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Roles of macrophage migration inhibitory factor in polymyositis: Inflammation and regeneration

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Abstract

Objective: To elucidate the clinical significance of macrophage migration inhibitory factor (MIF) serum concentration in patients with polymyositis.

Methods: Thirty-six patients with polymyositis were enrolled. Serum samples were obtained and stored to detect MIF and interleukin (IL)-6 using commercially available enzyme-linked immunosorbent assay kits. The relationships between these cytokines and clinical data were analyzed.

Results: The serum MIF concentration was significantly lower in patients in remission (34.74 ± 17.75) and in healthy controls (38.87 ± 9.30 ng/ml) than that in patients with active polymyositis (50.04 ± 23.84 ng/ml). There were no significant differences between healthy controls and patients in remission. The serum IL-6 concentration in patients with active polymyositis (19.67 ± 7.16 pg/ml) was significantly higher than that in patients in remission (15.81 ± 4.00 pg/ml) and controls (8.14 ± 3.71 pg/ml). The serum IL-6 concentration was negatively correlated with the serum MIF concentration ($r = -0.283$). No relationship was found between the serum MIF concentration and glucocorticoid dose. The MIF concentration peaked twice during treatment when the creatine kinase concentration was decreasing.

Conclusion: MIF and IL-6 play important roles in the inflammation associated with polymyositis. MIF might also be involved in the early stage of regeneration in polymyositis. MIF may thus serve as a biomarker of disease activity and outcome.

Keywords

Polymyositis, macrophage migration inhibitory factor, interleukin-6, biomarker, inflammation, creatine kinase

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Introduction

Polymyositis (PM) is an autoimmune inflammatory myopathy. It is mainly characterized by proximal and symmetrical

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muscle weakness and often has internal organ involvement. The main pathophysiological mechanism of PM is infiltration of CD8+ T cells and macrophages into muscle fibers, leading to lysis of the muscle fibers.[1] In clinical practice, we still depend on the physician's clinical assessment, mainly including manual muscle strength testing and measurement of serum muscle enzyme levels, to evaluate the disease activity and prognosis. The serum levels of many inflammatory factors are reportedly increased in patients with inflammatory myopathies,[2,3] and IL-6 may be a biomarker of disease activity or outcome. Macrophage migration inhibitory factor (MIF) is a potent and pleiotropic cytokine that is secreted by activated T cells and macrophages and plays a critical role in inflammatory and autoimmune diseases. It has anti-apoptotic, proproliferative and pro-inflammatory effects and may act as a modulator in cytokine responses.[4] MIF plays important roles in many inflammatory diseases. Interestingly, MIF can induce the cytokines IL-6 and tumor necrosis factor-α (TNF-α), which are known as the most useful biomarkers in patients with PM.[3] MIF inhibitor can significantly decrease IL-6 and TNF-α production.[5] Moreover, MIF is a modulator of glucocorticoid sensitivity and may thus improve the status of high-dose glucocorticoid therapy in patients with PM. The concentrations of MIF in skeletal muscle are reportedly higher in patients with inflammatory myopathies.[6] However, the significance of the serum MIF level in patients with PM is still unknown. In this study, we explored clinical significance of the serum MIF level in patients with PM.

Materials and methods

Patients

In total, 36 inpatients with PM were enrolled in Zhejiang Provincial People's Hospital from August 2010 to December 2014. Patients with PM satisfied the criteria proposed in 1975 by Bohan and Peter[7,8] as well as the consensus guidelines for PM in China. We excluded patients with sporadic inclusion body myositis, muscular dystrophy, and other myopathies. All patients underwent muscle magnetic resonance imaging. Patients with overlapping syndromes were excluded. According to the established disease activity tools described by the International Myositis Assessment and Clinical Studies Group, we used the measures of global activity, muscle strength, physical function, and laboratory assessment. Twenty-five patients had an active disease status and 12 patients were in remission when serum samples were obtained (two serum samples from one patient were obtained: one during active disease and one during remission). Detailed clinical and laboratory data were collected. Patients with active disease were initially administered 1.0 to 1.5 mg/kg/d of glucocorticoids, and the glucocorticoids were gradually tapered according to the clinical assessment. Two patients were followed during treatment, and their serum samples were obtained when related laboratory data were checked every week. Serum samples were voluntarily obtained from 10 healthy age- and sex-matched controls. All serum samples were stored at −80°C prior to analysis. The study was approved by the local ethics committee, and informed consent was obtained from patients and controls.

Methods

Enzyme-linked immunosorbent assay (ELISA) for MIF. The serum MIF concentration was detected by a commercially available ELISA kit (Quantikine ELISA for human MIF; R&D Systems, Minneapolis, MN, USA). According to the protocol, all of the reagents, working standards, and

serum samples were prepared before detection. A total of 100 µl of Assay Diluent RD1-53 was added to each well of a 96-well microplate, and 50 µl of standard, control, or serum samples was then added to each well. The microplate was incubated for 2 hours at room temperature on a horizontal orbital microplate shaker. The microplate was then washed four times with wash buffer. MIF conjugate (200 µl) was added to each well of the microplate, which was incubated for another 2 hours. Another four washes were repeated. Next, 200 µl of substrate solution was added to each well, and the microplate was incubated for 30 minutes at room temperature. Finally, 50 µl of stop solution was added to each well, and the optical density was determined within 30 minutes using a microplate reader set to 450 nm.

ELISA for IL-6. The serum IL-6 concentration was detected by a commercially available ELISA kit (Quantikine ELISA for human IL-6; R&D Systems). According to the protocol, all of the reagents, working standards, and serum samples were prepared before detection. A total of 100 µl of Assay Diluent RD1W was added to each well of a 96-well microplate, and 100 µl of standard, control, or serum samples was then added to each well. The microplate was incubated for 2 hours at room temperature on a horizontal orbital microplate shaker. After washing four times with wash buffer, 200 µl of IL-6 conjugate was added to each well of the microplate, and incubation was performed for another 2 hours. The microplate was washed another four times. A total of 200 µl of substrate solution was added to each well, and the microplate was incubated for 20 minutes at room temperature. Finally, 50 µl of stop solution was added to each well, and the optical density was determined within 30 minutes using a microplate reader set to 450 nm.

Statistical analysis

The serum MIF concentration is expressed as the mean ± standard deviation. Statistical differences between two groups were evaluated by an independent-samples t test. Spearman's correlation test was used to evaluate the relationship of the MIF concentration and laboratory data or glucocorticoid dose. A p value of <0.05 was considered statistically significant. All data were analyzed with the statistical software package SPSS 17.0 (SPSS Inc., Chicago, IL, USA).

Results

In total, 36 patients with PM were enrolled, comprising 29 women and 7 men with a mean (±standard deviation) age of 56.13 (±11.10) years. The mean serum MIF concentration in 25 patents with active PM was 50.04 ± 23.84 ng/ml. The mean concentration in the 12 patients in remission was 34.74 ± 17.75 ng/ml, which was significantly lower than that in the patients with active disease ($p = 0.037$). The mean serum MIF concentration in the healthy controls was 38.87 ± 9.30 ng/ml, which was significantly lower than that in patients with active PM ($p = 0.045$). However, there was no significant difference between the healthy controls and patients in remission (Figure 1). The mean serum IL-6 concentration was 19.67 ± 7.16 pg/ml in the patients with active PM and 15.81 ± 4.00 pg/ml in patients in remission. These concentrations were significantly higher than those in controls (8.14 ± 3.71 pg/ml, $p < 0.001$). Furthermore, the mean IL-6 concentration in patients with active PM was significantly higher than that in patients in remission ($p = 0.043$).

The serum MIF concentration was negatively correlated with the serum IL-6 concentration ($r = -0.283$, $p = 0.046$) (Figure 2). When the serum samples were obtained, no significant difference in the

serum MIF concentration was found between patients with active PM who were and were not receiving glucocorticoid therapy. Additionally, no relationship was found between the serum MIF concentration and glucocorticoid dose. Other parameters, such as the erythrocyte sedimentation rate and serum levels of creatine kinase (CK), lactate dehydrogenase, aspartate transaminase, alanine transaminase, and C-reactive protein, showed no significant correlation with the serum MIF concentration.

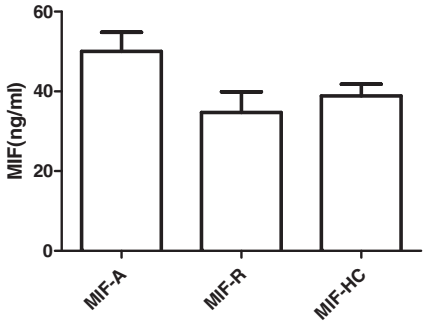

Figure 1. Serum levels of macrophage migration inhibitory factor (MIF) in patients with active polymyositis (MIF-A), patients in remission (MIF-R), and healthy controls (MIF-HC).

Two patients were followed up during treatment. Both patients were newly diagnosed and given 1 mg/kg/d of a glucocorticoid for treatment. Patient 1 had muscle weakness for 1 month when the treatment began, while Patient 2 had muscle weakness for 6 months. In Patient 1, serum samples began to be obtained after nearly 1 month of treatment. In Patient 2, the first serum sample was obtained before treatment. We observed two peaks in the serum MIF concentration during treatment when the CK levels were descending, and the levels decreased when the CK levels normalized (Figures 3 and 4).

Discussion

MIF acts as both a potent cytokine and a hormone-like molecule. Many researchers have found that MIF can induce TNF-α in monocytes, IL-6 and IL-12 in peritoneal macrophages, and IL-6 and IL-8 in synovial fibroblasts.[9–12] However, Kudrin et al.[13] found no induction of TNF-α, IL-6, or IL-12 release by synovial fibroblasts or macrophages using very highly purified MIF. MIF can be produced by many cell types, mainly by activated T cells and

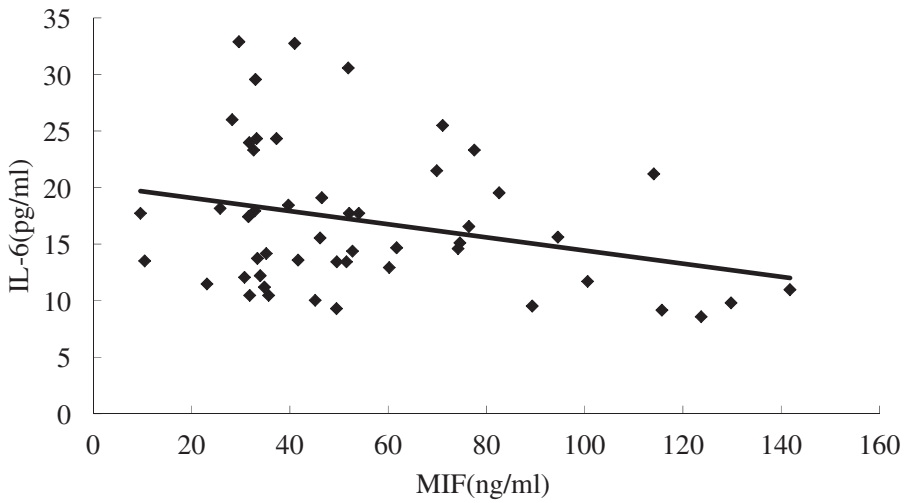

Figure 2. Relationship between macrophage migration inhibitory factor (MIF) and interleukin-6 (IL-6).

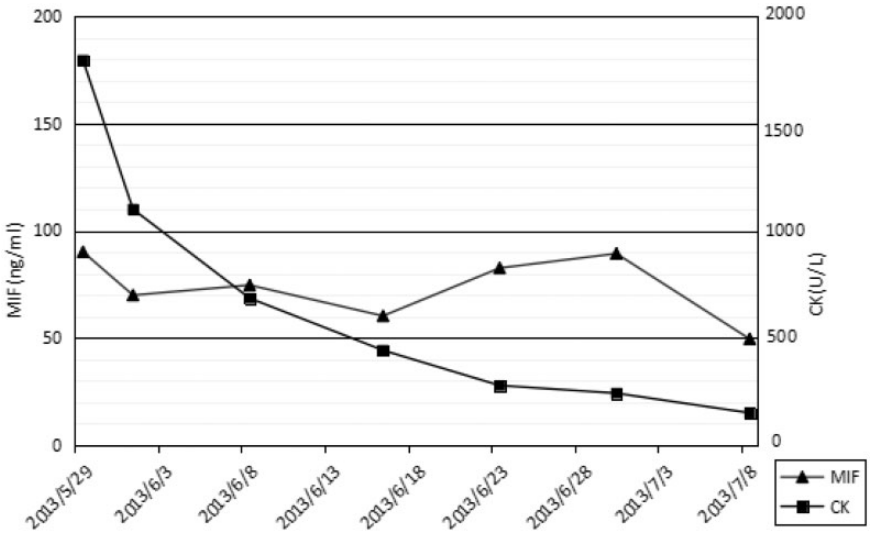

Figure 3. Clinical course of Patient 1.
MIF, macrophage migration inhibitory factor; CK, creatine kinase.

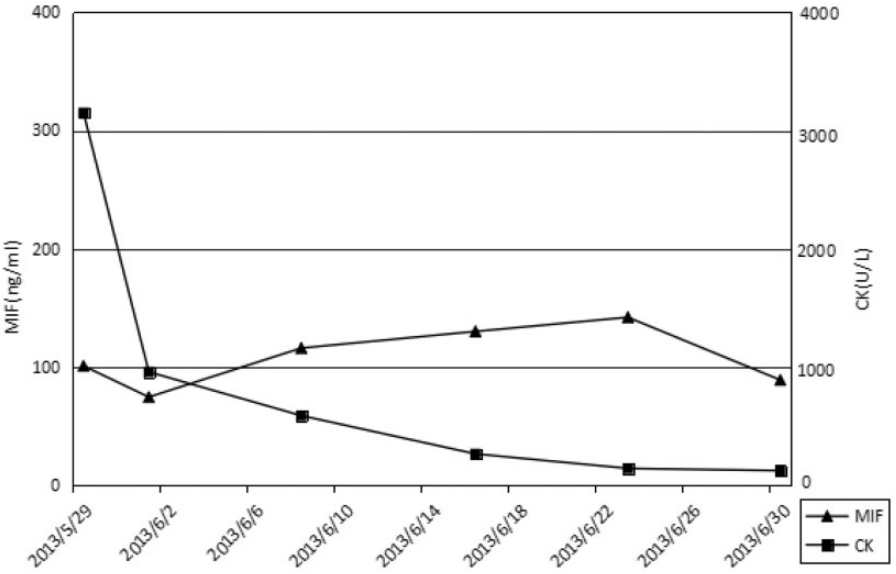

Figure 4. Clinical course of Patient 2.
MIF, macrophage migration inhibitory factor; CK, creatine kinase.

macrophages.[14] Furthermore, MIF is involved in the processes of autophagy and autophagic cell death.[15] The pathogenesis of PM involves cytotoxic CD8+ T cells attacking skeletal muscle fibers followed by invasion of non-necrotic fibers by these T cells and macrophages.[1] Additionally, macrophages are important cells in the

regeneration of inflammatory disorders of skeletal muscle, while the random migration of macrophages can be inhibited by MIF. Few studies have focused on MIF in relation to skeletal muscle. In the present study, we found that the MIF concentration was higher in patients with active PM. However, there were no significant differences between patients in remission and healthy controls. Reimann et al.[6] also found that the MIF concentrations in protein lysates were higher than in controls. This may suggest that MIF plays a role in patients with active PM.

The serum IL-6 level may be a sensitive biomarker of disease activity in dermatomyositis[16,17] and is reportedly an important proinflammatory cytokine in the inflammatory process of PM. Therefore, we evaluated the serum IL-6 concentration in the present study. Although IL-6 and MIF are both inflammatory factors, we unexpectedly observed that the serum IL-6 concentration was negatively correlated with the serum MIF concentration in patients with PM. A previous study showed that MIF was detected not only in inflammatory cells but also in muscle fiber membranes, suggesting that MIF is also involved in the response to muscle fiber damage.[6] Whether a balance in MIF exists between inflammation and regeneration in patients with PM requires further research. In an in vitro experiment, glucocorticoids affected MIF production in a bimodal way: low concentrations of glucocorticoids induced MIF production, and high concentrations of glucocorticoids inhibited MIF production.[9] Another in vivo study showed that MIF could be up-regulated by endogenous glucocorticoids in rats with adjuvant-induced arthritis.[18] In humans, the serum MIF concentration is influenced by exogenous glucocorticoids even after adjusting for disease activity variables.[19] We found no significant differences in the serum MIF concentration between patients with active PM who were and were not receiving glucocorticoid therapy as well as no relationship between the serum MIF concentration and glucocorticoid dose. Exogenous glucocorticoids are not the main factor impacting the MIF concentration in patients with active PM. Consequently, unlike IL-6 as a proinflammatory factor, MIF may play another role in PM.

We also followed up two patients during their treatment. In these patients, glucocorticoids were given at 1 mg/kg/d, and the dose was not changed until the CK level had normalized to exclude the effects of the glucocorticoids. While the CK level was descending, we observed two peaks in the MIF concentration. MIF, which has anti-apoptotic, pro-proliferative, and macrophage-attracting functions,[4] may have another effect in the regeneration after muscle injury. MIF was detected at muscle fiber membranes, at the borders of infiltrates, or in necrotic fibers in the skeletal muscle of patients with PM. Focal sarcoplasmic reactivity was also observed, especially in fibers showing sarcolemmal MIF immunoreactivity.[6] The MIF concentration decreased when the CK level normalized. These data suggest that MIF plays a role not only in the inflammatory process of PM but also in the early stage of the regeneration response. Further studies involving more patients should be performed to confirm this result.

In conclusion, MIF and IL-6 play important roles in the inflammation associated with PM. MIF acts as a potent and pleiotropic cytokine and may also be involved in the early stage of regeneration in PM. MIF may be a biomarker of disease activity and outcome.

Declaration of conflicting interests

The authors declare that there is no conflict of interest.

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