Respiratory Medicine 141 (2018) 7–13

Contents lists available at ScienceDirect

Respiratory Medicine

journal homepage: www.elsevier.com/locate/rmed

Role of IL-15 in interstitial lung diseases in amyopathic dermatomyositis with anti-MDA-5 antibody

Toshinori Takada[a,b,*], Kazumasa Ohashi[a], Masachika Hayashi[b], Katsuaki Asakawa[b], Takuro Sakagami[b], Toshiaki Kikuchi[b], Shinji Sato[c]

[a] Uonuma Institute of Community Medicine, Niigata University Medical and Dental Hospital, Minami-uonuma, Japan
[b] Division of Respiratory Medicine, Niigata University Medical and Dental Hospital, Niigata, Japan
[c] Division of Rheumatology, Department of Internal Medicine, Tokai University School of Medicine, Isehara, Japan

ARTICLE INFO

Keywords:
IL-15
Interstitial lung disease
Dermatomyositis
Anti-MDA-5 antibody
IL-10

ABSTRACT

Background: Anti-MDA-5 antibody is closely associated with interstitial lung disease (ILD) in amyopathic dermatomyositis (ADM). Patients with ADM with anti-MDA-5 antibody sometimes develop fatal ILD in spite of intensive immunosuppressive therapy. However, an initial decrease after treatment in anti-MDA-5 antibody titers may not be predictive of subsequent better survival of the disease.

Methods: To clarify immunoregulatory features of deadly ILD in ADM with the anti-MDA-5 antibody, we retrospectively examined clinical records of consecutive patients with anti-MDA-5 antibody positive ADM-ILD with preserved serum since 2000. Serum cytokine/growth factor (GF) protein concentration was measured using a cytokine panel analysis. We compared concentrations of each cytokine/GF between survivors and non-survivors and further examined changes in cytokines/GF levels during treatment in some patients.

Results: Twenty-six patients were enrolled in the study. Nine out of 26 patients did not respond to intensive immunosuppressive therapy and died due to respiratory failure. We compared cytokine/GF concentrations and found that serum IL-15 before treatment was significantly elevated in non-survivors than in survivors ($p < 0.05$). 11 out of 17 responders and 6 of 9 dead patients had preserved serum taken more than one time. We then calculated rates of change per day (slopes) in each cytokine/GF concentration. Comparison of slopes of cytokine/GF protein over the treatment duration showed that the slopes in non-survivors were significantly increased in IL-10 and IL-15 ($p < 0.01$).

Conclusions: IL-15, as well as IL-10, may play a key role in the progression of the patients with ADM-ILD with anti-MDA-5 antibody positive.

1. Introduction

Polymyositis and dermatomyositis (PM-DM) are forms of idiopathic inflammatory myositis. DM is identified by a characteristic rash accompanying proximal skeletal muscle weakness and evidenced muscle inflammation. In addition, if a patient has the typical DM rash but no or little muscle weakness or abnormal muscle enzymes, the clinical condition is termed amyopathic DM (ADM) [1]. Patients with ADM appear to have a greater risk of developing interstitial lung disease (ILD) and tend to follow a fulminant disease with rapid progression of radiographic changes and respiratory failure similar to acute interstitial pneumonia [2].

An autoantibody which was associated with ADM was identified [3]. Since the antibody recognizes an antigen of an RNA helicase encoded by melanoma differentiation-associated gene 5 (MDA-5), it is now referred to as anti-MDA-5 antibody [4]. The antibody may be associated with a more aggressive form of ILD with a worse prognosis [5]. Findings of a recent meta-analysis suggested that anti-MDA-5 antibody could be used as a biomarker in the clinical diagnosis of ADM and that the presence of the antibody was also associated with a poor prognosis of the patients with DM [6]. Moreover, the antibody appeared to be a noninvasive biomarker in the diagnosis of rapidly progressive ILD in DM patients [7]. Ferritin, IL-18 concentration, alveolar-arterial oxygen difference, and right middle lobe ground glass opacity score were identified as poor prognostic factors as well as the anti-MDA-5 antibody for the patients with anti-MDA-5 antibody-positive DM-ILD [8,9]. The role of anti-MDA-5 antibody in the pathogenesis of ADM-ILD is still unknown. We previously showed that a chemokine CX3CL1 might be involved in the development of anti-MDA-5 antibody positive ADM-ILD using a multiplex immunoassay system [10]. However, a recent report

* Corresponding author. Uonuma Institute of Community Medicine, Niigata University Medical and Dental Hospital, 4132 Urasa, Minami-uonuma, Niigata 949-7302, Japan.
E-mail address: ttakada@med.niigata-u.ac.jp (T. Takada).

https://doi.org/10.1016/j.rmed.2018.06.012
Received 27 March 2018; Received in revised form 6 June 2018; Accepted 13 June 2018
Available online 15 June 2018
0954-6111/

Abbreviations list

ADM	amyopathic DM
AE	adverse event
CMV	cytomegalovirus
CyA	cyclosporine
GF	growth factor
ILD	interstitial lung disease
IVCY	intravenous cyclophosphamide
MDA-5	melanoma differentiation-associated gene 5
MMF	mycophenolate mofetil
mPSL	methyl PSL
PM-DM	Polymyositis and dermatomyositis
PSL	prednisolone

indicated that the anti-MDA-5 antibody titer declined similarly between the survivors and the non-survivors from disease onset to 2 months afterward [11]. The results suggest that an initial decrease in anti-MDA-5 antibody titers after treatment may not be indicative of subsequent better survival and that progression of ADM-ILD might be determined by other factors irrelevant to treatment including glucocorticoid and immunosuppressive agents.

Combination immunosuppressive therapy consisting of systemic glucocorticoids, calcineurin inhibitors, and intravenous cyclophosphamide (IVCY) is often selected to prevent patients with ADM with anti-MDA-5 antibody from developing fatal ILD, but sometimes in vain [10]. Thus, in order to establish effective treatment, we focused our study on understanding the immunoregulatory changes which may affect the progression of ILD in the disease. We examined differences in serum cytokine/growth factor (GF) concentrations before and during treatment between survivors and non-survivors using a multiplex immunoassay system.

2. Materials and methods

We retrospectively collected clinical records and preserved serum available before and during treatment of consecutive patients with ADM-ILD treated in the Niigata University Medical and Dental Hospital and the Uonuma Kikan Hospital from 2000 to May 2017. ADM was diagnosed with findings as follows: characteristic rashes of DM including Gottron's papules and the heliotrope eruption confirmed by dermatologists without considering onset of skin disease, within mildly elevated creatinine kinase levels (less than 700 IU/L), and normal or only mildly reduced muscle strength consistent with age, sex, and severity of systemic illness. ILD was diagnosed by clinical findings as follows: fine crackles, exertional dyspnea, nonproductive cough, and reticular shadow on chest radiographs and ground-glass opacity on chest high-resolution computed tomography. Patients who were found to be with anti-MDA-5 antibody negative were excluded from this study. This study was conducted in accordance with the amended Declaration of Helsinki. The Committee of Ethics, Niigata University, approved the protocol and written informed consent was obtained from all living patients.

2.1. Anti-MDA-5 antibody titer measurement

An ELISA for anti-MDA-5 using recombinant MDA-5 established previously was used to measure the titer of anti-MDA-5 antibody in preserved serum available before and during treatment of the patients with ADM-ILD [4]. Briefly, 96-well polyvinyl plates (Sumilon multiwell plates, H type; Sumitomo Bakelite, Tokyo, Japan) were coated with purified recombinant MDA-5 protein dissolved in phosphate-buffered saline (PBS, 0.5 μg/mL) at 4 °C for 12 h, followed by incubation with patients' sera diluted 1:250. All samples were examined in duplicate, and the antibody units were calculated from optical density at 450 nm by reference to a standard curve constructed using serial concentrations of a serum sample containing a high titer of the anti-MDA-5 antibody. A cutoff level of anti-MDA-5 antibody was set at 15 Unit.

2.2. Cytokine/growth factor protein level measurement

A panel of cytokines/growth factors was measured using the Milliplex Map Human Cytokine/Chemokine Kit (Merck Millipore, Darmstadt, Germany) in accordance with the instructions of the manufacturer at GeneticLab Co., Ltd. Sapporo, Japan (http://www.genelab.com) according to procedures previously described [10]. All samples were diluted by the addition of an equal amount of saline, and 15 μL of the diluted samples were used for this assay. It is a multiplexed, particle-based, flow-cytometric assay named Luminex systems (Luminex Corporation, Austin, TX, USA) which utilizes anti-cytokine monoclonal antibodies linked to microspheres incorporating distinct proportions of two fluorescent dyes. The system was customized to detect and quantify following 38 cytokines/growth factors: epidermal growth factor (EGF), fibroblast growth factors-2 (FGF-2), eotaxin, transforming growth factor-α (TGF-α), granulocyte-colony stimulating factor (G-CSF), FMS-like tyrosine kinase-3 (Flt-3) ligand, granulocyte macrophage-CSF (GM-CSF), chemokine (C-X3-C motif) ligand 1 (CX3CL1), interferon-α2 (IFN-α2), IFN-γ, growth related oncogene (GRO), monocyte chemotactic protein-1 (MCP-1), MCP-3, macrophage-derived chemokine (MDC), sCD40L, interleukin-1α (IL-1α), IL-1β, IL-1ra, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12 (p40), IL-12 (p70), IL-13, IL-15, IL-17A, IFN-γ inducible protein 10 (IP-10), macrophage inflammatory protein-1α (MIP-1α), MIP-1β, tumor necrosis factor-α (TNF-α), TNF-β, and vascular endothelial growth factor (VEGF).

2.3. Statistical analysis

Comparisons of the categorical data were made with chi-square or Fisher's exact test. Nonparametric numeric data were compared by the Mann-Whitney *U* test. The rate of change (slope) in each cytokine/growth factor concentration measured in pg/mL per day over the course of treatment was compared between the survivor and the non-survivor groups as well as anti-MDA-5 antibody titers. *P*-values less than 0.05 were considered statistically significant.

3. Results

Twenty-seven patients were diagnosed as ADM-ILD with anti-MDA-5 antibody positive. 10 out of 26 patients did not respond to intensive immunosuppressive therapy and died due to respiratory failure but one case. One non-survivor was excluded from the study because she died of thrombotic thrombocytopenic purpura with multiple organ failures but without respiratory insufficiency [12]. Finally, 26 patients were enrolled into the study. 12 out of 26 patients were included in the previous study [10]. One survivor was published as a case report [13].

In the patient background, survivors were younger, with lower levels of serum ferritin, and with higher P/F ratio before treatment (Table 1). Anti-MDA-5 antibody titers and serum Krebs von den Lungen 6 levels were not different between the groups. All of the patients were empirically treated with immunosuppressive agents: cyclosporine (CyA) with a trough levels usually at 100–150 ng/mL, tacrolimus with trough at 5–10 ng/mL, IVCY (500 mg/body), and mycophenolate mofetil (MMF) 1500 mg daily as well as glucocorticoid. Disease duration from the initial administration of corticosteroids to the discharge from