Dopamine suppresses Th17-cells function in multiple sclerosis.

M. Melnikov\(^*,\) A. Sviridova, M. Pashenkov, A. Boyko
Department of Neurology, Neurosurgery and Medical Genetics of Pirogov Russian 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.\(^2\) All patients were non-smoking and had no medical disorders, according to the Beck Depression Inventory and cognitive impairments, according to the 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 SSRI therapies 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.\(^1\)

**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 whereas cytokine 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\(^−7\) M whereas 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 (SCH33992) or D2-like (sluxipride) dopaminergic receptors (both from Sigma) at a concentration of 10\(^−7\) M whereas 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 nmol/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 Prism 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 (P<0.05) with blockade of 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-like 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**


**Disclosure**

The reported study was funded by RFBR according to the research project № 18-31-00439.