

Original article

Interleukin-18 is a key mediator in dermatomyositis: potential contribution to development of interstitial lung disease

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Abstract

Objective. To determine whether IL-18 is involved in the inflammation of DM and PM.

Methods. Thirty-three patients with DM were enrolled in this study, including 25 with interstitial lung disease (ILD). In addition, 16 patients with PM were enrolled, including 6 with ILD. All patients were admitted to our hospital as a result of their condition requiring treatment, and clinical laboratory data including serum IL-18 were recorded on admission.

Results. Serum IL-18 was significantly ($P < 0.0001$) higher in both DM and PM patients than in healthy controls ($n = 30$). Serum ferritin and IL-18 were significantly ($P = 0.003$ and 0.0044 , respectively) higher in DM than in PM patients. Additionally, ferritin and IL-18 were significantly ($P = 0.023$ and 0.034 , respectively) higher in DM patients with ILD than in DM patients without ILD. Significant positive correlations were found between creatine kinase (CK) and ferritin ($r_s = 0.39$, $P = 0.024$); CK and IL-18 ($r_s = 0.48$, $P = 0.005$); and IL-18 and ferritin ($r_s = 0.54$, $P = 0.0012$) in the DM group as a whole. These findings were different for the DM plus ILD subgroup: significant positive correlations were found between CK and ferritin ($r_s = 0.40$, $P = 0.047$); CK and IL-18 ($r_s = 0.63$, $P = 0.0008$); and IL-18 and ferritin ($r_s = 0.41$, $P = 0.042$).

Conclusion. Serum IL-18 was strikingly elevated in patients with DM and was associated particularly with disease activity and ILD complication in DM.

Key words: Dermatomyositis, Polymyositis, Interleukin-18, Ferritin, Macrophage activation.

Introduction

Inflammatory myopathies, characterized by muscle weakness and inflammation [1], fall into three major groups: PM, DM and sporadic IBM [1]. In general, DM is considered to arise from CD4⁺ T- and B-cell-mediated muscle inflammation. The complement system is activated, resulting in membrane deposition of an attack complex within muscle capillaries [2]. In PM, autoreactive cytotoxic T cells may mediate MHC I-restricted

cytotoxicity against autoantigens expressed on muscle [2]. Thus, the pathology differs between DM and PM.

We reported that serum ferritin predicted development and severity of acute interstitial lung disease (ILD) with DM [3]. Additionally, we have encountered several patients in whom levels of serum ferritin were high and correlated with DM disease activity (Gono T. and Kawaguchi Y., unpublished data). Ferritin is the major molecule for iron storage and plays a crucial role in the sequestration of potentially harmful molecules of reactive iron [4]. Ferritin can be secreted by the liver, T lymphocytes and macrophages. Very high serum levels of ferritin have been reported in systemic-onset JIA, adult-onset Still's disease and haemophagocytic syndrome related to CTDs, which is within the spectrum of secondary haemophagocytic lymphohistiocytosis [5–7]. Macrophage activation syndrome is now considered to be the specific term for a form of secondary haemophagocytic lymphohistiocytosis

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Submitted 15 April 2010; revised version accepted 26 May 2010.

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seen in the context of rheumatic disorders [5, 7]. The pathophysiology of macrophage activation syndrome involves lack of regulation of T lymphocytes and excessive production of cytokines, such as the TNF- α , IL-1 β , IL-6 and IL-18, resulting in macrophage activation [5]. Additionally, it has been reported that mRNAs for IL-18 and IL-12 are readily detected in Kupffer cells and activated macrophages, and that dendritic cells produce IL-18 in active inflammatory myopathies [8, 9]. Considering these results, we hypothesized that high levels of serum ferritin might reflect the aberrant production of IL-18 by activating T lymphocytes, macrophages and dendritic cells in DM. In the present study, we sought to determine whether IL-18 could contribute to the inflammation associated with DM and PM.

Materials and methods

Patients

This retrospective study included patients admitted to our hospital from 1 August 1992 to 31 January 2009. All the enrolled patients had skin rash, myopathy, respiratory symptoms or a combination thereof on admission and were diagnosed as having PM or DM based on the criteria of Bohan and Peter [10]. Patients with an overlapping syndrome such as SLE and SSc were excluded. Clinical data were obtained from medical records on admission. The present study was approved by the ethics committee in our institution according to the Declaration of Helsinki. Blood tests, including creatine kinase (CK), CRP, ferritin, ANA and anti-Jo-1 antibody, were measured by standard methods. Additionally, serum IL-18 was measured by ELISA (R&D systems, Minneapolis, MN, USA). Serum IL-18 was also investigated in 30 healthy controls whose age and gender were matched to the patients.

Statistical analysis

Statistical analyses were performed using the Fisher's exact test for the comparison of frequencies and the Mann-Whitney U-test for comparisons of median levels. Correlation coefficients were established using Spearman correlation coefficients. The data were analysed using JMP software (SAS Institute, Cary, NC, USA). $P < 0.05$ was considered to be statistically significant.

Results

Clinical manifestations in patients with DM and PM

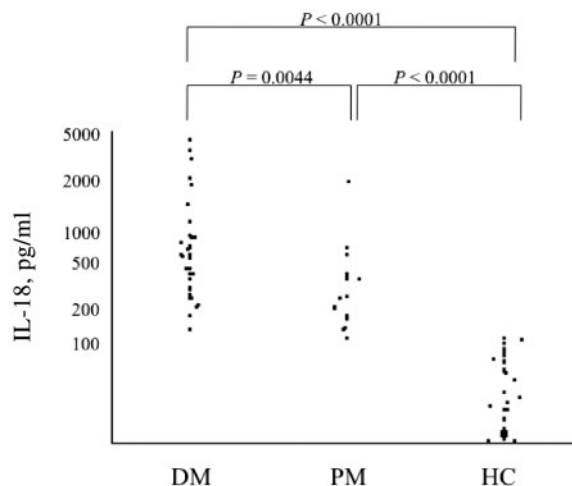
Thirty-three patients with DM were enrolled in this study, including 25 with ILD and 8 without ILD. In addition, 16 patients with PM were enrolled, including 6 with ILD and 10 without ILD. Table 1 shows results of the examinations upon admission. The age at onset was not significantly different between the DM and PM patient subsets. The level of CK was significantly ($P=0.01$) higher in patients with PM than in those with DM. Although CRP levels were not significantly different, ferritin was significantly ($P=0.003$) higher in DM than in PM patients. As shown in Fig. 1, the median levels (range) of

TABLE 1 Comparison in clinical data between DM and PM patients on admission

Variables	DM (n = 33)	PM (n = 16)	P-value
Age, years	48.1 (15.6)	51.7 (15.0)	0.51
CK, IU/ml	375 (35–11 167)	2173.5 (545–8802)	0.001
CRP, mg/dl	0.2 (0–6.5)	0.15 (0–3)	0.24
Ferritin, ng/ml	236 (23–2140)	55 (7–1400)	0.003
IL-18, pg/ml	552 (141–4850)	256.5 (119–2190)	0.0044
ANA \geq \times 160, n (%)	7 (22)	4 (25)	1
Jo-1, n (%)	4 (12)	2 (13)	1

The values of age indicate mean (s.d.), and those of CK, CRP, ferritin and IL-18 indicate median (range). The P -value was estimated using Mann-Whitney U-test or Fisher's exact test.

Fig. 1 The level of serum IL-18 in DM, PM and HC. The P -value was estimated using the Mann-Whitney U-test. HC: healthy controls.



IL-18 were 552 (141–4850), 256.5 (119–2190) and 50.5 (18–121) pg/ml in DM patients, PM patients and healthy controls, respectively. The levels of IL-18 were significantly higher in DM and PM patients than in healthy controls ($P < 0.0001$ for both comparisons). Additionally, the level of IL-18 was significantly ($P=0.0044$) higher in DM patients than in PM patients. The frequencies of ANA and Jo-1 positivity were not significantly different between DM and PM patients.

Comparison of laboratory data between DM with and without ILD

As shown in Table 2, ferritin and IL-18 levels were significantly ($P=0.023$ and 0.034 , respectively) higher in DM patients with ILD than those without ILD, but the CK and CRP levels were not significantly different. This result could indicate that the alveolar inflammation induced by IL-18 was more active in DM with ILD than in DM without ILD. In contrast, the median values of IL-18 were

TABLE 2 Comparison of clinical data between DM with and without ILD on admission

Variables	DM with ILD (n = 25)	DM without ILD (n = 8)	P-value
CK, IU/ml	375 (35–7641)	416.5 (61–11 167)	0.83
CRP, mg/dl	0.2 (0–6.5)	0.15 (0.1–1.9)	0.73
Ferritin, ng/ml	320 (52–2140)	81.65 (23–1100)	0.023
IL-18, pg/ml	625 (141–4850)	327.5 (225–655)	0.034

The values of CK, CRP, ferritin and IL-18 indicate median (range). The *P*-values were estimated using Mann–Whitney U-test.

382.5 and 234.5 pg/ml in PM patients with ILD and without ILD, respectively. There were no significant differences between PM with and without ILD for the measured parameters. Additionally, no significant difference was found in the median value of IL-18 among DM patients without ILD, PM patients with and without ILD. The median value of IL-18 was higher in DM with ILD than in other three groups. These findings can be contributed to the statistical difference between the whole DM and PM patients. On the other hand, the small number of PM patients with ILD may be contributed to no difference in the inflammatory parameters between PM patients with and without ILD.

Correlation coefficients between parameters in DM overall and DM with ILD

As shown in Table 3, correlation coefficients between parameters were established for DM overall and for DM with ILD. Although correlations between CRP and CK, CRP and IL-18, and CRP and ferritin were not found, significant positive correlations were found between CK and ferritin ($r_s = 0.39$, $P = 0.024$), CK and IL-18 ($r_s = 0.48$, $P = 0.005$) and IL-18 and ferritin ($r_s = 0.54$, $P = 0.0012$) in the DM group as a whole (r_s : correlation coefficient established employing Spearman correlation coefficients). These findings were especially notable in the DM with ILD subset. Significant positive correlations were found between CK and ferritin ($r_s = 0.40$, $P = 0.047$), CK and IL-18 ($r_s = 0.63$, $P = 0.0008$), and IL-18 and ferritin ($r_s = 0.41$, $P = 0.042$) in the DM with ILD subset. In contrast, there was no significant correlation with PM (data not shown).

Discussion

We have demonstrated that the level of serum IL-18 is high in both DM and PM patients compared with controls. Additionally, significant correlations between serum IL-18 and ferritin, and serum IL-18 and CK, were revealed for the first time in the DM patients of the present study. Cytokines other than IL-18 were not investigated in the present study because of inadequate quantities of stored serum samples. Serum ferritin is an important laboratory hallmark in macrophage activation syndrome and haemophagocytic lymphohistiocytosis [7]. Additionally, IL-18 is an important cytokine in both

TABLE 3 Correlation coefficients in the entire group of DM and DM with ILD

Variables	DM (n = 33)		DM with ILD (n = 25)	
	r_s	P-value	r_s	P-value
CK vs CRP	0.27	0.13	0.3	0.15
CK vs ferritin	0.39	0.024	0.4	0.047
CK vs IL-18	0.48	0.005	0.63	0.0008
IL-18 vs CRP	0.33	0.052	0.39	0.057
IL-18 vs ferritin	0.54	0.0012	0.41	0.042
Ferritin vs CRP	0.26	0.14	0.2	0.33

r_s : correlation coefficient established employing Spearman correlation coefficients.

macrophages and Th-1 immune activation, two important pathogenic mechanisms in haemophagocytic lymphohistiocytosis. It has been reported that a severe IL-18/IL-18-binding protein imbalance results in macrophage and Th-1 lymphocyte activation, which escapes control by NK cell cytotoxicity and may allow for secondary haemophagocytic syndrome in patients with underlying diseases [6]. We speculated that this pathogenesis might be similar to that of DM–ILD with high concentration of serum ferritin. We found elevation of liver enzyme such as γ -GTP and hyperferritinaemia in acute ILD with DM [3]. Alveolar macrophages, which are activated by some antigens, microbes or autoimmune stimuli, are induced to produce leukotriene B4 and IL-8. These mediators stimulate neutrophils to induce fibrosis in the lung [11]. These results could indicate that macrophages such as Kupffer cells and alveolar macrophages are intensively activated and may cause injury to the liver and lungs with DM. Taken together, we investigated whether IL-18 was involved in DM. We did not obtain direct evidence of macrophage activation, and whether alveolar macrophages are activated must be investigated in DM with ILD.

Previous reports have described that the increase of ESR, CRP, fibrinogen and ferritin could be potential acute-phase reactants in patients with DM or PM [12]. In our study, although there were significant differences in the levels of IL-18 and ferritin between DM with and without ILD, we found no significant difference in the levels of CRP in these two DM subsets. Additionally, significant positive correlations were found between IL-18 and ferritin, but not between CRP and ferritin in the entire DM group and the DM with ILD subset. These findings suggest that the increase in serum ferritin concentration reflects overproduction of IL-18, rather than an acute-phase reactant such as IL-6 and CRP, in DM.

High concentrations of serum and muscular IL-18 suggest that the deregulated IL-18/IL-18R pathway may be pathogenetic in inflammatory myopathy and measurement of IL-18 might be predictive of the disease activity [9]. Although the pathohistology differed between DM and PM, IL-18 was demonstrated in muscles and was produced by both macrophages and dendritic cells surrounding perivascular and perimysium areas, and

endomysium in DM and PM, respectively [9]. Although this report showed that serum IL-18 levels correlated with the muscle enzymes in six DM or PM patients, whether IL-18 levels differ between DM and PM has not been investigated previously. Thus, we revealed the difference between serum IL-18 levels in DM and PM. Our study demonstrated that serum IL-18 level was significantly ($P=0.0044$) higher in DM than in PM, although CK level was higher in PM than in DM. These results may indicate that IL-18 is involved more closely in DM than in PM.

On the other hand, serum IL-6 production and the Type 1 IFN gene signature were reported to be candidate biomarkers for disease activity in adult and JDM [13]. Type 1 IFN-regulated chemokines include monokine induced by IFN- γ , IFN- γ -inducible 10-kDa protein, IFN-inducible T-cell α -chemoattractant and monocyte chemoattractant protein 1 in this study. These Type 1 IFN-related chemokines were correlated with disease activity in DM [13]. Additionally, IL-18 attracts plasmacytoid dendritic cells and promotes Th-1 immune induction by plasmacytoid dendritic cells through IL-18 receptor expression [14]. Plasmacytoid dendritic cells are mediators of muscle inflammation in JDM [15]. Taken together, IL-18 may stimulate Type 1 IFN-regulated chemokines and plasmacytoid dendritic cells, which induce muscle inflammation in DM.

In the present study, ferritin and IL-18 were significantly higher in the DM with ILD than in the DM without ILD patients. This result could indicate that inflammation induced by IL-18 was more active in DM with ILD. We reported that serum ferritin predicted the development and severity of acute ILD with DM [3]. The cumulative survival rate was poor in DM with ILD showing hyperferritinaemia, especially at levels >1500 ng/ml [3]. Additionally, we have encountered several cases in which both serum ferritin and IL-18 were correlated with the activity of acute ILD with DM (Gono T. and Kawaguchi Y., unpublished data). We consider that both serum IL-18 and ferritin can be biomarkers in disease activity with DM-ILD. Blocking serum IL-18 and inhibiting Th-1 lymphocytes and macrophages may be a treatment strategy to consider for DM with ILD.

In conclusion, serum IL-18 was elevated in patients with DM and PM, and was associated particularly with disease activity and ILD complication in DM. Both serum IL-18 and ferritin can be useful markers for DM disease activity.

Rheumatology key messages

- IL-18 was elevated in patients with DM and PM.
- The concentrations of IL-18 were associated with disease activity and ILD complication in DM.
- Significant correlations between IL-18 and ferritin, and serum IL-18 and CK, were revealed in DM patients.

Acknowledgements

Funding: This study was supported in part by research grants from the Ministry of Health, Labour and Welfare in Japan.

Disclosure statement: The authors have declared no conflicts of interest.

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