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Increased IL-17A but Decreased IL-27 Serum Levels in Patients with Multiple Sclerosis

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ABSTRACT

Background: Effector $CD4^+$ T cell subsets play an important role in Multiple Sclerosis (MS). Interleukin-27 (IL-27) suppresses Th (Th1, Th2 and Th17) cells and dampens autoimmunity and tissue inflammation by promoting the generation of Type 1 regulatory T cells (Tr1). **Objective:** To identify the relative levels of IL-27 and IL-17A in MS disease. **Method:** In a case-control study, venous blood was collected from forty MS patients and forty-three healthy subjects as control group. Serum levels of IL-27 and IL-17A were measured by ELISA method. **Results:** A significant difference between serum IL-17A concentration in patients (120.68 ± 209.85 pg/ml) and control group (67.26 ± 117.76 pg/ml, p=0.016) was found. Serum IL-27 levels of the MS patients (159.7 ± 581.4 pg/ml) were significantly lower than control subjects (180.35 ± 507.84 pg/ml, p=0.001). **Conclusion:** Our findings show decreased levels of IL-27 against increasing IL-17A levels in patients group which may suggest the suppressive role of IL-27 on inflammatory process of MS.

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Keywords: Interleukin-27, Interleukin-17A, Multiple Sclerosis

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INTRODUCTION

Autoimmune diseases are a main cause of morbidity and mortality in the industrial world, affecting 3-8% of the population. Autoimmunity develops after breaking tolerance of the immune system against self-antigens, a process that involves many different molecules and yet poorly understood (1). Multiple sclerosis (MS) is an autoimmune disorder which affects the central nervous system (CNS) with over two million sufferers worldwide. MS is the second trauma which causes neurological disability in young adults (2). The most common form of the disease is relapsing-remitting MS (RR-MS) that affects up to 80% of patients. Damage to the axonal myelin, by inflammatory cells, leads to defects in electrical signal transfer (3). This axonal damage is the essential cause of clinical disability and progress of the disease although the exact pathogenesis of MS is largely unknown (4,5). Cellular infiltration of macrophages/microglia linage and $CD8^+$ T lymphocytes (5), B cells and immunoglobulin G (IgG)-positive plasma cells, dendritic cells (6) and Th-17 cells (7), exist in trailing areas of complete demyelization (8). IL-17 and its secreting Th cell subset (Th17 cells) play an important role in MS (7). IL-17A is a member of the IL-17 family, which includes IL-17A to E (also called IL-25) and IL-17F (9,10). IL-17 main source cellular (Th17) are characterized by the expression of IL-17A, and also IL-17F, IL-6, TNF-α and GM-CSF but neither IFN-γ nor IL-4 (11,12). IL-17 increased expression in patients with a variety of allergic and autoimmune diseases, such as rheumatoid arthritis (RA), MS (17), inflammatory bowel disease (IBD), and asthma, suggests the contribution of IL-17 to the induction and/or development of such diseases (13). Increasing IL-17 mRNA expression is shown in blood (17,18) and cerebrospinal fluid of MS patients and joint fluid in patients with arthritis (7). Our previous study on
MS patients showed increased IL-17A and IL-17F mRNA expression in peripheral blood mononuclear cells (17). Serum levels of IL-17A and IL-17F were also higher than normal controls in our study (unpublished data). Moreover, Th17-related cytokines have essential role in MS pathogenesis (7,11,12,15,16). Increased expression of IL-23 has been shown both in the level of protein and mRNA in CD4 positive activated T lymphocytes and activated dendritic cells in patients with Relapsing Remitting (RR) MS (17,6).
IL-27 which is a member of the IL-6/IL-12 family of cytokines is a heterodimer cytokine composed of two subunits called p28 and Epstein Barr Virus-induced gene 3 (EBI-3) (11,19). This family has an essential role in fundamental processes such as neuronal growth, bone maintenance, cardiac development and immune regulation (14). IL-27 receptor (IL-27R) is expressed by a variety of immune and non-immune cells, such as T cells, monocytes, dendritic cells, mast cells, hepatocytes, endothelial cells, neurons, B cells and NK cells which directly or indirectly affected immune responses (11,12,14,19,20,21). IL-27 plays its role on immune system with pro- and anti- inflammatory effects (14,19). The members of this family, particularly those signaling via gp130, suppress the pro-inflammatory properties of inflammatory responses (14). IL-27 has complex effects on Th17 immune responses (19) to break the normal activity of effector T cells leading to autoimmunity (12). IL-27 also plays an important role in the promotion of T regulatory (Treg) cells (22) but it is not essential for normal Treg cell function (11). IL-27 is similar with IL-12 in sequence, structural homology, and can promote Th1 responses (14). IL-27 can promote early Th1 development as well as suppressing late Th1 responses (19). IL-27 transcription in uterine NK cells, and lympho-

cytes that promote immune tolerance and placental development, can explain the role of this cytokine in cell migration and homeostatic immune regulation (14,23). IL-27 can augment proliferation and secretion of IFN-γ by naive $CD4^-$ T cells, and when it's effect combined with IL-12, can synergize the induction of IFN-γ production by human NK cells (14,25). In an opposite activity, IL-27 inhibits IFN-γ expression by fully differentiated Th1 cells (19). IL-27 sensitizes lymphocytes to IL-12 (26), but suppresses IL-2 production by wild type cells (11,19). IL-27 uses STAT-1, STAT-4 (19, 26), and STAT-3 or STAT-5 signaling pathway (19). IL-27 suppresses Th2 cells differentiation (27) by blocking the GATA-3 in the STAT-1 pathway and can induce IL-10 and suppress Th17 differentiation in a STAT-1 dependent way (3,19,22,11). In the absence of STAT-1, IL-27 can induce Th17 development because the suppressive effect of IL-27 requires to STAT-1 signaling pathway (11). The levels of IL-17 and Th17 cells in the absence of IL-27 signaling are reported to increase (11). IL-27 promotes the generation of Tr1 cells. Inflammation in autoimmunity dampens by Tr1 cells through the immunosuppressive IL-10 secretion. IL-27 requires IL-10 for anti-inflammatory effects on Th1-mediated EAE (19). Mouse studies have demonstrated that increasing numbers and/or function of Tr1 cells could improve the course of autoimmune diseases (19). Therefore, aim of this study was evaluating IL-27 and IL-17 serum levels to clarify the role of these cytokines in MS disease. In a parallel study, we analyzed IL-17A gene polymorphisms in the same population of MS patients (unpublished data).

MATERIALS AND METHODS

Sampling: The subjects who enrolled in this study were the MS patients referring to the Neurology Department and Neurology Research Center of Imam Reza Hospital, Tabriz University of Medical Sciences in a ten months period, October 2011- September 2012. The patients were carefully examined by a neurologist and a definitive diagnosis of MS was established based on MacDonald criteria concerning their clinical signs and symptoms and adjunctive diagnostic tools such as MRI. Samples were collected from two groups: forty MS patients; twenty-three female and seventeen male (33.75 ± 7.67 years) and forty-three age and sex matched healthy subjects; twenty-six female and seventeen male (30.57 ± 6.37 years) as controls. All patients had relapsing-remitting MS and had not received any immunosuppressive therapy. Controls were persons without any history of autoimmune disease or inflammatory disorder such as asthma, arthritis, colitis and other gastro-intestinal or organ specific chronic inflammatory disease even migrant, smoking and common cold. Ethics approval for this study was obtained by Tabriz University of Medical Sciences ethical committee. Informed written consent was obtained from every patient participating in this study. Venous blood samples of cases and controls were collected and after serum separation were stored at -80°C.

Cytokine Analysis: IL-27 and IL-17A serum levels were evaluated by ELISA method; applying human IL-27 (eBioscience, Cat. No. 88-7278) and human IL-17A (eBioscience, Cat. No. 88-7176) ELISA kits. In the first step capture antibody diluted in 1X coating buffer and 100 µl/well of capture antibody were applied for coating ELISA plates [non irradiated microtiter plates [NUNC Maxisorp flat-bottom (Cat. No. 44-2404)]. The sealed plates were incubated overnight at 4°C. After washing the plates with washing buffer; phosphate buffer saline (PBS) and 0.05% Tween-20, pH=7.4, wells were blocked with 200 µl/well of 1X assay diluents. The ready plates used for cytokine

measurement according to the instructions. OD reading was performed at 450 nm and resulting data were analyzed according to the standard curve. This curve receives from 8 different dilutions of recombinant standard in 1X Assay diluent buffer.

Statistics analysis: Data were analyzed with descriptive statistics (mean ± SD and N (%)); Independent samples *t–test* and chi-square test were performed to compare results between the groups. All statistical tests were two-sided. $p<0.05$ was considered statistically significant. Kolmogorov-Smirnov test was used for evaluation of distributions. Statistical analysis was performed using the statistical software SPSS16.

RESULTS

In the present study serum levels of IL-27 and IL-17A were evaluated in forty MS patients and forty-three healthy controls. Mean age of the patients and controls were 33.75 ± 7.67 years and 30.58 ± 6.37 years, respectively. Age range was 18-35 for MS patients and 20-43 years for controls. There was no significant difference in the mean age of case and control groups. Female/male was 23/17 in MS patients, and 26/17 in control group (Table 1).

Table 1. Age and gender distribution of MS patients and control group (p> 0.05).

Group / Variable	Patient (N=40)	Control (N=43)
Sex (F/M ratio)	23/17	26/17
Age (yrs)	33.75 ± 7.67	30.58 ± 6.37
Age range (yrs)	(18-53)	(20-43)

Variations in the Serum levels of IL-17A and IL-27 in MS patients and the healthy controls. The results revealed increase in the serum levels of IL-17A in MS patients (120.68 ± 209.85 pg/ml) compared to healthy controls (67.26 ± 117.76 pg/ml), p=0.016. The serum levels of IL-27 in MS patients (159.7 ± 581.4 pg/ml) was significantly lower than healthy controls (180.35 ± 507.84 pg/ml), p= 0.001 (Table 2).

DISCUSSION

The results of our study showed lower serum levels of IL-27 in MS patients compared controls suggesting IL-27 potential to be an effective response modifier for immuno-

therapy of autoimmune diseases like MS, which are partly dependent on IL-17 activity, through IL-27 anti-inflammatory properties.

Disregulated and hyper-reactive T cell responses can lead to autoimmunity (12). The cells and cytokines which are effective in inflammatory process such as T lymphocytes, IL-1, IL-6, TGF-β, IL-21, IL-23, and IL-27 play an essential role in MS. These cells are responsible for general and specific signs of inflammation. Autoreactive CD4 positive T lymphocytes, especially Th17, Th1, and CD8 positive T cells have vital role in multiple sclerosis pathogenesis (30). Some cytokines can inhibit effector cells and play this role in different ways. IL-27 can block early Th17 development but fully differentiated Th17 cells may become resistant against inhibitory effects of IL-27 (19). This cytokine blocks the expression of Th17 transcription factors RORC and RORγt/RORα, but not in the presence of IL-23 (3,19). Therefore, completely differentiated Th17 cells resist against IL-27 suppressive effects (3,12).

Table 2. Serum levels of IL-17A and IL-27 (pg/ml) in MS patients and controls (p=0.016) and (p=0.001) respectively.

Group Variable	Patient (N=40)	Control (N=43)
IL-17A	120.68 ± 209.85	67.26 ± 117.76
IL-27	159.7 ±581.4	180.35 ±507.84

IL-23, a survival factor for Th17, limits the IL-6 negative regulation and SOGS3, and induces IL-17 production in STAT3 dependent way. But IL-27 can induce SOGS3 in STAT1 dependent way and suppress the IL-17 production (11). IL-27 can induce Treg1 and IL-10 secretion in Th1, Th2 and Th17 (3). Neufert et al. demonstrated that TGF-β-induced Treg cells activity can be inhibited by IL- 27, in a STAT-1 independent manner (22). Huber et al. study showed the suppressive effect of IL-27 on Tregs partially related to STAT-3 signaling (31). Furthermore, Pot et al. reported that IL-27 promotes the differentiation of IL-10-producing Tr1 cells which have potent immunomodulatory functions (30). Neufert et al. addressed whether suppression of IL-2 by IL-27 is responsible for the inhibitory effects of IL-27 on Tregs (22). Neutralizing anti-p28, one of the IL-27 subunits, causes anti-inflammatory effects which lead to a rapid suppression of inflammatory autoimmune diseases such as MS. IL-17A neutralization with blocking antibodies reduced the severity of EAE (11,28). In experimental models, the mice with IL-27Rα deficiency has shown increased severity of inflammation and demyelination, high IL-17 levels in CNS, T cell proliferation and Th17 differentiation, that suggested suppressive role of IL-27 on EAE progression. IL-27Rα is commonly expressed on the cells of immune system, and T cells, B cells and myeloid cells are responsive to IL-27.

Happel et al. reported that IL-17 clearly plays a role in acute models of autoimmunity, and treatment with IL-17 blocking agents has been shown to reduce symptoms after onset of disease (32). Batten et al. findings has supported these data by showing that the number of Th17 and the level of IL-17 and other inflammatory mediators are high in the patients with MS (11). Kunzl and Ibrahim reported that IL-17 mRNA-positive mononuclear cells (MNCs) increase in peripheral blood of 40% of MS patients compared to healthy controls (1). Wraith and Matusevicius reported increase in the number of IL-17 mRNA expressing mononuclear cells in both blood and cerebrospinal fluid of MS patients (7,18). We showed significant increase in expression of IL-17A and IL-17F in peripheral blood mononuclear cells (PBMCs) of MS patients (17). Vaknin-Dembinsky et al. showed this raise in the brain of MS patients (6) while Wraith et al. suggested correlation of IL-17 mRNA high expression with severity of MS clinical signs (7). Our findings in this and a previous study supported these studies by showing that concentration of IL-17A in MS patients is higher than healthy subjects (17). The high levels of IL-17A can be to some extent responsible for MS pathogenicity. The anti-inflammatory effects of IL-27 can suppress the inflammatory effects of IL-17A and cause partial suppression in clinical symptoms and development of MS disease.

On the other hand, IL-27, by promotion of IL-10 secretion, can inhibit the inflammatory response. In the absence of IL-27 suppressive effects inflammatory immune response, which usually occurs in MS patients, develops mainly through IL-17A dependent activity. These findings may explain high activity of IL-17A in the absence of suppressive factors. This imbalance can be dependent on Th17 activity, IL-17 mRNA expression and inflammatory process in relapsing MS (7,17,18).

The results of our present study confirm previous findings on critical role of IL-17 in MS disease and support other findings of IL-27 suppressive effects on inflammatory process and therapeutic potential of IL-27 in MS. Further studies on more MS populations and also, in vitro assessment of IL-17A/IL-27 interaction should evaluate our results.

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