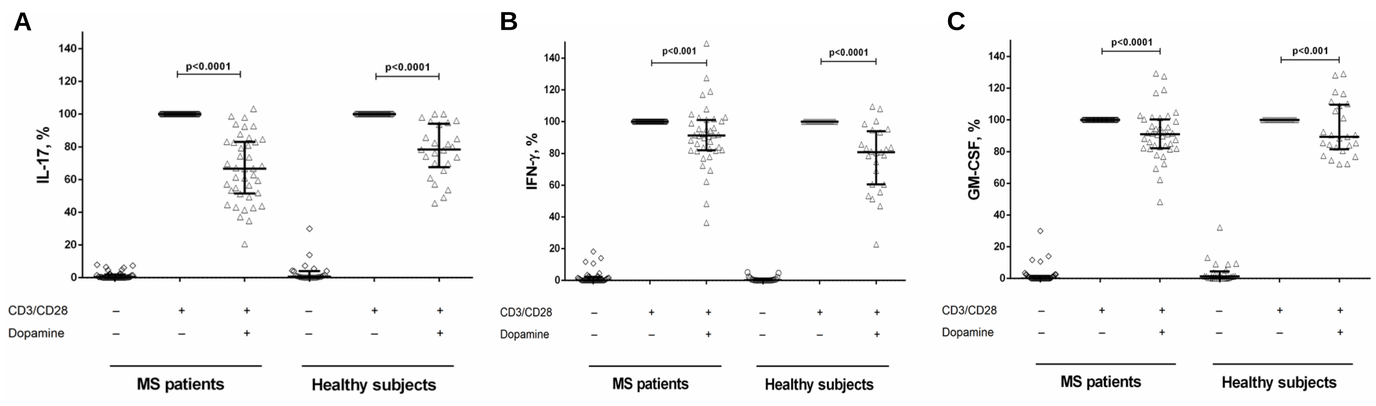

Figure 1. The influence of dopamine on IL-17 (A), IFN-γ (B), and GM-CSF (C) production by stimulated CD4+ T-cells in MS patients and healthy subjects. Horizontal lines at the graphs correspond to the median and whiskers indicate to 25th and 75th percentiles.

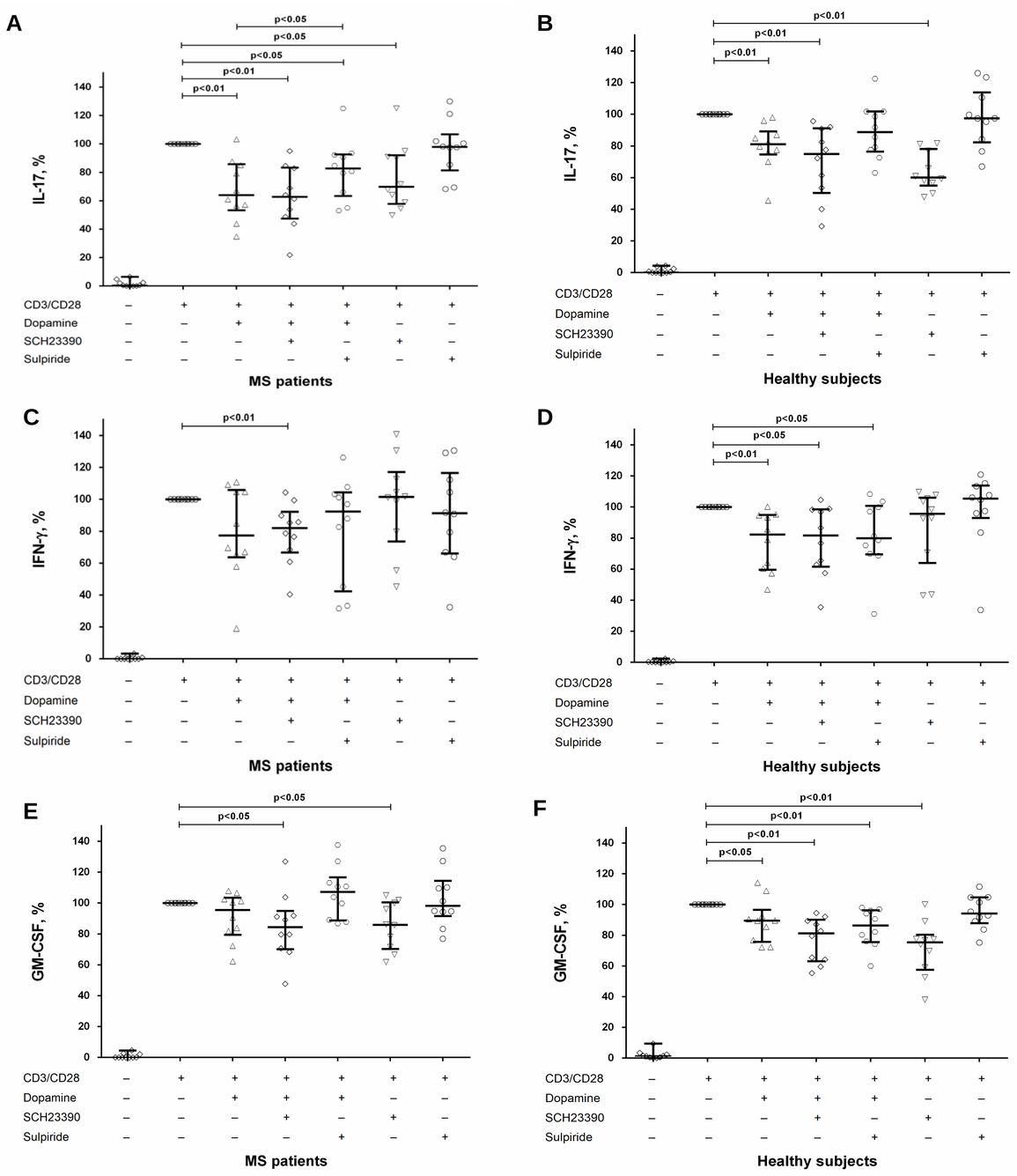

Figure 2. The role of D1- and D2-dopaminergic receptors in dopamine-mediated suppression of IL-17 (A and B) IFN-γ (C and D), and GM-CSF (E and F) production by stimulated $CD4^+$ T-cells in MS patients and healthy subjects. Horizontal lines at the graphs correspond to the median and whiskers indicate to 25^{th} and 75^{th} percentiles. The median values of MS and control groups were compared and the p values are indicated at the figure.