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Serum Levels of Interleukin-27 and Interleukin-33 in Patients With Dermatomyositis and Polymyositis Compared to Healthy Control Subjects

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Abstract

Background: In some autoimmune diseases such as rheumatoid arthritis (RA), Interleukin-27 (IL-27) and IL-33 levels are increased. These observations suggest that IL-27 and IL-33 may have a role in the pathogenesis of polymyositis/dermatomyositis (PM/DM).
Objectives: The aim of this study was to assess IL-27 and IL-33 levels in PM/DM patients compared to healthy control subjects.
Patients and Methods: Twenty patients with DM and nine patients with PM were recruited in this study. Twenty-nine healthy controls whose age and gender were matched with the patients were also recruited. Serum IL-27 and IL-33 was measured by the Enzyme-Linked Immunosorbent Assay (ELISA).
Results: The serum levels of IL-27 in patients with DM and PM were higher than those of healthy controls. There were no significant differences in serum levels of IL-33 in patients with DM and PM compared to the healthy control group.
Conclusions: These data indicate that IL-27 might be selectively involved in the pathogenesis of DM and PM. However, IL-33 does not appear to be influenced.

Keywords: Dermatomyositis, Polymyositis, Interleukin-27, Interleukin-33

1. Background

Dermatomyositis (DM) and Polymyositis (PM) are rare diseases related to a group of acquired, systemic, connective tissue disorders, called Idiopathic Inflammatory Myopathy (IIM). Clinical symptoms consist of sub-acute onset, proximal symmetric muscle weakness and inflammatory cells in filtration into muscle tissues. The etiology of these muscle disorders is not well understood and is believed to involve components of the cellular and humeral immune systems (1, 2). The expression of different cytokines, such as Tumor Necrosis Factor (TNF)-α, IL-1α, IL-β and IL-15 has been reported in inflammatory foci (3, 4). The present study was carried out to investigate the serum levels of Interleukin-27 (IL-27) and Interleukin-33 (IL-33) in patients with DM/PM in comparison with healthy control subjects.

Interleukin-27 is a member of the IL-6/IL-12 family that consists of two subunits: Epstein-Barr virus-induced molecule 3 (EBI3) subunit joined with the p28 subunit that signals through a receptor complex composed of IL-27Ra and glycoprotein 130 (gp130) subunits (5, 6). Interleukin-27 is mainly produced by activated antigen-presenting cells including monocytes, endothelial cells and dendritic cells, and induces the development of naive T cells into Th1 cells and also has synergistic effects with IL-12 to trigger the synthesis of Th1 cytokine (5, 7).

Interleukin-33 is a member of the IL-1 family of cytokines, which plays its role after binding to its specific cell membrane receptor. Involvement of IL-33 in certain acute and chronic inflammatory and auto-immune diseases has been previously reported. Interleukin-33 is found in the nuclei of the producing cells like epithelial and smooth muscle cells and is widely expressed in tissues, yet specifically located within cell types such as epithelial linings and smooth muscle cells (8). The biological effect of the presence of IL-33 in the nucleus is not well recognized, and at least two different roles have been explained. First, storage of IL-33 in the nucleus may act as an alarm in, which is released after cell destruction to modify the immune response and the surrounding tissue. Second, IL-33 was found to participate with heterochromatin and it was proposed that nuclear IL-33 negatively alters gene transcription by an intracrine, non-IL-33 receptor-mediated pathway by a yet to be known molecular mechanism. Therefore, IL-33 is a dual function cytokine, which acts as an intracellular modulator of gene expression and also as an alarm in agent that begins inflammation in response to cellular necrosis (9, 10).

In some auto-immune diseases such as Rheumatoid Arthritis (RA) IL-27 and IL-33 levels are increased (11, 12). These observations suggest that IL-27 and IL-33 may have a

role in the pathogenesis of PM/DM. However, the roles of IL-27 and IL-33 in PM/DM disease are unknown and there is no data on IL-27 and IL-33 cytokines, and the relationship between these cytokines and risk factors of PM/DM. At present, laboratory parameters related to disease activity in PM/DM are still lacking. Potentially, serum levels of IL-27 and IL-33 could be disease biomarkers.

2. Objectives

The purpose of this study was to assess IL-27 and IL-33 serum levels in patients with PM/DM, and to determine the serum levels of IL-27 and IL-33 compared to healthy control subjects.

3. Patients and Methods

3.1. Patients

Twenty patients with DM (15 females and five males) and nine patients with PM (seven females and two males) were recruited in this study. Diagnosis was based on both clinical and laboratory parameters and on muscle biopsy specimens according to the Bohan and Peter criteria (13, 14). Twenty-nine healthy controls whose age and gender were matched with the patients were also recruited. This study was approved by the ethics committee of Zahedan University of Medical Sciences. All patients provided written informed consent for participation in the present study. Patients with infections, allergies, history of autoimmune disorders, or other medical conditions that might be related to change of inflammatory status were excluded.

3.2. Sample Collection and Cytokines Assays

Five milliliters of peripheral blood was collected from the patients and healthy controls. After separating the serum from the blood, all samples were stored at -80°C until examination. Sera IL-27 and IL-33 were measured by the Enzyme-Linked Immunosorbent Assay (ELISA) according to the manufacturer's instructions (eBioscience, Vienna, Austria). The optical density of each well was determined using a microplate reader set to 450 nm. The concentrations of interleukins were calculated according to the optical density values. The detection limits were, 0.2 pg/mL for IL-33 and 9.5 pg/mL for IL-27. Clinical chemistry blood tests, including Creatine Kinase (CK), Lactate Dehydrogenase (LDH) and C-reactive Protein (CRP), were performed by standard methods.

All of the patients were medically treated with corticosteroids (prednisone 1 mg/kg/day, administered orally). The following drugs were used in combination for corticosteroid sparing: azathioprine (2 mg/kg/day), methotrexate (15 - 25 mg/week), cyclophosphamide (1 - 2 mg/kg/day), and folic acid (1 mg/day).

4. Results

Data were processed to determine the mean ± Standard Deviation (SD). Statistical differences were analyzed with the Mann-Whitney U-test with a non-Gaussian population. Spearman's correlation coefficient was used to test the correlations between two variables using the SPSS 20 (SPSS Inc., Chicago, IL) software.

Age of the patients was between 7 and 69 years and age of the control subjects was between 9 and 65 years. The serum levels of IL-27 in patients with DM (1462 ± 69.82 pg/mL) and PM (1300.44 ± 113.05 pg/mL) were higher than that of the healthy controls (767.13 ± 22.27 pg/mL) ($p = 0.000$, $p = 0.000$, respectively). There were no significant differences in serum levels of IL-33 in patients with DM (71.50 ± 8.60 pg/mL) and PM (76.59 ± 7.59 pg/mL) compared to the healthy control group (71.08 ± 4.68 pg/mL) ($p = 0.61$ and $p = 0.41$, respectively) (Tables 1 and 2).

Table 1. Serum Interleukin-27 and Interleukin-33 Levels in Patients With Dermatomyositis and Healthy Controls

Interleukin	Control Group (A)	DM Group Before Treatment (B)	DM Group Three Months After Treatment (C)	Comparison Between A and B	Comparison Between A and C	Comparison Between B and C
IL-27, pg/mL	767.1 ± 22.2	1462 ± 69.8	1442 ± 69.3	0.000	0.000	0.58
IL-33, pg/mL	71 ± 4.6	71.5 ± 8.6	70.8 ± 8.2	0.61	0.60	0.84

Table 2. Serum Interleukin-27 and Interleukin-33 Levels in Patients With Polymyositis (PM) and Healthy Controls

Interleukin	Control Group (A)	PM Group Before Treatment (B)	PM Group Three Months After Treatment (C)	Comparison Between A and B	Comparison Between A and C	Comparison Between B and C
IL-27, pg/mL	767.1 ± 22.2	1300.4 ± 113	1261 ± 118.6	0.001	0.001	0.60
IL-33, pg/mL	71 ± 4.6	76.5 ± 7.5	72.7 ± 7.8	0.41	0.56	0.48

Serum levels of IL-27 in patients with DM (1442 ± 69.37 pg/mL) and PM (1261 ± 118.69 pg/mL) three months after treatment were not significantly changed compared to the patients before treatment (p = 0.583, p = 0.605, respectively). Three months after medical treatment, no significant differences in IL-33 levels were found in patients with DM (70.83 ± 8.21 pg/mL) and PM (72.73 ± 7.82 pg/mL) (p = 0.841 and p = 0.489, respectively) (Tables 1 and 2). Demographic data and blood parameters are shown in Table 3.

Table 3. Clinical Data of the Patients With Dermatomyositis and Polymyositis

	Dermatomyositis	Polymyositis	Control
Number	20	9	29
Gender			
Female	15	7	20
Male	5	2	9
CK, U/L	877.5 ± 79.3	683.7 ± 107.1	81.8 ± 7.9
LDH, U/L	1399 ± 86.5	1250.8 ± 139.2	231.6 ± 18.4
CRP, mg/L	19 ± 11.35	13.1 ± 11.2	5.2 ± 3.2

5. Discussion

Dermatomyositis and Polymyositis are chronic autoimmune diseases with clinical symptoms of muscle weakness, in which inflammatory cells invade muscle tissue. Studies have repeatedly demonstrated that inflammatory cells, including CD8+, CD4+ and T lymphocytes, are activated in patients with DM/PM, both in the skeletal muscle and peripheral blood (15-17). Interleukin-27 is a member of the IL-12 family, which plays a key role in regulating the proliferation and function of T cells. Furthermore, IL-27 may exert both pro- and anti-inflammatory properties by modulating the differentiation of Th1 and Th17 cells (18). Therefore, it is necessary to find whether IL-27 exerts a pathologic or protective function in DM and PM.

In the present study, serum IL-27 levels were significantly increased in patients with DM and PM in comparison to the healthy control group. The study of Shen et al. agreed that the level of IL-27 was significantly higher in patients with DM and PM compared with those of healthy control subjects. They observed significant correlations between serum IL-27 levels and CK levels, in a high CK level group of patients with IIM, particularly, in disease of PM Whereas no correlation was found between IL-27 and ESR, CRP serum levels (19). Studies have reported the involvement of IL-27 in collagen-induced arthritis. In another study, work of Cao et al. (20) showed that IL-27 is involved in the development of arthritis by inducing the differentiation of naive T cells to Th1 cells (20, 21). All these observations along with the results of the present study support the pro-inflammatory effect of IL-27, which maybe exerted through inducing differentiation of naive T cells to Th1 cells.

Our study confirmed the existence of higher levels of IL-27 in patients with PM/DM compared to healthy controls as previously reported by Shen et al. (19) in China. Our results add to previous observations and give further evidence in favor of the regulatory role of IL-27 in these diseases.

Interleukin-33 as a pro-inflammatory cytokine can mediate various immune responses. Previous studies have shown increased serum levels of IL-33 in patients with rheumatoid arthritis compared to healthy control subjects (12, 22). It seems that synovial fibroblasts are one of the major sources of IL-33 in RA, generating huge amounts of IL-33 in the presence of TNF-α stimulation in vitro. Additionally, in vivo data demonstrated that administration of IL-33 increased the severity of experimental arthritis (23). Surprisingly, in the present study, serum IL-33 levels had no significant changes compared to the control group. Also there were no significant alterations in serum IL-33 levels in patients with DM and PM three months after treatment compared to the patients before treatment. In addition, serum IL-33 levels had no correlation with other clinical parameters inpatients with DM and PM. The reason for this may be that elevated levels of ST2 can bind to IL-33 and decrease the serum levels of IL-33 in patients with DM and PM. Dermatomyositis and Polymyositis are rare diseases, therefore, the number of subjects was not large enough to explore the relationship of cytokine levels and other serum parameters such as CK and CRP.

In conclusion, high serum levels of IL-27 provide further evidence for activation of inflammatory and immune response in patients with DM/PM. This suggested that IL-27 may have a pathological function in DM/PM and will be a valuable marker for diagnosis of DM/PM, and anti-IL-27 may be a useful agent for the treatment of DM/PM, which should be considered by future investigations.

Footnotes

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