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Immunomodulatory effects of testosterone on inflammatory cells in patients with multiple sclerosis.

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Background

Multiple sclerosis (MS) is a chronic, autoimmune disease of the central nervous system (CNS) affecting young adults. MS affects females twice as often as men, but the reason still remains elusive. One of the most promising hypotheses is that sex hormones acting as immunomodulatory and neuroprotecting agents could play a key role in modulation of MS course. Male hypogonadism is a risk factor for MS. Moreover, low testosterone levels negatively modulate the course of the disease suggesting a pivotal role of this hormone in MS pathogenesis.

Hypotheses

1. Testosterone can modulate immune response during the course of MS.
2. Testosterone exerts its effects through lymphocytes T CD4+.
3. Secretion cytokines and chemokines is sex-dependent.

Main aims

Evidence suggests that testosterone modulates lymphocyte secretion profile, regulates inflammatory response and stimulates oligendrocyte proliferation thus affecting three main components of MS: inflammation, demyelination and neurodegeneration. Therefore the main aims of the study are:

1. Evaluation of influence of blood serum testosterone concentration on clinical course of the disease during treatment with immunomodulatory drugs
2. Assessment of the effect of testosterone on secretion of pro-inflammatory (IL-17, IFN-γ and CCL-5) and anti-inflammatory cytokines (IL-10) from CD4(+) T-cells isolated from patients with multiple sclerosis

Table 1. Correlation of blood serum testosterone concentration and selected clinical parameters.

Testosterone vs:	Whole group		Females		Males	
	R	p	R	p	R	p
EDSS	0,11	0,57	-0,17	0,46	-0,61	0,14
Disease duration	-0,06	0,77	-0,19	0,39	-0,41	0,36
ΔSDMT	0,18	0,35	0,01	0,96	-0,47	0,28
ΔFSMC- cognitive	0,00	0,99	0,04	0,87	0,09	0,85
ΔFSMC- motor	-0,08	0,69	0,17	0,46	-0,29	0,53
ΔFSMC- total	-0,01	0,94	0,14	0,55	-0,09	0,85
ΔMoCA	-0,22	0,24	-0,31	0,16	-0,62	0,14
ΔBDI	-0,17	0,38	-0,15	0,51	-0,39	0,38
ΔMFIS	0,22	0,24	0,39	0,07	-0,11	0,81

Table 2. Secretion of selected interleukins after T-stimulation in selected patients.

T10	MS	controls	p-value
IL-10	359,79	540,64	0,013
IL-17	178,58	16,91	0,001
IFN-γ	215,06	24,61	0,001
CCL-5	103,24	126,35	0,013

Conclusions

1. We didn't find signifficant correlation between motor as well as cognitive performance and baseline testosterone concentration in patients treated with immunomodulatory drugs.
2. Secretion of anti-inflammatory cytokines is not to be not affected by testosterone, however more research is needed to establish the exact role of testosterone in pathogenesis of MS.
3. Concentration of pro-inflammatory cytokines (IFN-γ, IL-17) in MS patients is possibly influenced by patient's sex.
4. Testosterone in MS patients with preserved secretion of chemokines/interleukins results in higher expression of proinflammatory and lower expression of anti-inflammatory proteins in comparison to control group implicating possible testosterone-dependent regulation of inflammation in patients with MS.

Materials and Methods

A total of 33 patients (24 females and 9 males) with RRMS treated with immunomodulatory drugs (11 patients on fingolimod and 22 on natalizumab) participated in clinical tests (neurological and cognitive evaluation): EDSS, SDMT, FSMC, MoCA, BDI evaluation and had serum testosterone measurement in 2 time points (year 0 and 1 of observation). Mean age of study group was 40,18 years and mean disease duration was 12,03 years.
A total of 21 with RRMS patients and 10 age-matched patients with non-inflammatory neurological diseases (control group) were enrolled for in vitro studies.
Peripheral blood samples were taken according to the protocol shown in Fig. 1. Expression profile of IL-10, IL-17, IFN-γ and CCL-5 was assessed after testosterone (T), flutamide (F), testosterone and flutamide (T+F) administration, as well as in mediums: DMEM and ethanol (EtOH). Enzyme-linked immunosorbent assay (ELISA) was performed to evaluate the secretion of IL-10, IL-17, IFN-γ and CCL-5. Statistical analysis was performed using the GraphPad Prism 9 software and Statistica 13. A p value below 0.05 was deemed significant.

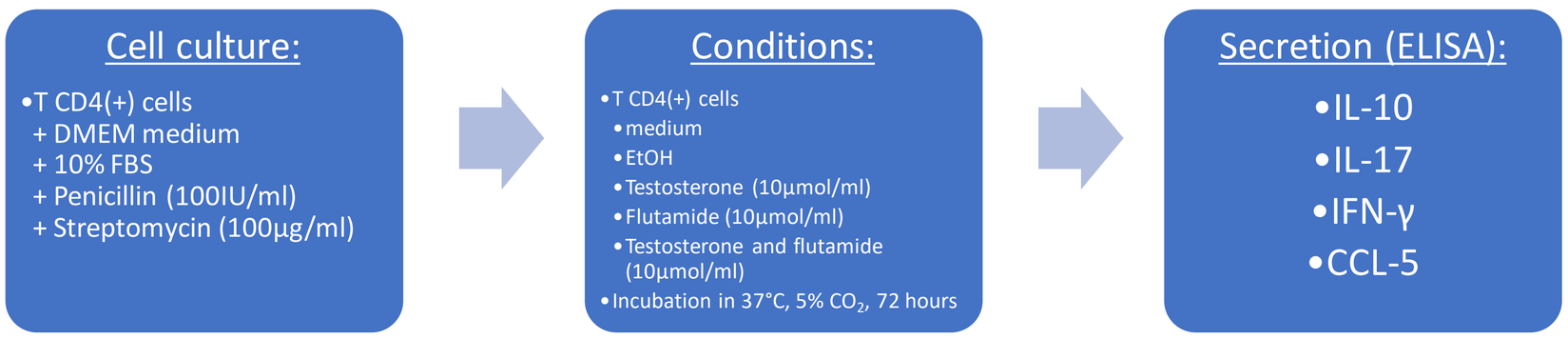

Figure 1. Laboratory protocol.

Results

1. IL-10 concentration was signficantly lower in study group compared to controls after exposure to testosterone (Fig. 2A).
2. CCL-5 concentration was significantly higher in study group compared to controls in medium (Fig. 2D)
3. No differences in cytokine/chemokine secretion after exposure to testosterone has been observed in both males and females.
4. In selected patients (secreting) IFN-γ and IL-17 secretion after testosterone stimulation is significantly higher in study group, while IL-10 and CCL-5 is significantly lower compared to controls (Tab. 1).
5. No correlation between baseline testosterone concentration and selected clinical parameters has been found for both males and females (Tab. 2)

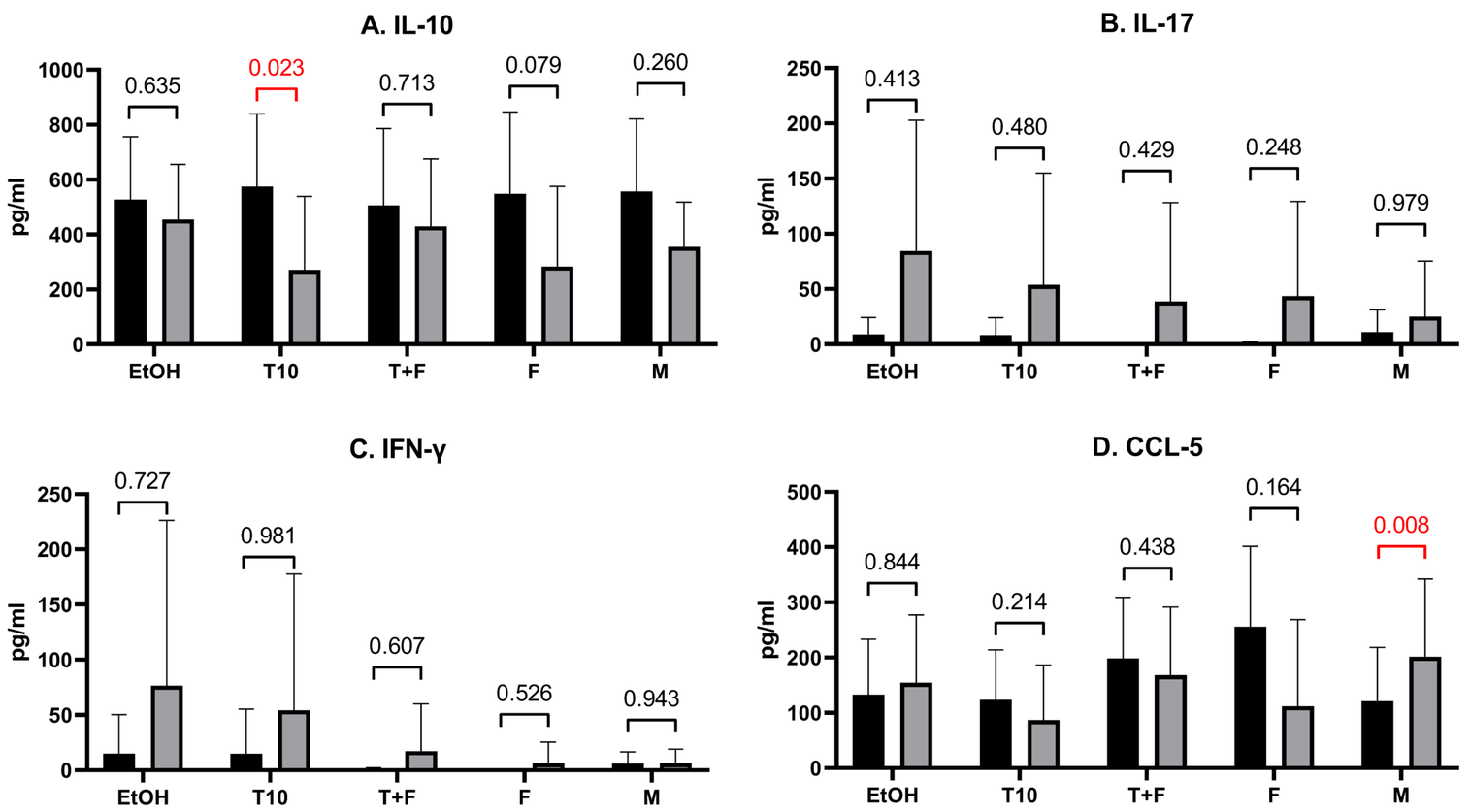

Figure 2. Secretion of studied cytokines and chemokines in different conditions.

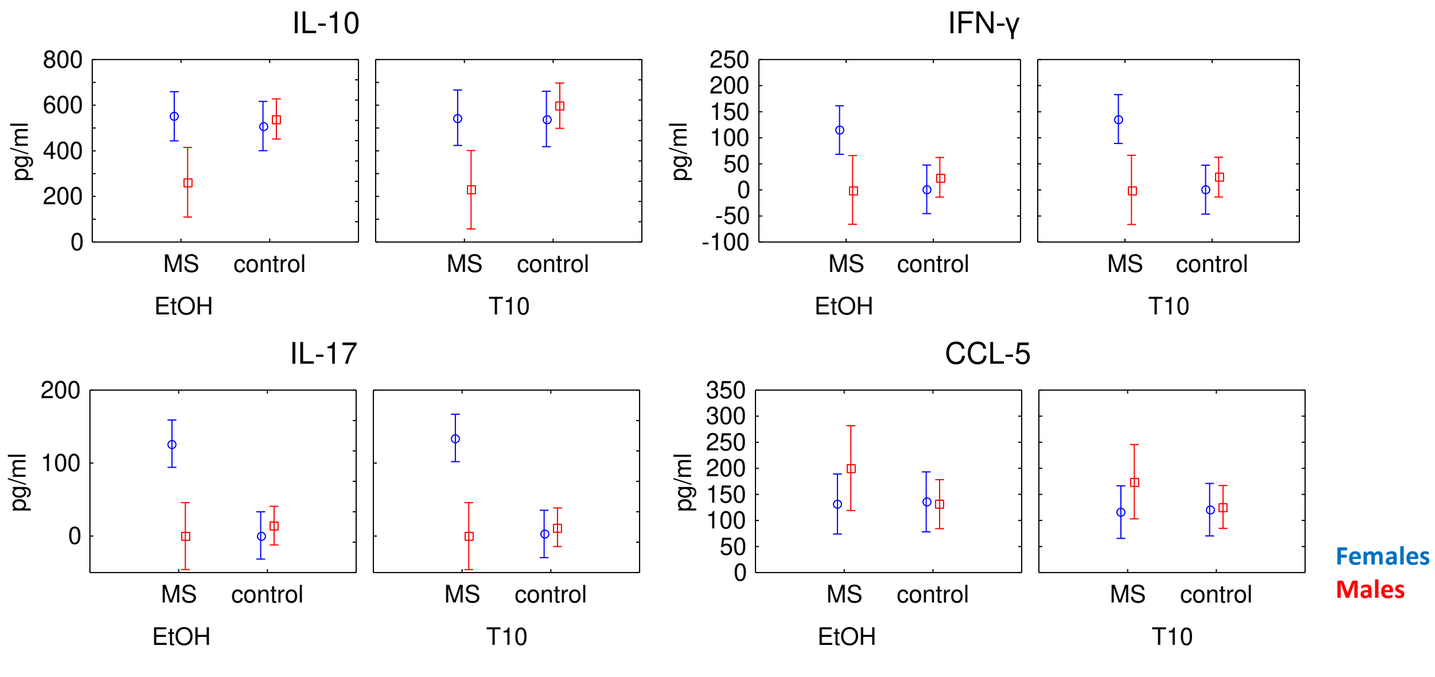

Figure 3. Comparison of cytokine/chemokine secretion among sexes under the influence of testosterone.