

Potential role of type I interferon/IP-10 axis in the pathogenesis of anti-MDA5 antibody-positive dermatomyositis

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Abstract

Objective

Dermatomyositis (DM) patients with anti-melanoma differentiation-associated protein 5 (MDA5) antibodies are known for poor prognosis. This study was designed to identify humoral factors that are readily detectable in the disease and may reflect its activity and pathophysiology.

Methods

We first quantified the serum level expression of 28 cytokines in the serum of patients with collagen vascular diseases using bead-based multiplex immunoassays. We completed these evaluations at hospital admission and followed up with three DM patients with anti-MDA5 antibodies during hospitalisation. We also performed an immunohistochemical analysis of skin samples obtained from two patients.

Results

The serum level of interferon gamma-induced protein 10 (IP-10) was significantly higher in DM patients with anti-MDA5 antibodies than in those without the antibody, decreasing drastically upon treatment. Interestingly, this time course paralleled not that of interferon (IFN)- γ , which was originally reported to be the inducer of IP-10, but that of IFN- α 2. Immunohistochemical analysis revealed that most of the IP-10-positive cells were macrophages. Furthermore, monocytes stimulated with type I IFN in vitro produced IP-10 in a dose-dependent manner.

Conclusion

IP-10 is a potentially useful disease activity marker of DM with anti-MDA5 antibodies, correlating more with IFN- α 2 than IFN- γ . IP-10 released from macrophages might prompt the infiltration of macrophages themselves. Thus, the type I IFN/IP-10 axis may play a pivotal role in the pathogenesis of this intractable disease.

Key words

dermatomyositis, chemokines, interferons, melanoma differentiation-associated protein 5, monocytes/macrophages

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Introduction

Dermatomyositis (DM) is an inflammatory disease characterised by muscle weakness and a distinctive skin rash (1), often associated with the development of other serious complications, like interstitial lung disease (ILD). This is especially concerning in the case of rapidly progressing ILD (RP-ILD) which is one of several complications of DM that determine its prognosis. DM with little or no symptoms of myositis is referred to as “clinically amyopathic DM” (CADM) (2, 3), and patients with CADM tend to develop RP-ILD (3, 4). In 2005, Sato *et al.* identified that an autoantibody for a 140 kD polypeptide is specific for CADM (5). Its autoantigen was later identified as melanoma differentiation-associated protein 5 (MDA5) (6, 7). Among patients with CADM, those of Asian descent have a high frequency of the anti-MDA5 antibody and a strong association with RP-ILD (8). MDA5 is an intracellular virus sensor that recognises picornaviruses, like the coxsackie virus, which induce the type I interferon (IFN) response upon activation (9–11). More recent studies have shown that DM is one of several “interferonopathies”, along with systemic lupus erythematosus (SLE) (12). Thus, it is intriguing that MDA5, a key inducer of type I IFN, is also an autoantigen in CADM. This indicates that the presence of anti-MDA5 antibodies may not only be an outcome of the disease but can also be involved in its pathogenesis.

As CADM with RP-ILD is refractory to most treatment strategies, more facilities have come to use a combination of a corticosteroid, cyclophosphamide, and calcineurin inhibitors, such as cyclosporine A, to manage this condition (13). It has substantially improved the prognosis of these patients, making it a common intervention in CADM (14). However, it also strongly suppresses immunity and thus opportunistic infections are a frequent concern in these patients. In addition, there are still some cases that are resistant to this approach, forcing the evaluation of various other strategies including plasma exchange (15) with varying degrees of success. Recent reports

include an evaluation of Janus kinase inhibitors (JAKi) in the treatment of this condition, supporting its further investigation (16, 17). Rituximab has also been administered in a small number of cases but with limited success (8, 18). A deeper understanding of the pathophysiology of this condition will be beneficial for the development of more effective strategies with less immunosuppression. In this regard, specific activity markers for this disease may elucidate their pathogenesis.

Several markers have been proposed for monitoring the disease activity of RP-ILD with anti-MDA5 antibody, including serum KL-6, which is a general marker of ILD (19), and serum ferritin, which is relatively specific for this condition (20). However, they do not always respond promptly to disease activity change, sometimes leading to overtreatment and unwanted drug side effects. In addition, the relationship between those markers and type I IFNs remains obscure. Thus, novel activity markers of the disease may shed more light on its pathogenesis. Here, we first compared the profile of proteins in the serum of anti-MDA5 antibody-positive and negative DM patients upon admission, and identified a chemokine interferon gamma-induced protein 10 (IP-10) as a potential biomarker for anti-MDA5 positive DM. We also revealed a correlation between IFN- α 2 and IP-10; both factors decreased in response to therapeutic intervention. We then performed immunohistochemical analysis of a skin lesion to examine the cells that colocalised with IP-10. We also performed *in vitro* experiments to analyse whether IP-10 is induced by the stimulation of type I IFN in monocytes derived from peripheral blood.

Materials and methods

Patients

Peripheral blood was obtained from patients admitted to Jichi Medical University Hospital between November 2019 and March 2021. DM patients were diagnosed based on the 2017 European League Against Rheumatism/American College of Rheumatology criteria (21) and we included patients with SLE, who met the 1997 ACR cri-

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teria (22, 23), as our control population. Our study complied with the Declaration of Helsinki and was approved by the Institutional Review Board of Jichi Medical University (A21-042). Written informed consent was obtained from each patient.

Bead array assay

The serum levels of the 27 cytokines were quantified using a Bio-Plex 200 system (Bio-Rad) according to the manufacturer's instructions. These cytokines included: eotaxin, fibroblast growth factor basic (FGF basic), granulocyte colony-stimulating factor (G-CSF), granulocyte macrophage colony-stimulating factor (GM-CSF), IFN- γ , interleukin 1 β (IL-1 β), IL-1 receptor antagonist, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12 (p70), IL-13, IL-15, IL-17A, IP-10, monocyte chemoattractant protein 1 (MCP-1), macrophage inflammatory protein 1 α (MIP-1 α), MIP-1 β , platelet-derived growth factor BB (PDGF-BB), regulated on activation, normal T cell expressed and secreted (RANTES), tumour necrosis factor α (TNF- α), and vascular endothelial growth factor (VEGF), all of which were included in the Bio-Plex Pro Human Cytokine 27-plex Assay set. We also evaluated IFN- α 2 and monokine induced by gamma interferon (MIG) using single-plex assays from the same supplier (Bio-Rad). The values below the lowest standard curve points (under the lower limit of quantification, ULOQ) were treated as equal to ULOQ.

Immunohistochemical analysis

Immunofluorescent double labelling was performed by incubating 4 mm frozen tissue sections blocked with Background Sniper (BioCare medical Inc., 10 min at room temperature), with appropriate dilutions of the relevant primary antibodies for 1 hour at room temperature. IP-10 rabbit monoclonal antibody (clone JA10-82, Thermo Fisher Scientific Inc.) was combined with CD4 mouse monoclonal antibody (clone 4B12), CD8 mouse monoclonal antibody (clone C8/144B), or CD68 (clone KP1); all the mouse monoclonal antibodies

Table I. Clinical characteristics of the cohort.

	SLE n=14	DM (MDA5 Ab ⁻) n=6	DM (MDA5 Ab ⁺) n=9
Age (mean \pm SD), years	38.2 \pm 9.4	60.8 \pm 14.7	53.4 \pm 13.1
Female, n (%)	13 (92.9)	5 (83.3)	4 (44.4)
CK (U/mL)	43 (25-74)	323 (96-660)	233 (122-418)
KL-6 (ng/mL)	291 (178-428)	528 (169-972)	738 (587-1085)
Ferritin (ng/mL)	159 (154-681)	29 (21-450)	316 (90-845)
PSL (mg/day)	5 (1-10)	2.5 (0-40)	0 (0-2)

The age values are reported as the mean \pm standard deviation (SD). The laboratory markers and prednisolone (PSL) doses are described using their median values (interquartile range) and the groups are delineated based on diagnosis. CK: creatine kinase.

were from Dako Denmark A/S. In the following detection step, a mixture of goat anti-mouse IgG (H + L) secondary antibody conjugated with Alexa Fluor 488 and goat anti-rabbit IgG (H + L) secondary antibody with Alexa Fluor 568, both from Thermo Fisher Scientific Inc., was applied for 30 min at room temperature in the dark. Samples were mounted with ProLong Gold Antifade Reagent with DAPI (Thermo Fisher Scientific Inc.) and a confocal quantitative image cytometer, CQ1 (Yokogawa), was used to take immunofluorescence photos.

Purification of monocytes

Human peripheral blood mononuclear cells (PBMCs) derived from healthy donors were separated by Ficoll-Paque gradient centrifugation. Monocytes were then sorted from PBMCs by an immunomagnetic selection using autoMACS Pro Separator and the Human Pan Monocyte Isolation Kit (Miltenyi Biotec) according to the manufacturer's protocol. Sorted monocytes and non-monocytes were cultured in α -minimum essential medium (Gibco Invitrogen) supplemented with 10% foetal bovine serum in the presence or absence of 1 or 10 ng/mL interferon α A (PBL Assay Science) for 15 hours. The levels of IP-10 in the culture supernatants were quantified with a Human CXCL10/IP-10 Quantikine ELISA Kit (R&D systems). To analyse the purity of monocytes and non-monocytes, they were incubated with anti-CD14 antibodies conjugated with Alexa Fluor 488, anti-CD16 antibodies conjugated with Alexa Fluor 647, and antibodies conjugated with anti-CD86 conjugated with phycoerythrin in the

presence of FcR Blocking Reagent (all the reagents were from BD Bioscience). After washing the cells, they were analysed by FACSCanto II (BD Bioscience). Dead cells were excluded from the analysis by 7-Amino-Actinomycin D labelling (Miltenyi Biotec). FlowJo software (BD Bioscience) was used for data analysis. This experiment was approved by the Institutional Review Board of Jichi Medical University (21-041) and three healthy donors were recruited.

Statistical analysis

Clinical characteristics and measurements were compared using Fisher's exact test for proportions, and a Mann-Whitney U-test for continuous variables. A *p*-value <0.05 was considered statistically significant and the Bonferroni correction was used for multiple comparisons. R (version 3.6.2) was used for statistical analysis.

Results

Clinical characteristics of anti-MDA5 antibody-positive patients

The clinical characteristics of all patients enrolled in this study are summarised in Table I. Our cohort included 14 SLE patients, nine DM patients with anti-MDA5 antibodies, and six DM patients without anti-MDA5 antibodies. The patients in the SLE cohort were significantly younger than DM patients. There was no statistical difference in ages between DM patients with or without anti-MDA5 antibodies. We observed statistically significant difference in female-to-male ratio, which was lower in the DM patients with anti-MDA5 antibody, compared with that of the SLE cohort.

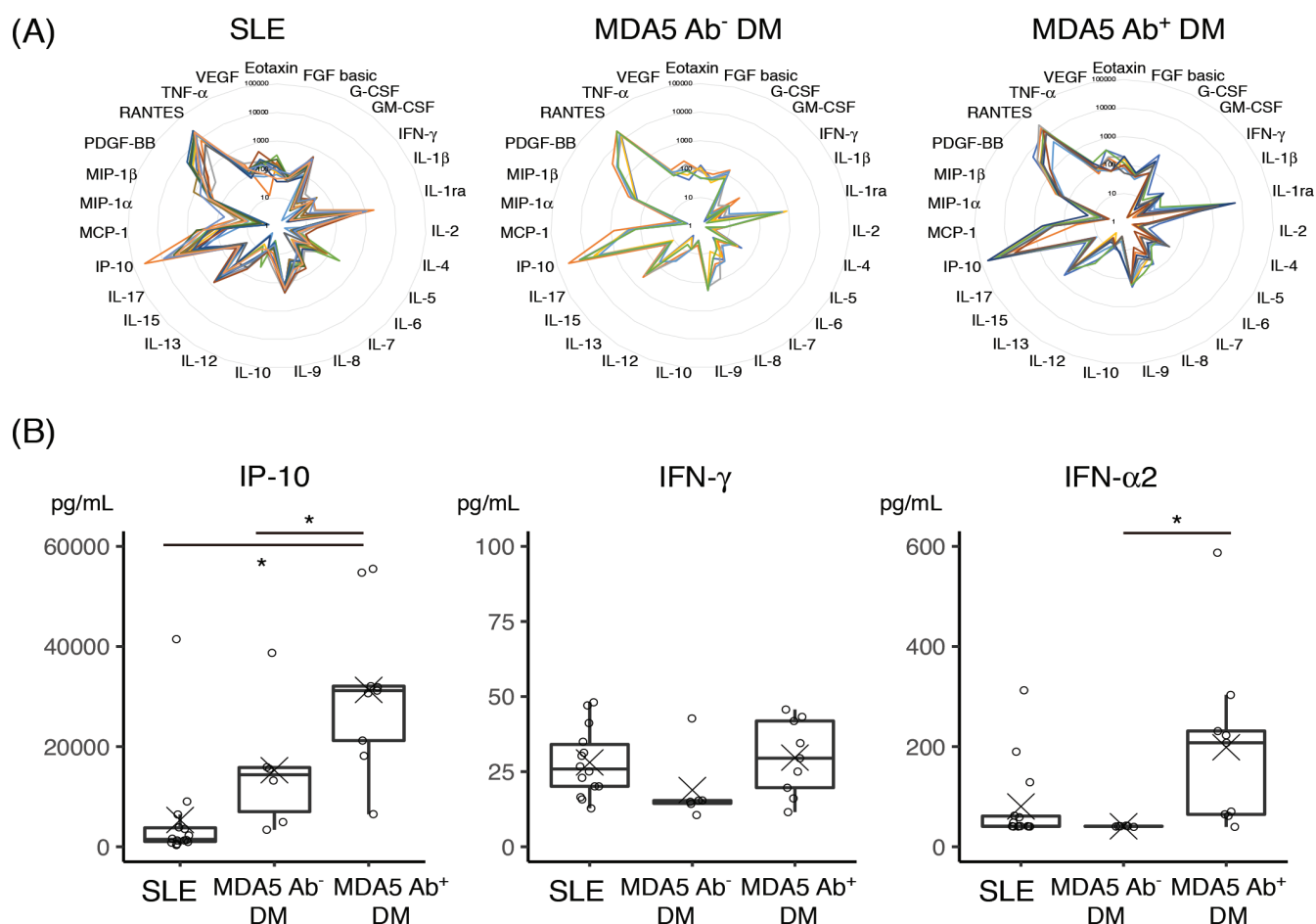


Fig. 1. Cytokine profiles of SLE and DM patients with or without anti-MDA5 antibodies.

A: Radar charts describing the serum cytokine levels quantified using 27-plex assay in SLE and DM patients with or without anti-MDA5 antibodies at the time of admission. Note that the cytokine levels are shown in logarithmic scale (pg/mL).

B: Box plots overlaid with dot plots for IP-10, IFN-α2, and IFN-γ expression.

Any values below the lowest standard curve points (the lower limit of quantification, ULOQ) were treated as equal to the ULOQ. A Mann-Whitney U-test followed by Bonferroni correction was used to assess the statistical differences; * $p < 0.05$.

Comparison of cytokines of the SLE and DM patients with or without anti-MDA5 antibodies on admission

Figure 1A shows the radar charts or the 27-plex cytokine evaluations for each patient at the time of admission, which reveal a substantially increased IP-10 expression in DM patients with anti-MDA5 antibodies. In fact, there was a statistically significant difference between the DM patients with the anti-MDA5 antibody and those without the antibody or SLE patients (Fig. 1B, left panel). IP-10 was originally identified as being induced by IFN-γ (24). However, there was no significant difference in the levels of serum IFN-γ, although it appeared to increase in the serum of anti-MDA5 antibody-positive patients, compared to the antibody-

negative patients. As IP-10 has also been shown to respond to type I IFN signalling (25), we went on to evaluate a difference in IFN-α2 expression between anti-MDA5 antibody-positive and negative DM patients. IFN-α2 expression was significantly higher in the anti-MDA5 antibody-positive DM patients than in the antibody-negative DM patients (right panel). In fact, serum IFN-α2 was under the limit of detection in all the anti-MDA5 antibody-negative patients in our cohort.

Clinical course of three DM patients with anti-MDA5 antibodies and the level of known activity markers and IP-10, MIG, and type I and II IFNs

Figure 2 represents the clinical course in three of the anti-MDA5 antibody-

positive patients, which was evaluated to determine if these profiles were static or responded to clinical intervention in any predictable way. We treated the patients using ferritin as a main clinical parameter (20), because the results were available on the same day of the test. Evaluation of the clinical course of these patients revealed that potent immunosuppressive therapy allowed for each patient to return home alive and that their improvements corresponded to substantial decreases in serum IP-10 expression, which was quantified post hoc. Time course evaluations of the serum expression levels of IFN-α2, IFN-γ, IP-10, and MIG, which is also identified as a chemokine induced by IFN-γ (26), are shown in Figs. 3A-C. While IFN-α2 promptly fell below the limit of detection in these assays, neither IFN-γ nor

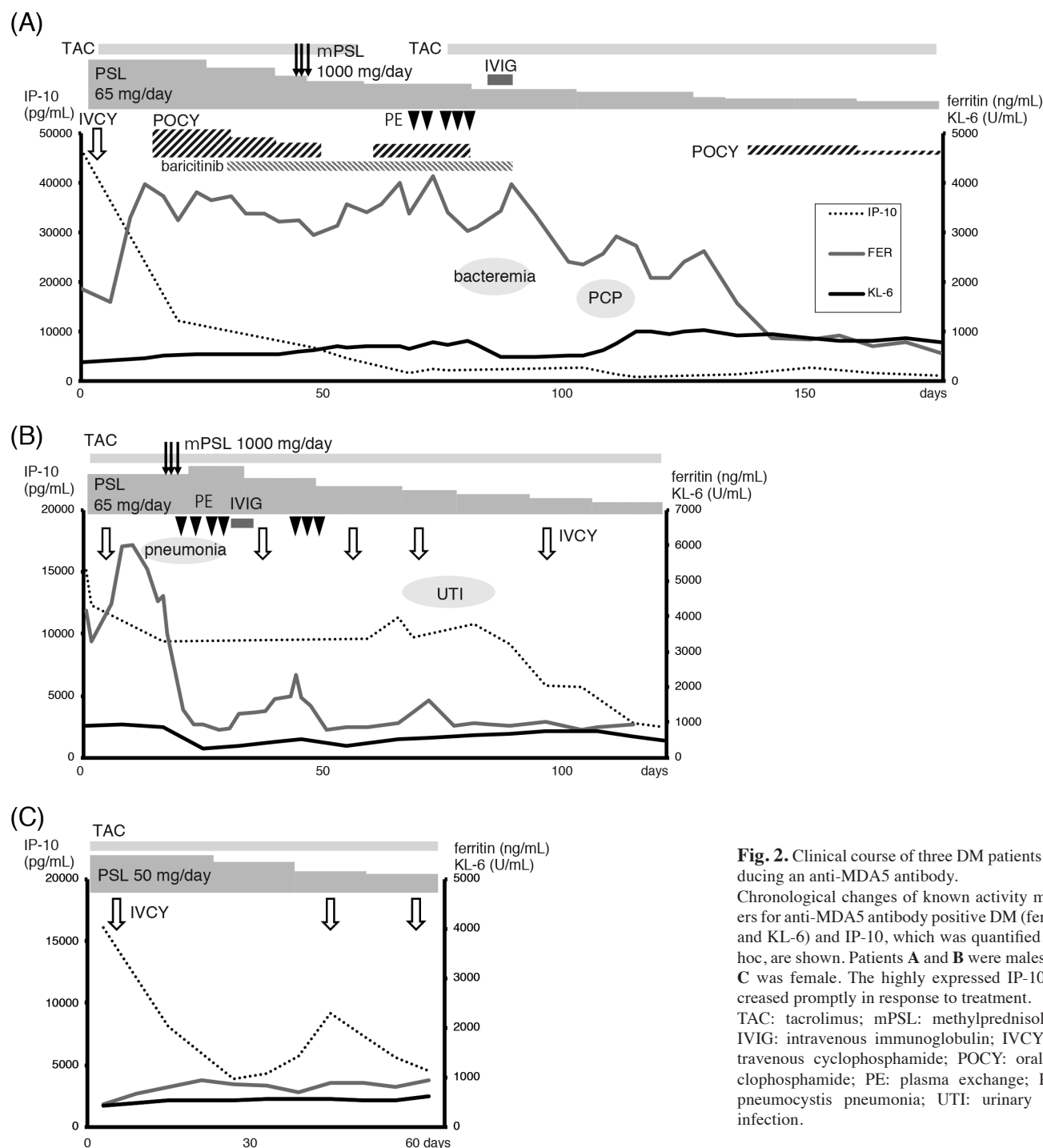


Fig. 2. Clinical course of three DM patients producing an anti-MDA5 antibody.

Chronological changes of known activity markers for anti-MDA5 antibody positive DM (ferritin and KL-6) and IP-10, which was quantified post hoc, are shown. Patients **A** and **B** were males and **C** was female. The highly expressed IP-10 decreased promptly in response to treatment.

TAC: tacrolimus; mPSL: methylprednisolone; IVIG: intravenous immunoglobulin; IVCY: intravenous cyclophosphamide; POCY: oral cyclophosphamide; PE: plasma exchange; PCP: pneumocystis pneumonia; UTI: urinary tract infection.

MIG decreased substantially during the course of treatment. Thus, the expression patterns of two chemokines IP-10 and MIG, both of which have been thought to be the targets of IFN- γ , were quite different. The correlation between IP-10 and IFN- α 2 seemed much stronger than that of IP-10 and IFN- γ , suggesting that type I IFN, including IFN- α 2, is more likely to be the inducer of IP-10 than type II IFN (IFN- γ).

We also had six more paired serum samples at admission (pre) and discharge (post) from the patients with anti-MDA5 antibodies; all of whom were discharged from the hospital within a month (11–23 days, median=16.5 days). When we compiled the data (nine pairs), IP-10 and IFN- α 2 showed statistically significant decreases between pre and post, despite the limited number of the samples, whereas IFN- γ did not (Fig. 3D). IFN- α 2

and IP-10 revealed a positive correlation, and the logarithmic approximation seemed to be more appropriate than the linear approximation (coefficient of determination $R^2=0.53$ and $p=0.023$ vs. $R^2=0.33$ and $p=0.10$; Fig. 3E).

Immunohistochemical analysis of skin lesions from two patients with anti-MDA5 antibodies

We next completed a representative

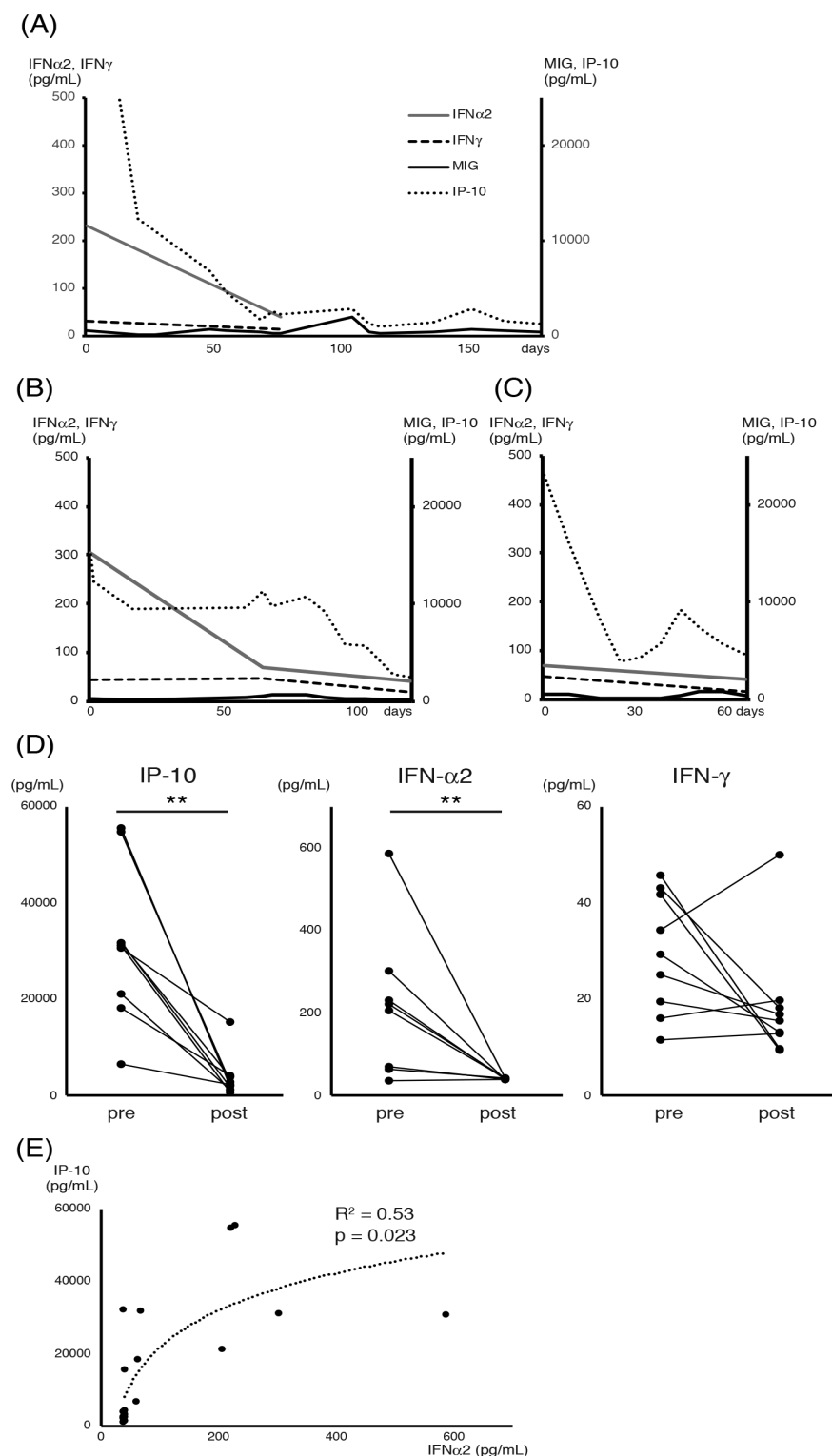


Fig. 3. Time course showing changes in serum IP-10, MIG, IFN- α 2, and IFN- γ expression, before-and-after comparison, and the relationship between IFN- α 2 and IP-10.

Patients A-C are the same as those in Fig. 2. Although both IP-10 and MIG were initially identified as IFN- γ -inducible chemokines utilising the same receptor CXCR3, their expression patterns were quite different. Compared to IP-10, MIG was not highly expressed and did not decrease substantially in response to treatment. The levels of IFN- γ also did not seem to respond promptly to the treatment.

D: Before-and-after comparison of IP-10, IFN- α 2, and IFN- γ (n=9). A Wilcoxon signed-rank test was used. ** $p < 0.01$.

E: Scatter plot of IFN- α 2 and IP-10. The logarithmic approximation curve is also shown.

R^2 : coefficient of determination.

immunohistochemical analysis of an erythematous rash on the left elbow (Gottron's sign) of one patient (Pt. (A), Fig. 4A). In the case of psoriasis, another inflammatory skin disease, IP-10 protein was reported to be expressed in keratinocytes (27) and this chemokine has been implicated in the infiltration of various mononuclear cells in skin lesions. Our evaluations revealed that most of the IP-10 positive cells in the dermis were CD68 positive, and while a small number of cells in the dermis were CD8 positive, very few of them were also IP-10 positive, which means that there were substantially more CD68⁺IP-10⁺ cells than CD8⁺IP-10⁺ cells. And there were almost no CD4 positive cells in these samples. Similar analysis was performed on another patient (Pt. (B), skin from the right elbow, Fig. 4B), which also revealed the double staining of IP-10 and CD68 (Fig. 4B). CD4 and CD8 positive cells were almost absent in the sample. Thus, in anti-MDA5 antibody-positive DM, the main producers of IP-10 are likely to be monocyte/macrophage lineage cells rather than keratinocytes and the mononuclear cells that are attracted into the skin lesions are also monocyte/macrophage lineage cells rather than T cells. In order to assess whether monocytes are capable of producing IP-10 by stimulation with type I IFN, we sorted monocytes from PBMCs. The purity of monocytes (CD14 or CD16 positive and CD86 positive) was ~80% (Fig. 5A). After culturing the monocytes and non-monocytes *in vitro* in the presence or absence of type I IFN, the levels of IP-10 in the culture supernatants of monocytes revealed a dose-dependent response, whereas that of non-monocytes revealed a substantially low response (Fig. 5B).

Discussion

Our study detected high levels of IP-10 expression in the serum samples of DM patients positive for anti-MDA5 antibodies. This agreed with a recent study from Asakawa *et al.* who reported that IP-10 was the cytokine most significantly associated with amyopathic DM-ILD in anti-MDA5 antibody-positive samples compared with

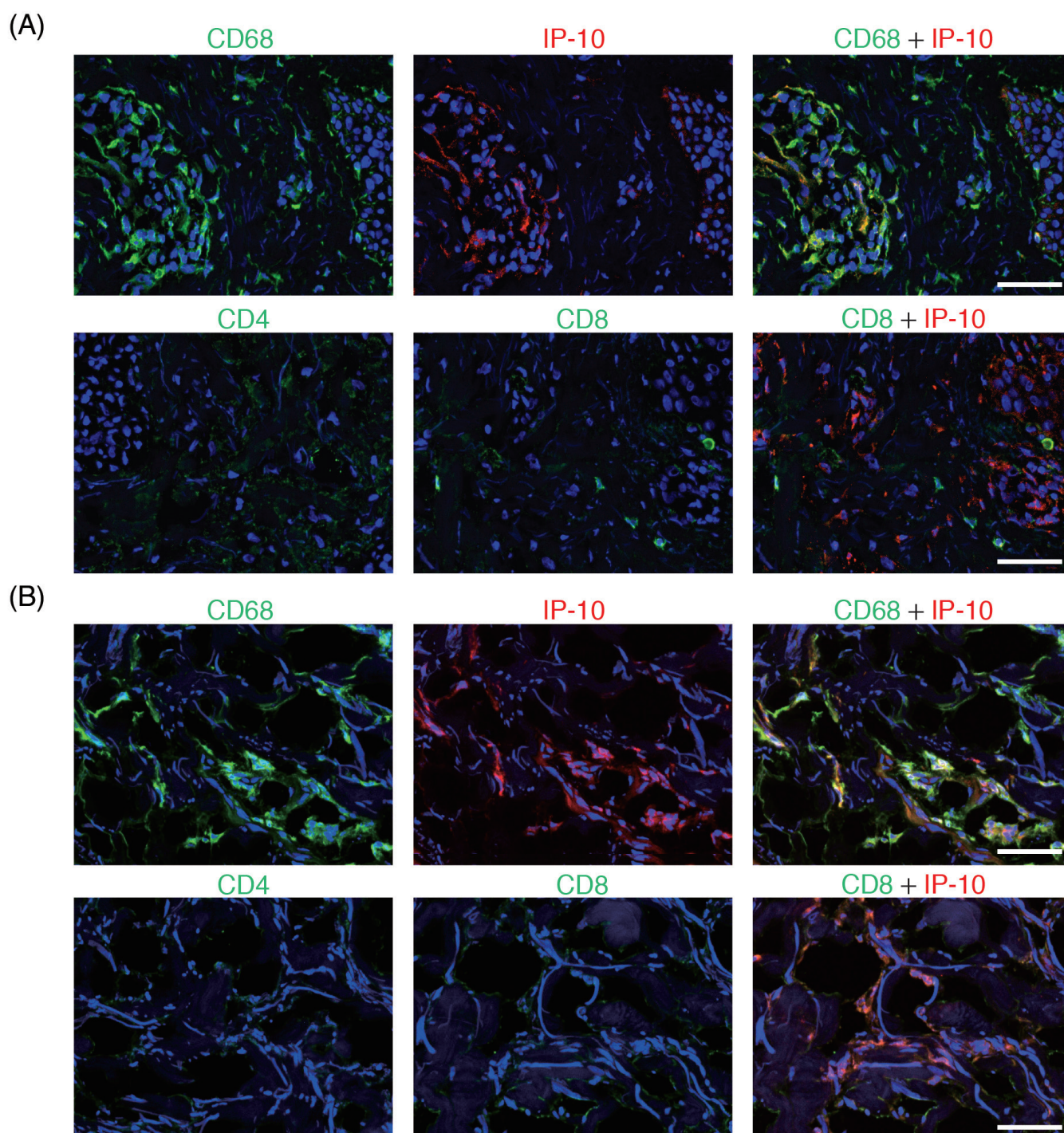


Fig. 4. Immunohistochemical analysis of the Gottron's signs in two patients (Patients A and B).

Tissue sections derived from erythematous rashes on the elbow (Gottron's sign) of two patients were double stained with IP-10 (red) and either CD68, CD4, or CD8 (green). Most of the IP-10-positive cells in the dermis were also CD68-positive, and CD8⁺IP-10⁺ cells were scarce. In addition, there were almost no CD4⁺ cells. Scale bars = 50 μ m.

anti-synthetase antibody-positive ones when evaluating a panel of 38 known cytokines (28). The high expression of IP-10 suggests that this chemokine can function as an activity marker of the disease. Indeed, IP-10 rapidly decreased in response to treatment in all clinical cases evaluated in our study,

suggesting that it is closely related to the pathogenesis of the disease (Figs. 2, 3).

IP-10 has been implicated in the pathogenesis of psoriasis along with MIG (29, 30). As their names suggest, they were identified as factors induced by IFN- γ , and have been categorised as

Th1 chemokines (31). In addition, they both utilise CXCR3 as common receptors. However, the expression level of MIG was not as high as IP-10 upon disease onset, and it did not change much in the course of the treatment, suggesting that the regulations of these closely related chemokines are quite different

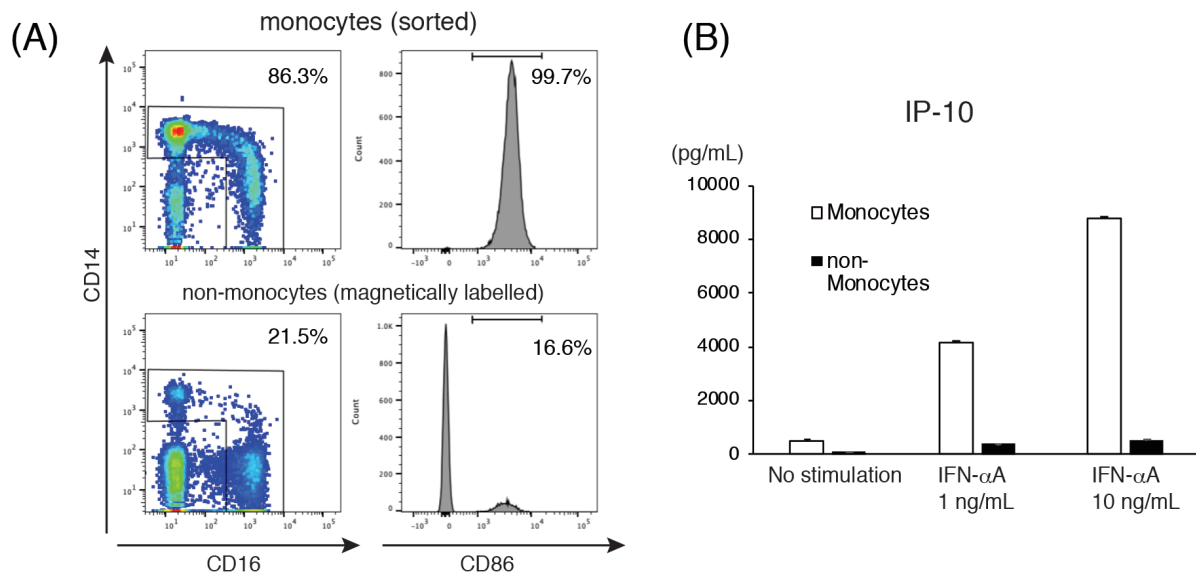


Fig. 5. Sorted monocytes from peripheral blood produced IP-10 in response to type I IFN *in vitro* in a dose-dependent manner.

A: Monocytes were sorted from PBMCs derived from healthy donors. The purity of monocytes was analysed by flow cytometry. CD14- or CD16-positive cells were ~80% in the sorted (magnetically unlabelled) cells and most of them were also CD86-positive. On the other hand, ~20% cells were CD14- or CD16-positive in the remaining magnetically labelled cells, and among them, less than 20% of cells were CD86-positive, indicating that more than 96% cells were non-monocytes in this fraction.

B: Monocytes cultured in the presence of type I IFN *in vitro* produced IP-10 in a dose-dependent manner, whereas non-monocytes barely produced IP-10. Error bars indicate SD. The data were representative of three independent experiments.

in this disease (Figs. 3A-C). Moreover, the level of IP-10 did not parallel that of IFN- γ , which did not decrease substantially. In contrast, there was a substantial decrease in IFN- α 2 expression over the same time period, suggesting that IP-10 expression may trend more with IFN- α 2, a type I IFN, than with IFN- γ , a type II IFN, in DM patients with anti-MDA5 antibodies. In accordance with this, the relationship between type I IFN and anti-MDA5 antibody-positive DM has attracted more and more attention (32, 33).

Anti-MDA5 antibody-positive DM patients are characterised by marked skin inflammation across various parts of the body, and the Gottron's phenomenon is most well recognised. Given this, we used samples with the Gottron's sign to complete the immunohistopathological analysis of IP-10 expression described in our results. These evaluations revealed that most of the IP-10-positive cells in the skin are likely to be of monocyte/macrophage-lineage (Fig. 4). This is distinct from the report on psoriasis in which keratinocytes are the main producers of IP-10 (27). Wenzel *et al.* analysed the expression of IP-10 in DM skin lesions in the context of the type I IFN-driven immune response (34).

Although the anti-MDA5 antibody had not been identified at that time, their patients included those with CADM. IP-10 was moderately expressed in all investigated samples, especially in the areas of the upper dermis. Although the numbers of CD3⁺ cells (T cells), CD20⁺ cells (B cells), and CD68⁺ cells in DM skin lesions were significantly more abundant than in healthy controls, they were substantially lower than those in chronic discoid lupus erythematosus (CDLE) samples. Moreover, the number of CD68⁺ cells outnumbered CD4⁺ and CD8⁺ cells in the DM skin lesions; in contrast, the total numbers of CD4⁺ and CD8⁺ cells were more than that of CD68⁺ cells in the case of CDLE. Thus, their data are compatible with ours. Notably, however, while CD4⁺ and CD8⁺ T cells are candidate cells to migrate towards the site of inflammation in response to IP-10 (30), our samples contained a few CD8⁺ cells and almost no CD4⁺ cells. As Wenzel *et al.* did not perform double staining immunohistochemistry, they did not characterise the IP-10 producing cells. Judging from our data (Fig. 4), both the producing cells and target cells of IP-10 are likely to be monocyte/macrophage-lineage cells in DM with anti-MDA5

antibodies. Indeed, we demonstrated that monocytes derived from peripheral blood produced IP-10 in response to type I IFN in a dose-dependent manner (Fig. 5B).

Of course, we should not confuse correlation with causation. The level of IP-10 seemed to decrease with cyclophosphamide (CY) treatment; however, it is difficult to know whether the decrease was caused indirectly by the reduction of type I IFN due to the cytotoxic effect of CY on plasmacytoid dendritic cells (formerly called natural IFN- α producing cells), or directly by the effect of CY on monocytes. On the other hand, the apparent lack of reduction in IFN- γ and MIG values might be explained by the mechanism of action of CY, which, in some cases, may potentiate the action of natural killer cells (35). It could be useful to explore the use of anifrolumab, a monoclonal antibody against type I IFN receptor subunit 1 that has been recently introduced in the treatment of SLE (36), against anti-MDA5 antibody-positive DM; this might be helpful for deciphering the type I IFN/IP-10 axis *in vivo*, although it would be an off-label use.

IP-10 has been implicated as a useful marker for predicting the progressive

severity of coronavirus disease-2019 (COVID-19) (37, 38). Considering that type I IFNs are cytokines induced by viral infection, this finding also suggests the direct link between type I IFN and IP-10. More recently, Gono *et al.* also advocated that cytokine storm, which is similar to the one caused by antiviral proinflammatory response, occurs in anti-MDA5 antibody-related ILD based on multi-OMICS analysis of circulating monocytes from three patients with anti-MDA5 antibody-associated ILD (39). They also mentioned COVID-19 in their discussion as an example of virus-related ILDs that resembles anti-MDA5 antibody-positive ILD. The apparent difference between their data and ours is that they detected serum IFN- β , not IFN- α , in the serum of patients with anti-MDA5 antibody-associated ILD. In fact, IFN- β was not detectable in our samples (data not shown), which may be due to the different detection method. As COVID-19 is also a disease in which treatment-refractory pneumonitis is problematic, it is likely that IP-10 is detectable in the lungs of both COVID-19 patients and RP-ILD patients with anti-MDA5 antibodies. Given the risks associated with bronchoscopic examination, however, it may not be easy to confirm this hypothesis. Serum ferritin is a known prognostic marker of RP-ILD in DM. Gono *et al.* reported that a serum ferritin level over 1500 ng/mL was closely associated with poor prognosis (20). The same group also reported that persistently high levels of serum IL-18 are a useful predictor of poor prognosis in anti-MDA5 antibody-positive DM RP-ILD (40). Notably, adult-onset Still's disease (AOSD) is another condition in which both IL-18 and ferritin are significantly upregulated in the serum (41). We previously reported that IL-18, in cooperation with IL-6, induced ferritin production in human monocytes *in vitro* (42), even though AOSD is not recognised as a type I interferonopathy. This suggests that both the IL-18/ferritin and type I IFN/IP-10 axes may co-regulate anti-MDA5 antibody-positive DM determining both outcome and severity. Given that, it is important to determine which axis is more critical

to the pathogenesis of the disease. The fact that JAK inhibitors can be an effective therapeutic in CADM with RP-ILD (16, 17) may provide some hints as to this question, since JAK inhibitors block type I IFN signal transduction by inhibiting JAK1 and TYK2 (43) but do not interfere with IL-18 pathway. It is intriguing that JAK inhibitors are also listed in a guideline against COVID-19 (44). Of course, the relationship between the two axes is still enigmatic and further evaluation of these questions is still necessary. The limitation of our study is that this is a small, single-institutional investigation. Analysis of more patients, including treatment non-responders, might establish IP-10 as a prognostic factor for anti-MDA5 antibody-positive DM.

In conclusion, in this study, the serum analysis of DM patients with anti-MDA5 antibodies revealed that the type I IFN/IP-10 axis may play an important role in the pathogenesis of the disease. Moreover, the infiltration of monocyte/macrophage-lineage cells into the skin lesions could have been mediated by autocrine release of IP-10, leading to the exacerbation of inflammation. Thus, IP-10 may not only be a useful indicator of disease activity, but further analysis could establish it as a therapeutic target for this life-threatening disease.

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