Fluoxetine modulates macrophages-induced Th17-immune response in multiple sclerosis.

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Introduction

In multiple sclerosis (MS), psychological stress may enhance the production of pro-inflammatory cytokines and promote exacerbations of the disease. Serotonin (5-hydroxytryptamine (5-HT) is one of the key neurotransmitters in CNS that is involved in developing psychological and cognitive impairments. Fluoxetine is a selective serotonin reuptake inhibitor (SSRI), which also has an immunomodulatory effect.[1]

Objective

The aim of this study was to clarify the influence of fluoxetine on Th17-cells and macrophages-induced Th17-immune response, which plays a critical role in MS pathogenesis.

Materials and methods

Patients

Thirty 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 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).

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

Factor	MS patients, n=30	Healthy subjects, n=20
Age, years	29 (24; 32)	30.0 (28; 35)
Men/women (% women)	10/20 (66.7)	8/12 (60)
Duration of MS, years	2 (2; 5)	NA*
EDSS score	1.5 (1.5; 2)	NA*

* NA – not applicable

CD4+ T-cells cultures and stimulation.

CD4+ T-cells were isolated from PBMCs by magnetic cell sorting and stimulated with anti-CD3/anti-CD28-microbeads for 72 hours whereafter culture supernatants were collected and stored at –70° C. To assess the effect of fluoxetine on Th17-cells function, CD4+ T-cells were cultured in the presence of fluoxetine (10^{-6}) and stimulated with anti-CD3/anti-CD28 microbeads. To study the involvement of 5-HT_{2B}-receptor in fluoxetine-mediated modulation of cytokine production, some samples of CD4+ T-cells were pre-incubated with antagonist (RS 127445) or agonist (BW 7233C86) of 5-HT_{2B}-receptor (both at 10^{-6} M) whereafter fluoxetine and anti-CD3/anti-CD28 microbeads were added to the cultures.

Macrophages generation and stimulation.

To generate human immature macrophages, monocytes were isolated from PBMCs by magnetic cell sorting. Then monocytes were cultured for 6 days in CCM supplemented with 40 ng/ml recombinant human granulocyte-macrophage colony-stimulating factor (GM-CSF). To assess the effect of fluoxetine on macrophages-induced Th17-immune response, monocyte-derived macrophages were incubated in the presence of fluoxetine (10^{-6}) whereafter lipopolysaccharide (LPS) were added to the cultures. Then, LPS-activated macrophages were co-cultured with autologous CD4+ T cells (in the presence of staphylococcal enterotoxin B) for 72 hrs.

Cytokine evaluation

Levels of IL-17, IFN-γ, and IL-6 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 fluoxetine and antagonist or agonist of 5-HT^{2B}-receptor.

Statistical analysis

The statistical analysis of the results was performed using Prizm 6 software. The nonparametric Mann-Whitney U-test or Wilcoxon signed-rank test were used to compare two groups. One-way ANOVA followed by Bonferroni correction was used for multiple comparisons. Differences were considered statistically significant if $p<0.05$.

Results

The cytokine production by CD4+ T-cells (**Table 2**) and macrophages (data not shown) were comparable between the groups. Fluoxetine suppressed cytokine production by CD4+ T-cells (**Fig. 1**) and macrophages in both groups (data not shown) without affecting cell viability. The blockade of 5-HT_{2B}-serotoninergic receptor with specific antagonist reduced the inhibitory effect of fluoxetine on cytokine production in MS patients (**Fig. 2)**, while activation of 5-HT_{2B}-serotoninergic receptor had no effect on fluoxetine-mediated cytokine suppression **(Fig. 2)**. Finally, fluoxetine reduced IL-17 production by CD4+ T-cells activated with LPS-stimulated macrophages in both groups (**Fig. 3**).

Conclusions

Fluoxetine has an anti-inflammatory effect in MS by the direct suppression of Th17-cells function and macrophages-induced Th17-immune response. The direct inhibitory effect of fluoxetine on Th17-cells in MS could be mediated by the activation of 5-HT_{2B}-serotoninergic receptor.

References

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

Cytokine	Stimulation	MS patients, n=30	Healthy subjects, n=20
IL-17, pg/ml	None	0 (0; 4)	4 (0; 9)
	Anti-CD3 / anti-CD28	732 (321; 1201)	696 (105; 984)
IFN-γ, pg/ml	None	7 (0; 24)	6 (0; 34)
	Anti-CD3 / anti-CD28	4934 (2776; 7377)	4781 (2964; 5519)

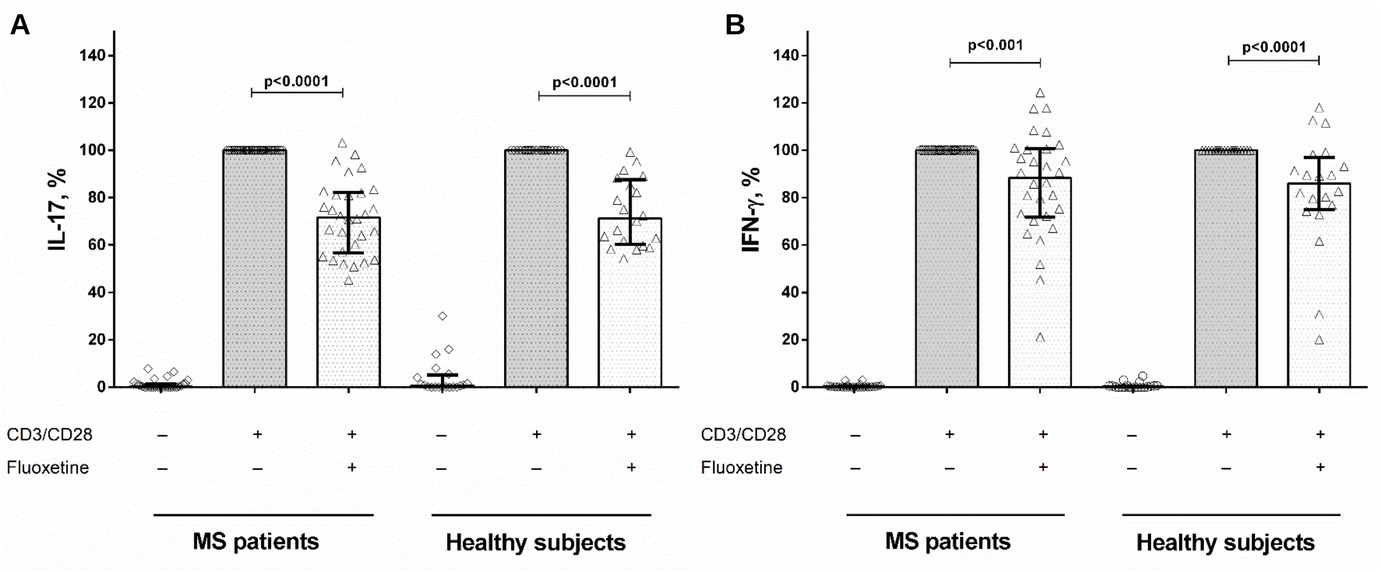

Figure 1. The influence of fluoxetine on IL-17 (A) and IFN-γ (B) production by stimulated CD4+ T-cells in MS patients and healthy subjects. Horizontal lines at the graphs correspond to the median and whiskers indicate to 25th and 75th percentiles. The median values of MS and control groups were compared and the p values are indicated at the figure.

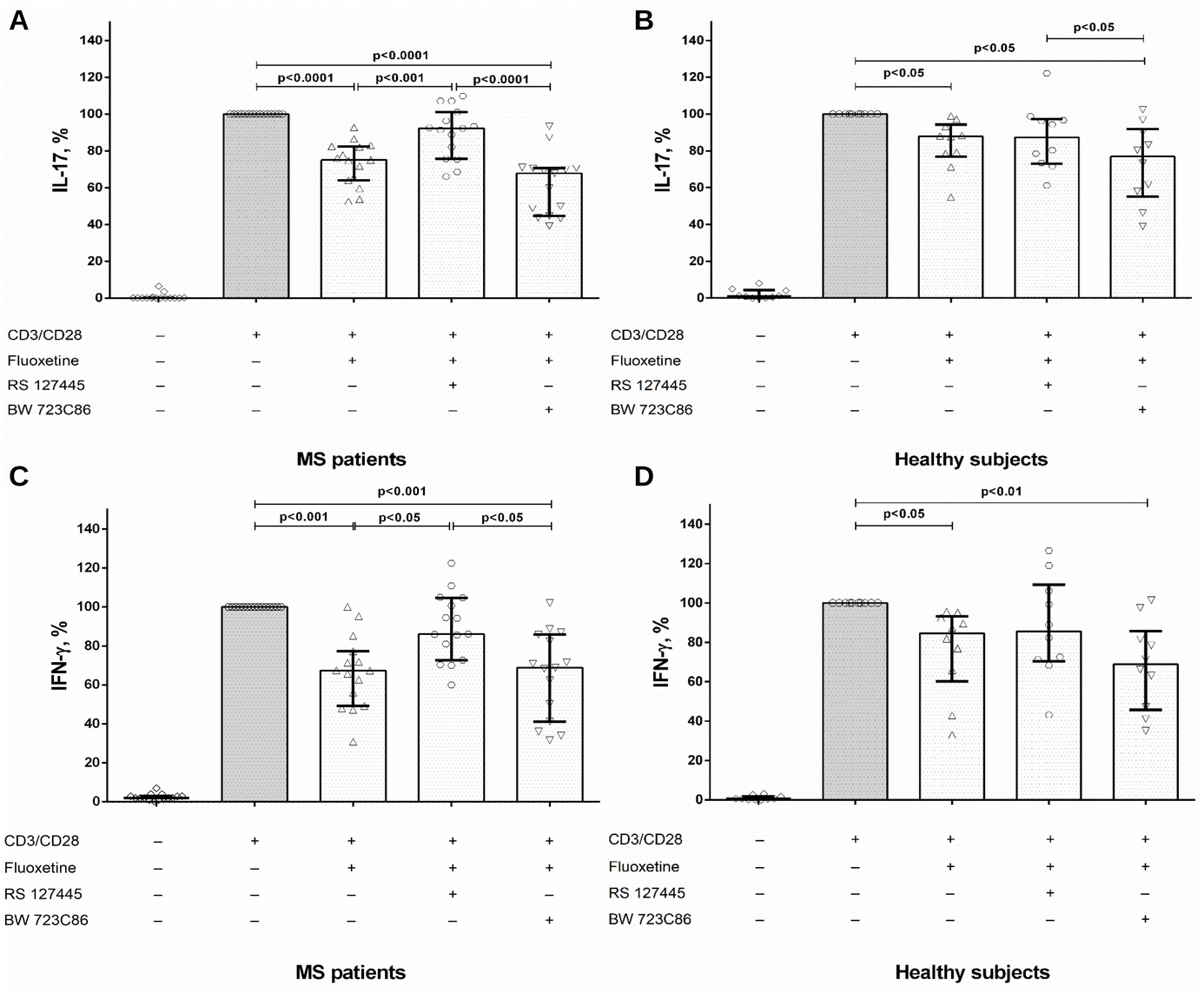

Figure 2. The involvement of 5-HT_{2B}-receptor in fluoxetine-mediated of IL-17 (A and B) and IFN-γ (C and D) suppression in MS patients and healthy subjects..

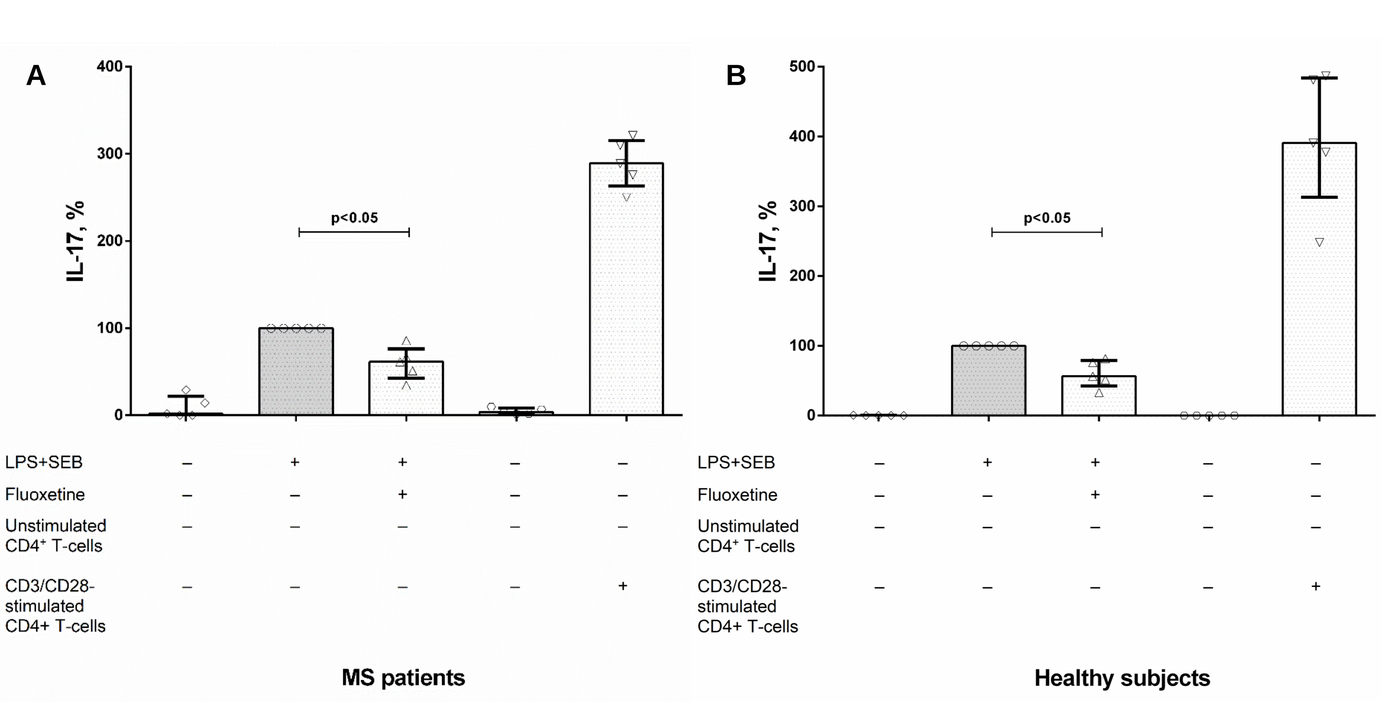

Figure 3. The influence of fluoxetine on IL-17 production CD4+ T-cells stimulated with LPS-activated macrophages in MS patients (A) and healthy subjects (B).

Disclosure

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