Review

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Utility of dermatomyositis-specific autoantibodies for diagnosis and clinical subsetting

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Autoantibodies directed against nuclear or cellular elements are present in patients with dermatomyositis (DM). With a few exceptions, most of these autoantibodies are found exclusively in patients with this condition. Antibodies against aminoacyl tRNA synthetases, signal recognition particle or Mi-2 are well-established polymyositis- and/or DM-specific autoantibodies. Recently, additional autoantibodies specific for DM have been reported by several investigators. These novel DM-specific autoantibodies have proven useful for diagnosis, treatment selection, prognosis and classification of DM patients into distinct subsets. This article reviews the clinical characteristics and immunological findings in patients with these DM-specific autoantibodies as well as their utility in the clinical setting and for attempts to classify DM patients into distinct clinical phenotypes.

Keywords: autoantibodies • clinically amyopathic dermatomyositis • dermatomyositis • dermatomyositis-specific antibodies • interstitial lung disease • juvenile dermatomyositis • malignancy • rapidly progressive interstitial lung disease

Dermatomyositis (DM) is an idiopathic inflammatory myopathy (IIM) that presents with chronic inflammation of the muscle accompanied by typical skin manifestations such as heliotrope rash or Gottron's papule. Interstitial lung disease (ILD) and malignancy are well known as the main complications of this condition. Similar to other connective tissue diseases (CTD), a distinct immunological characteristic of DM is the presence of autoantibodies that target different nuclear or cellular components [1]. Most of these targeted nucleic acids and/or proteins have important functions for maintaining biological activities such as gene transcription, protein synthesis and translocation, and for responses to infections. These autoantibodies found in patients with DM have proven clinically useful for diagnosis and disease subtype classification because patients with the same antibodies exhibit relatively homogeneous clinical features [2]. However, despite this clinical utility, until recently, autoantibodies found in DM have not attracted a great of attention because of their low frequencies.

In the last decade, however, the discovery of a succession of new autoantibodies with distinct clinical and immunological characteristics in DM patients has rekindled interest in the field. In this article, we summarize recent advances regarding autoantibodies found in DM patients in relation to their specificity and clinical phenotypes and consider how understanding of these autoantibodies could be applied in daily clinical practice. Development of commercially available kits for measuring a full panel of such autoantibodies is under way. We also consider the immunological significance of these autoantibodies and the autoantigens they target in the context of their possible involvement in disease pathogenesis.

Dermatomyositis-specific autoantibodies

A spectrum of serum autoantibodies is detected in patients with IIM. These are

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divided into two main classes. One is specific to IIM patients but the other is also found in other DM and CTD as well as IIM. Targoff *et al.* proposed to designate these two types of autoantibodies 'myositis-specific antibodies: MSAs' and 'myositis-associated antibodies: MAAs,' respectively. Classically observed autoantibodies such as those against aminoacyl transfer RNA synthetases (ARS), anti-signal recognition particle (SRP) and Mi-2 antibodies are classified as MSAs, especially anti-Mi-2 antibodies are thought to as DM-specific autoantibodies. Recently, some novel autoantibodies specific for DM were described. Interestingly, MSAs (including DM-specific autoantibodies) are distinct and usually mutually exclusively present. Autoantibodies detected in IIM (both MSAs and MAAs) are listed and summarized in Table 1.

Anti-aminoacyl transfer RNA synthetase antibodies

ARSs are the enzymes that catalyze the binding of amino acids to their cognate tRNAs. Anti-aminoacyl-tRNA synthetase (anti-ARS) autoantibodies react with these cytoplasmic ARS enzymes and have been detected in patients with polymyositis (PM)/DM [2]. Six anti-ARS autoantibodies have been described traditionally: anti-histidyl (anti-Jo-1), anti-threonyl (anti-PL-7), anti-alanyl (anti-PL-12), anti-glycyl (anti-EJ), anti-isoleucyl (anti-OJ) and anti-asparaginyl (anti-KS) tRNA synthetase antibodies [3]. Precisely anti-ARS antibodies are not DM-specific but PM/DM-specific. However, since previous studies discuss clinical or immunological features of anti-ARS autoantibodies without any distinction between PM and DM. Therefore, in this section, we reviewed mainly the association between clinical or immunological features of PM/DM (not DM specific) and anti-ARS antibodies.

The first anti-ARS antibody, anti-Jo-1, was reported by Nishikai *et al.* in 1980 [4]. As the first described anti-ARS and because of its relatively high frequency in PM/DM patients, anti–Jo-1 autoantibodies are the best understood. In earlier studies, anti-Jo-1 was found in approximately 20–30% of IIM patients. Other anti-ARS autoantibodies are usually much less common with frequencies estimated at between 1 and 5%. Recently, two novel anti-ARS antibodies have been described. Hashish *et al.* reported an autoantibody against tyrosyl-tRNA synthetase (anti-Ha) in 2005 [5] and an autoantibody recognizing phenylalanyl-tRNA synthetase (anti-Zo) was found by Betteridge *et al.* in 2007 [6]. Thus, to date a total of eight different anti-ARS autoantibodies have been detected in PM/DM patients. Interestingly, with few exceptions, only a single type of anti-ARS antibody is generally present in each patient, and finding more than one such antibody in the same patient's serum is rare [7].

Related clinical features

Different anti-ARS antibodies have been reported to be associated with similar clinical characteristics. This is referred to as the 'anti-synthetase syndrome,' characterized by myositis with a higher proportion of ILD, shrinking lung, polyarthritis, fever, Raynaud's phenomenon and mechanic's hands (a hyperkeratosis along the sides of the fingers, mainly the radial sides) compared with the overall myositis population [8]. Although two classes of newly identified anti-ARS autoantibodies were only found in one case each, the patient with anti-Ha had muscle weakness, skin manifestations, arthritis and ILD, whereas the patient with anti-Zo had muscle weakness, fever, Raynaud's phenomenon, polyarthritis and mechanic's hands. These are indications that these two patients had typical clinical features of antisynthetase syndrome. Joint involvement (anti-ARS arthropathy) is one of characteristic clinical features associated with the presence of anti-ARS antibodies. Recently, Kaneko and colleagues reported that anti-ARS arthropathies could be categorized into three groups (nonerosive arthritis, erosive arthritis with anti-citrullinated peptide [CCP] or rheumatoid factor, subluxation of the thumbs and periarticular calcification without anti-CCP or rheumatoid factor exclusively found in anti-Jo-1-positive patients). They concluded that antibody profiles were useful for classification of arthropathy [9].

The presence of any of the anti-ARS antibodies in PM/DM patients with ILD complications usually indicates a chronic-type of the latter (slowly progressive over long periods or hardly progressive, or even asymptomatic), with a few exceptions [10]. Histopathologically, nonspecific interstitial pneumonia is the most characteristic pattern. Less frequently, usual interstitial pneumonia and organizing pneumonia are found and diffuse alveolar damage rarely detected [11].

As alluded to above, although anti-ARS syndromes have common clinical symptoms, further examination reveals distinct differences in the clinical characteristics depending on which anti-ARS antibodies are present. It has been reported that anti-Jo-1 antibodies are closely associated with myositis [2] and the same seems to be true of anti-PL-7 autoantibodies. However, Sato *et al.* reported the close association between anti-PL-7 antibodies and PM-systemic sclerosis (SSc) overlap, although numbers of patients were limited in that study [12]. Yamasaki *et al.* reported that PM/DM patients with anti-PL-7 autoantibodies had milder myositis with lower creatine kinase levels than those with anti-Jo-1 antibodies [13]. On the other hand,

Table 1. Myositis-specific autoantibodies and associated autoantibodies.

Autoantibody	Targeted antigen	Function of antigen	Clinical characteristics	Estimated frequency (%)	
Myositis-specific antibodies				**ADM**	**JDM**
Anti-ARS	Aminoacyl-tRNA synthetases				
Anti-Jo-1	Histidyl tRNA synthetase	Translation and synthesis of protein	Antisynthetase syndrome (myositis, chronic interstitial pneumonia, polyarthritis, mechanic's hand, Raynaud's phenomenon, fever, shrinking lung)	~25–40 (including PM)	0–3 (including PM)
Anti-PL-7	Threonyl tRNA synthetase				
Anti-PL-12	Alanyl tRNA synthetase				
Anti-EJ	Glycyl tRNA synthetase				
Anti-OJ	Isoleucyl tRNA synthetase				
Anti-KS	Asparaginyl tRNA synthetase				
Anti-Ha	Tyrosyl tRNA synthetase				
Anti-Zo	Phenylalanyl tRNA synthetase				
Anti-Mi-2	NuRD helicases	Transcriptional factor	Typical DM	~5–20	3–7
Anti-SRP	Signal recognition particle	Protein translocation in ER	Necrotizing myopathy		
Anti-CADM-140/ MDA5	MDA5	Defense for virus infection	DM/CADM, RP-ILD	~7–60	~7–40
Anti-p155/ TIF-1γ	TIF1-γ	Transcriptional factor	DM/malignancy, JDM	~10–40	~20–40
Anti-MJ/NXP-2	NXP-2	Transcriptional factor	DM/malignancy, JDM/calcinosis	1-5	~10–30
Anti-SAE	SAE	After translational modification	DM/ present with CADM first	1–9	No data
Anti-200/100	HMGCR	Cholesterol synthesis	HMGCR inhibitor-associated myositis		
Anti-cN1A	cN1A	Hydrolysis of nucleoside	Inclusion body myositis		
Myositis-associated antibodies					
Anti-Ku	DNA-PK regulatory subunit	DNA repair	PM/SSc, SLE, DM		
Anti-PM-Scl	Nucleolar protein complex	RNA processing	PM/SSc		
Anti-U1RNP	U1 small nuclear RNP	Splicing of pre-mRNA	OL, MCTD		
Anti-SSA/Ro	RNA hY(hY1, hY3, hY5)	Not well known	SjS, SLE, PM/DM, SSc		

ADM: Adult dermatomyositis; ARS: Aminoacyl tRNA synthetase; CADM: Clinically amyopathic dermatomyositis; cN1A: Cytosolic 5-nucleotidase 1A; DM: Dermatomyositis; ER: Endoplasmic reticulum; HMGCR: 3-hydroxy-3-methylglutaryl-coenzyme A reductase; JDM: Juvenile dermatomyositis; MCTD: Mixed connective tissue disease; MDA5: Melanoma differentiation-associated gene 5; NPX-2: Nuclear matrix protein-2; NuRD: Nucleosome remodeling and deacetylase; OL: Overlap syndrome; PM: Polymyositis; RNP: Ribonucleoprotein; RP-ILD: Rapidly progressive interstitial lung disease; SAE: Small ubiquitin-like modifier activating enzyme; SLE: Systemic lupus erythematosus; SRP: Signal recognition particle, SSc: Systemic sclerosis; TIF-1γ: Transcriptional intermediary factor-1γ.

patients with anti-OJ, anti-PL-12 or anti-KS autoantibodies are more likely to have associated ILD without clinical evidence of myositis, often seen in patients with ILD alone or clinically amyopathic dermatomyositis (CADM) [7]. Anti-EJ autoantibodies are more closely associated with DM than PM [7]. Differences in degree of association with clinical entities in each anti-ARS antibody are summarized in Table 2.

Anti-ARS autoantibodies can be detected in patients with juvenile PM/DM although at relatively low frequency. Previous reports showed approximately 0 to 3% of juvenile PM/DM patients had anti-ARS autoantibodies [14,15]. Clinical features of juvenile DM (JDM) with anti-ARS are similar to adult PM/DM patients with anti-ARS syndrome [14,15].

Immunological aspects

Many studies have implicated the possible involvement of anti-ARS autoantibodies and their target antigens in the pathogenesis of PM/DM. Traditionally, studies focused on the association between ARS antigen and different environmental factors such as viruses as triggers of autoimmune responses. Molecular mimicry of ARS antigens and viruses, or the production of ARS antigen and virus complexes as cryptic epitopes, were proposed as potentially breaking tolerance to self antigens [16]. Casciola-Rosen *et al.* previously showed that histidyl tRNA (Jo-1 antigen) could be cleaved by granzyme B and that these Jo-1 antigen fragments then induced autoimmune responses [17]. Several studies revealed that specific ARS fragments cleaved by proteases have cytokine-like or chemokine-like properties for inflammatory cells and this might induce further development of an autoimmune response [18]. In addition, results showing that Jo-1 cleavage products are abundant in alveolar epithelial cells and that Jo-1 antigen was highly expressed in regenerating muscle cells might explain why muscle and lung are preferential target organs in PM/DM [19]. In animal models, Katsumata *et al.* reported that mice immunized with murine Jo-1 antigen and adjuvant developed muscle and lung inflammation [20]. Soejima *et al.* showed that immunization only with murine Jo-1 induced autoimmune responses in mice [21]. These results suggested that autoimmune responses to the Jo-1 antigen might induce muscle and lung injury in PM/DM patients in a similar fashion. Together, these findings strongly support the notion that exposure to ARS autoantigens or their fragments plays an important role in the pathogenesis of PM/DM.

As with anti-ARS autoantibodies, Eloranta *et al.* reported that anti-Jo-1 autoantibody immune complexes acted as endogenous IFN-α inducers, and suggested that IFN-α production in plasmacytoid dendritic cells might also be important for the pathogenesis of PM/DM [22]. Stone *et al.* suggested that anti-Jo-1 antibody levels correlated with muscle and joint symptoms in patients with IIM [23]. However, whether the production of anti-ARS autoantibodies is directly involved in DM pathogenic mechanisms remains an open question.

Anti-Mi-2 antibody

Anti-Mi-2 autoantibodies were first reported by Targoff and Reichlin in patients with myositis, mainly detected in sera from adult DM patients (21% thereof) [24]. The target autoantigen consists of a complex of at least eight proteins (240, 218, 150, 75, 65, 63, 50 and 34 kDa) of which the main target is thought to be the Mi-2 protein (Mi-2α and Mi-2β, 240kDa and 218 kDa, respectively). This nuclear helicase protein Mi-2β autoantigen is a component of a protein complex with histone deacetylases (nucleosome remodeling deacetylase: NuRD) that is involved in gene transcription by histone acetylation and nucleosome remodeling activities [25]. Anti-Mi-2 antibodies were also found in patients with JDM although their frequency was lower (3 to 7%) than in adult DM (5 to 20%) [14,26].

Table 2. Degree of association with polymyositis/dermatomyositis or interstitial lung disease in each anti-aminoacyl-tRNA synthetase antibody.

Autoantibody	Degree of association with each clinical entity
Anti-Jo-1 (anti-histidyl tRNA synthetase)	PM/DM > ILD
Anti-PL-7 (anti-threonyl tRNA synthetase)	PM/DM > ILD
Anti-PL-12 (anti-alanyl tRNA synthetase)	PM/DM < ILD, found in ILD alone
Anti-EJ (anti-glycyl tRNA synthetase)	PM/DM > ILD, DM > PM
Anti-OJ (anti-isoleucyl tRNA synthetase)	PM/DM < or = ILD, found in ILD alone
Anti-KS (anti-asparaginyl tRNA synthetase)	PM/DM < ILD, found in ILD alone
Anti-Ha (anti-tyrosyl tRNA synthetase)	DM and ILD (only one case report)
Anti-Zo (anti-phenylalanyl tRNA synthetase)	PM and ILD (only one case report)

DM: Dermatomyositis; ILD: Interstitial lung disease; PM: Polymyositis.

Related clinical features

In general, anti-Mi-2 autoantibodies are disease specific in both adult and JDM. These antibodies are associated with typical DM with cutaneous manifestations (i.e., Gottron's signs or papules, heliotrope rash, cuticular overgrowth and V-neck sign and Shawl sign rashes). Clinically, patients with anti-Mi-2 autoantibodies had milder muscle inflammation with good response to treatment with corticosteroids for myositis, with the exception of exacerbation during prednisolone dose tapering [24,27]. Moreover, these patients had a lower risk of complications like ILD or malignancy. Thus, DM with anti-Mi-2 autoantibodies had a relative good prognosis. Most studies using immunodiffusion or immunoprecipitation methods for detection indicated that the presence of anti-Mi-2 autoantibodies is closely associated diagnostically with both adult and JDM. However, antibody testing by ELISA revealed that their disease distribution was slightly different; thus, when Hengstman *et al.* identified anti-Mi-2 autoantibodies using ELISA, it was found that 50% of anti-Mi-2-positive patients had DM but the remaining 40 and 8% had PM and inclusion body myositis, respectively. Therefore, typical DM symptoms such as Gottron's sign or heliotrope rash were less frequent in this report [28]. However, analyses using the gold standard immunoprecipitation assay revealed anti-Mi-2 antibodies were found exclusively in patients with DM. Therefore, careful attention is required in the interpretation of results obtained by the different methods used for autoantibody detection.

Immunological aspects

Environmental factors are believed to be important in relation to the pathogenesis of anti-Mi-2-positive DM patients. A significant correlation between ultraviolet radiation exposure and the onset of DM and positivity for anti-Mi-2 autoantibodies has been reported [29]. Burd *et al.* noted that ultraviolet radiation stimulated greater upregulation of the Mi-2 protein relative to other molecules of the NuRD complex in human keratinocytes, further highlighting potential disease mechanisms [30]. Kashiwagi *et al.* demonstrated that the Mi-2b protein was necessary for the development and normal differentiation of basal epidermis in mice [31]. Similar to anti-ARS antigen, expression of the Mi-2 protein was markedly upregulated during muscle regeneration in both a mouse model and in human DM myofibers. High expression of Mi-2 correlated with proliferation of myoblasts, suggesting that it might participate in the regulation or modulation of myoblast differentiation [32].

Anti-transcription intermediary factor-1γ antibody

Targoff *et al.* [33] and Kaji *et al.* [34] reported autoantibodies against 155 kDa protein with a weaker 140 kDa band. Later, the 155 kDa autoantigen was identified as transcription intermediary factor-1γ (TIF-1γ) [35]. The TIF1 family is composed of tripartite motif-containing (TRIM) proteins, known to have at least three isoforms, TIF-1α (TRIM24), TIF-1β (KAP1, TRIM28) and TIF-1γ (TRIM33). Fujimoto *et al.* identified the 140 kDa target autoantigen as TIF-1α and also found that the 110 kDa TIF-1β protein was a target autoantigen in DM patients [36]. Therefore, autoantibodies to all three isoforms of the TIF1 family proteins are detected in DM patients.

Related clinical features

In previous reports, anti-TIF-1γ antibodies were shown to be specific for both adult DM and JDM, found in 10 to 40% [33,34,36–44] and 20 to 40% of patients [33,38,45], respectively. Anti-TIF-1γ autoantibodies are often found together with anti-TIF-1α autoantibodies. It is well known that DM patients often suffer from malignancy. The most distinct clinical characteristic of anti-TIF-1γ autoantibodies is indeed a significant association with malignancy [33,34,36–39]. According to a systematic review and meta-analysis by Trallero-Araguas and colleagues, the sensitivity of anti-TIF-1γ autoantibodies for diagnosing cancer-associated DM was 78% and specificity was 89% [39]. Almost 80% of patients with cancer-associated myositis tested positive for anti-TIF-1γ, indicating its strong association with cancer [39]. Aging is a major risk factor for malignancy, and despite the presence of anti-TIF-1γ autoantibodies, no JDM or young adult DM patients presented with cancer [36–38]. However, patients with anti-TIF-1γ autoantibodies had more severe extensive skin manifestations with a higher frequency of typical DM skin lesions such as Gottron's papules, heliotrope rash and V-neck sign, in both adult DM and JDM [36–38]. Nonetheless, the degree of calcinosis seemed to be lower than in anti-nuclear matrix protein-2 (NXP-2)-positive DM patients. Because the severity of muscle symptoms is variable, these antibodies are detected in both classic DM and CADM. In contrast to anti-ARS or anti-melanoma differentiation-associated gene 5 (MDA5)-positive DM patients, the frequency of ILD is low, especially rapidly progressive ILD (RP-ILD) [33,34,36–45]. Recently, Fiorentino *et al.* reported that rheumatic symptoms such as Raynaud phenomena or arthritis are also present at low frequencies in this group [44]. Unlike patients with anti-TIF-1γ autoantibodies, those with anti-TIF-1β autoantibodies seem to have no strong preponderance of malignancy. A clinical feature of patients with anti-TIF-1β autoantibodies reported so far is relatively mild myopathy [36,46].

From genetic analyses, Targoff *et al.* reported that Caucasian patients who had HLA-DQA1*0301 were at higher risk of having anti-TIF-1γ autoantibodies [33].

Immunological aspects

TIF-1γ, a member of the tripartite motif family, is a nuclear protein involved in DNA transcription. TIF-1γ is thought to be involved in regulation of TGF-β signaling via the Smad-4 pathway. Therefore, the TIF-1 protein could play pivotal role in promoting or suppressing malignant cell growth and differentiation.

It can be proposed that changes in conformation or expression of TIF-1 protein lead to the formation of malignant cells as well as the induction of autoimmune response that trigger the pathogenesis of cancer-associated DM. Casciola-Rosen *et al.* showed that expression of myositis-specific autoantigens is increased in cancer tissues similar to regenerating muscle fibers of myositis, and that autoimmune responses to cancer tissues cross-reacted with muscle fibers causing persisting muscle tissue injury [19]. Interestingly, there was a report that TRIM proteins and IFN, thought to be closely associated with DM pathogenesis, mutually regulate each other [47]. Based on these observations, it is proposed that TIF-1 proteins and/or autoantibodies against these are implicated in the pathogenesis of cancer-associated DM. However, this hypothesis remains to be confirmed.

Anti-melanoma differentiation-associated gene 5 antibody

Anti-MDA5 autoantibodies were first identified in sera from CADM patients by Sato *et al.* in 2005 [48]. CADM is a distinct subset within the PM/DM spectrum characterized by typical DM rashes but clinically little or no evidence of myositis. At first, these autoantibodies were designated anti-CADM-140 because they recognized a protein of approximately 140 kDa in CADM patient sera by immunoprecipitation assays [48]. Later, the autoantigen recognized by anti-CADM-140 autoantibodies was identified as MDA5, alternatively termed 'IFN-induced with helicase C domain protein 1: (IFIH1)' [49,50]. Although anti-MDA5 autoantibodies were first detected in Japanese patients with CADM, it became clinically evident that the antibody was also found in patients with classic DM especially in different ethnic groups [27,49–60]. Therefore, it is confirmed that anti-MDA5 autoantibodies are DM-specific antibodies found in both classic DM and CADM worldwide. Positivity for anti-MDA5 autoantibodies ranges from approximately 7 up to as much as 60% of patients [49–59].

Related clinical features

The most striking clinical characteristic of patients with anti-MDA5 autoantibodies is RP-ILD (Figure 1A) [48–54,57,60]. In patients with DM, especially those with CADM, it is known that RP-ILD sometimes develops during the clinical course, most commonly in eastern Asia. RP-ILD is a critical condition resistant to intensive immunosuppressive therapy, and is life threatening with a poor prognosis. Previous studies have documented that 14–58% of DM and RP-ILD patients with anti-MDA5 autoantibodies died of acute respiratory failure [49–60]. However, antinuclear antibodies or MSAs were rarely detected and no biomarkers have been identified for this condition so far. Because it is considered that early diagnosis and initiation of intensive treatment would be very important to rescue DM patients from this condition, new biomarkers for DM and RP-ILD are eagerly anticipated. In this context, anti-MDA5 autoantibodies could be a useful marker for early diagnosis of DM and RP-ILD. Moreover, recent studies indicated that the mean titer of anti-MDA5 antibody measured by ELISA before treatment could be useful for predicting the outcome in patients with DM and RP-ILD, with a possible close association between anti-MDA5 autoantibody titers and RP-ILD disease activity [51,54,61]. Histopathological findings of RP-ILD in patients with anti-MDA5 autoantibodies mainly show a diffuse alveolar damage pattern, although it is impossible to perform lung biopsy in most cases. Tanizawa *et al.* reported characteristic findings of high-resolution computed tomography images in patients with anti-MDA5 autoantibodies, in other words, a lower consolidation/ground-glass attenuation pattern at diagnosis and diffuse ground-glass attenuation and consolidation with air bronchograms present all over the lungs at severe respiratory failure [52]. Another distinct clinical feature of these patients is their skin manifestations. Fiorentino *et al.* first described skin ulceration (Figure 1B) and tender erythematous palmar papules as characteristic cutaneous features of patients with these antibodies [55]. Skin ulceration was typically seen in the lateral nail folds, over the extensor surfaces of joints (overlying the finger knuckles: Gottron papules, elbows, etc.). Skin biopsy showed vascular fibrin deposition with variable perivascular inflammation, suggesting vasculopathy. A recent study revealed an association between cutaneous ulcers and anti-MDA5 autoantibodies; patients with both cutaneous ulcers and these antibodies had an increased risk of ILD [62]. As other clinical features of patients with these antibodies, high frequencies of fever and/or polyarthritis were reported.

Anti-MDA5 autoantibody-positive DM patients with RP-ILD had high serum ferritin and IL-18 concentrations; these blood biomarkers were suggested to be useful for prognosis and evaluation of disease activity [51]. Recently, a concentration of serum IL-8

high enough to activate neutrophils was also reported in anti-MDA5-positive ILD patients [63]. Regarding immunogenetics, Gono *et al.* reported a correlation of the presence of anti-MDA5 autoantibodies with HLA-DRB1*0101/*0405 [64].

It is supposed that there is a certain racial or ethnic difference in the clinical characteristics of anti-MDA5-positive DM. The proportion of CADM patients is high in Japanese (most reports indicated >50%) [27,49–52] but lower in other eastern Asia and Euro-American populations (<50%) [54,56]. Regarding the coexistence of RP-ILD, east Asian cohorts have high frequencies [27,49–51,53] while the range is very wide in Euro-American cohorts [55–60].

Similar to adult DM patients, anti-MDA5 autoantibodies were detected in JDM. Frequencies of anti-MDA5 autoantibodies varied from 7 to 40% based on previous reports [65,66]. Clinical characteristics of JDM with anti-MDA5 are cutaneous ulcers and ILD [60], especially RP-ILD, in Japanese patients [66]. This is similar to adult DM patients with anti-MDA5 [65,66].

Immunological aspects

MDA5 (IFIH1) is the autoantigen recognized by anti-MDA5 antibody. This protein is the retinoic acid-inducible gene-I-like receptor involved in innate immune responses. MDA5 senses intracellular viral RNAs and plays an important role in antiviral responses through the induction of type I IFNs and inflammatory cytokines. This is very interesting in the light of the proposal that viral infection is an etiological agent in patients with IIM, and deserves more attention as a perspective on the pathogenesis of DM and RP-ILD. The recognition of viral components by MDA5 as an innate immune response possibly induces the production of anti-MDA5 autoantibodies and this would lead to the pathogenesis of DM and RP-ILD. Indeed, many previous studies reported possible associations between myositis and infection with coxsackievirus B, one of the picornavirus species recognized by MDA5. One hypothesis focuses on the overexpression of MDA5 in target tissues during viral infection [49]. A subsequent immune response triggers the production of IFN-α that would lead to apoptosis of cells infected by the virus. This condition would induce continuous release of MDA5 fragments cleaved by proteases and a complex of virus and MDA5 fragments. The autoimmune response to MDA5 would then be initiated and would damage target organ tissues. The elucidation of pathogenic roles of anti-MDA5 and/or its target antigen (MDA5) still remains an important challenge for the future in order to understand this condition and improve treatment.

Antinuclear matrix protein 2 antibody

Anti-NXP-2 autoantibodies were first described as anti-MJ antibodies by Oddis and colleagues in 1997. At that time, anti-MJ antibodies represented a new antibody specificity targeting a protein of approximately 140 kDa in patients with JDM. The frequency of anti-MJ in the JDM population was 18% [67]. In 2007, Targoff and colleagues identified the target MJ antigen as NXP2 [68]. NXP-2 (also known as MORC3) is a protein localized to the nuclear matrix and thought to be involved in transcriptional regulation. The frequency of this antibody in JDM is estimated as around 20% [67,69–72]. In 2009, Gunawardena *et al.* demonstrated that anti-NXP2 antibodies were the most common (23% of JDM) in a UK JDM cohort [69]. Espada *et al.* reported that anti-NXP-2 antibodies were found in 33% of an Argentinean JDM cohort [70]. Later, anti-NXP-2 antibodies were also identified in patients with adult DM and PM, although the frequency was low [72–74]. According to Gunawardena *et al.*, anti-NXP-2 antibodies were present in 11.6% of JDM and 0.8% of adult PM/DM (nine cases DM and one PM) [72]. Ichimura *et al.* identified anti-NXP-2 in seven in 445 (1.6%) of adult DM and one in 62 (1.6%) of adult PM [74].

Related clinical features

As mentioned above, anti-NXP-2 antibodies are found in sera from both adult DM and JDM patients. Interestingly, similar to the situation with anti-TIF-1γ antibodies, clinical features of JDM and adult DM with anti-NXP-2 antibodies differ markedly. The striking clinical feature of JDM with anti-NXP-2 antibodies is the association with calcinosis [67,69–72]. Gunawardena *et al.* reported that 54% of such JDM patients had calcinosis, significantly higher than in anti-NXP-2-negative or anti-TIF-1γ-positive patients [69]. Tansley *et al.* reported that anti-NXP-2 antibodies were associated with an increasing risk of calcinosis in parallel with disease severity, and with worse functional status. [71]. Occurrence of malignancy is rarely seen in JDM patients with these antibodies. In contrast, in adult DM patients, most previous reports revealed an association of anti-NXP-2 antibodies with malignancy. Fiorentino and colleagues reported an association between anti-NXP-2 antibodies and calcinosis cutis, malignancy and a benign course of skin disease; poor prognosis would therefore be expected [42,75]. On the other hand, coexistence of ILD has rarely been reported in patients with either JDM or adult DM with anti-NXP-2 antibodies.

Immunological aspects

It is still unknown whether NXP-2 or anti-NXP-2 antibodies are involved in the pathogenesis of the dis-

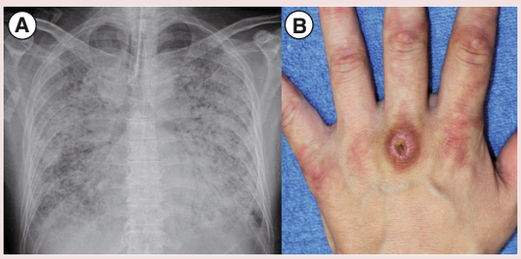

Figure 1. Distinct clinical features in dermatomyositis patient with anti-MDA5 autoantibodies. (A) Ground glass and infiltration shadow all over both lungs indicating rapidly progressive interstitial lung disease. **(B)** Skin ulceration of overlying a knuckle.
DM: Dermatomyositis.

ease. NXP-2 is assumed to regulate nuclear function in different ways (for instance, gene transcription) as well as nuclear proteins targeted by other DM-specific antibodies (anti-Mi-2 and anti-TIF-1γ antibodies). In connection with malignancy, it is interesting that a previous report noted that NXP-2 regulates the activation and subcellular localization of tumor suppressor gene p53. Finally, HLA-DRB1*08 was reported as a possible risk factor for anti-NXP-2 antibodies [69].

Anti-small ubiquitin-like modifier activating enzyme antibody

Betteridge and colleagues first identified novel autoantibodies that recognized 40 kDa and 90 kDa proteins in sera from adult DM patients. Serological examination revealed that the 40 kD target autoantigen is the small ubiquitin-like modifier-1 activating enzyme A subunit (SAE1), and that the 90 kDa protein is the B subunit (SAE2) [76]. Thus far, anti-SAE antibodies have been exclusively detected in patients with DM. In additional studies, the frequency of anti-SAE antibodies in DM patients was found to be 8.4% [77]. According to data from an Italian cohort, 7% of DM patients were positive for these antibodies [78]. Two Japanese groups reported anti-SAE-positive patients at frequencies of 1.6 and 1.8% of adult DM [79,80]. Another cohort from the Hungarian population indicated that 5.5% of adult DM patients had anti-SAE antibodies [81].

Related clinical features

The unique clinical characteristic of patients with anti-SAE antibodies is that cutaneous manifestations precede the appearance of muscle symptoms in most cases [77]. Therefore, anti-SAE-positive patients are commonly first diagnosed as having CADM and later rediagnosed with DM following the appearance of muscle weakness. Betteridge *et al.* reported that the duration between the appearance of cutaneous symptoms and myositis was approximately 3 months [77]. Another distinct feature of patients with these antibodies is a high frequency of dysphagia, although one study showed no such complication [78]. Complications like malignancy or ILD seem to be rare in patients with DM and anti-SAE antibodies, according to previous reports. One exception is the study of a Japanese cohort indicating that 71% of anti-SAE-positive DM patients had ILD [79]. Thus, the clinical features of patients with anti-SAE antibodies seem to be slightly different, possibly due to the smaller number of cases of anti-SAE-positive patients in each study. Further work is required to confirm unique clinical characteristics of patients with anti-SAE antibodies, including potential utility as a prognostic marker in CADM patients. Because screening for anti-SAE antibodies in JDM cohorts has not yet been carried out in detail, only a few cases of JDM with anti-SAE have been reported thus far [79].

Immunological aspects

Small ubiquitin-like modifier proteins are involved in post-translational modification and play an important role in supporting the function of other proteins by binding to them. SAE, the target protein of anti-SAE antibodies, is one of the enzymes that catalyze this reaction. Sumoylation is involved in different intracellular processing steps, such as nucleocytoplasmic transport, regulation of transcription, signal transduction and apoptosis. Although it has been assumed that anti-SAE or its autoantigen, SAE, would be involved in the pathogenesis of DM, the pathogenic role of SAE or anti-SAE antibodies is still unknown. A strong association of the presence of anti-SAE autoantibody with the HLA-DRB1*04 DQA1*03 DQB1*03 haplotype was reported as a genetic characteristic of patients with these antibodies [76].

Other myositis-specific or -associated autoantibodies

Autoantibodies specific for non-DM myositis

Anti-signal recognition particle antibody

Anti-SRP autoantibodies are one of the MSAs most often found in patients with PM. SRP, the target antigen, is a cytoplasmic RNA–protein complex (7SL-RNA with six proteins) that recognizes secretory or membrane proteins and is involved in the translocation of these synthesized proteins across the endoplasmic reticulum membrane. Although most patients who possess anti-SRP autoantibodies are diagnosed as having PM, previous studies also reported low frequencies of DM, SSc and anti-ARS syndrome patients with these antibodies. Anti-SRP autoantibodies are generally found in 5–10% of adult myositis patients,

whereas they are rarely found in patients with JDM, although the clinical characteristics are the same as in adult patients. Clinical characteristics of patients with anti-SRP antibodies are relatively acute onset, progressive disease course and severe muscle weakness with significantly elevated muscle enzymes. Patients are commonly resistant to treatment with corticosteroid and recurrence often occurs. ILD is less frequent in these patients and some early studies reported an association with cardiac involvement. Different from typical PM, distinctive histopathological features of patients with anti-SRP autoantibodies have been described as necrotizing myopathy with little or no inflammatory infiltrates. Thus, anti-SRP autoantibodies might be associated with a subtype of necrotizing myopathy. Suzuki *et al.* reported that patients with anti-SRP autoantibodies experienced chronic progressive weakness and atrophy of limbs and trunk muscles resembling muscular dystrophy [82].

Other than anti-SRP antibodies, anti-200/100 autoantibodies were found in patients with statin-associated autoimmune myopathy and anti-cN1A autoantibodies were detected in patients with inclusion body myositis, respectively.

Autoantibodies associated with myositis

Anti-Ku antibody

Anti-Ku autoantibodies were first described by Mimori *et al.* in patients with PM/SSc overlap syndrome [83]. The Ku autoantigen is a DNA-dependent protein kinase regulatory subunit that consists of 70/80 kDa heterodimers mainly involved in DNA repair, transcriptional regulation and replication. Previous reports indicated that anti-Ku autoantibodies are found not only in PM/SSc overlap but also in other disease subtypes such as systemic lupus erythematosus (SLE), SSc and overlap syndrome, particularly in Caucasian populations. According to a recent study of a European cohort, anti-Ku autoantibodies are frequently detected in undifferentiated connective tissue disease or SLE, as well as in SSc patients. Anti-Ku p70 antibodies were significantly associated with synovitis, erosive arthritis and articular contractures in female patients [84].

Anti-PM-Scl antibody

Anti-PM-Scl autoantibodies are found in sera from PM/SSc overlap syndrome patients. The PM-Scl autoantigen consists of a nucleolar and nucleoplasmic molecular complex containing 16 proteins of which the main antibody targets are 75 and 100 kDa proteins. Clinical features of patients with anti-PM-Scl autoantibodies are limited SSc associated with myopathy. Severe organ involvement is less frequent and a good response to corticosteroid therapy with better prognosis is then the usual course.

In recent studies, ILD in SSc patients with anti-PM-Scl autoantibodies also had a relatively mild disease

Table 3. Clinical distinct characteristics of each dermatomyositis-specific autoantibody (including polymyositis/dermatomyositis specific antibodies).

Clinical and laboratory findings	Anti-ARS	Anti-Mi-2	Anti-TIF-1γ	Anti-MDA5	Anti-NXP-2	Anti-SAE
Typical DM rash		•	•			•
V-sign or Shawl-sign		•	•			
Dysphagia						•
Fever	•			•		
Arthritis	•			•		
Calcinosis					•	
ILD						
Rapidly progressive ILD				•		
Chronic ILD	•					
Malignancy			•		•	
CADM	•		•	•		•†
Juvenile DM			•		•	
ANA positivity (%)		•	•		•	•

ANA: Anti-nuclear autoantibodies; ARS: Aminoacyl transfer RNA synthetases; CADM: Clinically amyopathic dermatomyositis; DM: Dermatomyositis; ILD: Interstitial lung disease; MDA5: melanoma differentiation-associated gene 5; NXP: Nuclear matrix protein; SAE: Small ubiquitin-like modifier activating enzyme; TIF: Transcriptional intermediary factor.
†CADM onset and later diagnosis as classic DM.

course and good outcome compared with SSc patients with anti-Scl-70. D'Aoust *et al.* also reported that patients with SSc and anti-PM-1α (major epitope of anti-PM-Scl) autoantibodies are younger, with myositis, calcinosis, arthritis and overlap disease, characteristics identical to other patients with anti-PM-Scl autoantibodies previously reported [85].

Anti-U1-RNP antibody

Anti-U1-ribonucleoprotein (RNP) autoantibodies are found in different CTD, especially mixed connective tissue disease, SLE, SSc PM/DM and overlap syndrome. Their target autoantigen is the U1 small nuclear RNP complex, the function of which is to assist in the splicing of messenger RNAs. Muscle symptoms of patients with anti-U1 RNP autoantibodies are relatively mild and the response to corticosteroid therapy is generally good.

Clinical subsetting of dermatomyositis using DM-specific antibodies

Many clinical studies have already demonstrated that DM-specific antibodies are useful for the diagnosis as well as clinical subsetting of DM because these antibody specificities have a close correlation with distinct clinical phenotypes. The overall frequencies of either MSA in DM patients ranged from approximately 41 to 79% in previous reports [27,34,44]. The discovery of new DM-specific antibodies, together with the already well-known ones, enables us to categorize more than half of DM patients into distinct clinical phenotypes. Clinical distinct characteristics of each subgroup of DM patients as classified by DM-specific antibodies (including anti-ARS) are summarized in Table 3 & Figure 2.

Conclusion

Our understanding of DM-specific autoantibodies has markedly improved in the last decade. This progress has been made possible by discoveries of newly identified DM-specific autoantibodies. These antibodies are useful for differential diagnosis, choice of treatment and prediction of outcome, as well as for classifying DM patients into clinical subgroups. In addition, recent studies relevant to immunological characteristics have suggested that these antibodies are involved in disease pathogenesis through the initiation or amplification of autoimmune responses. Further efforts to discover new

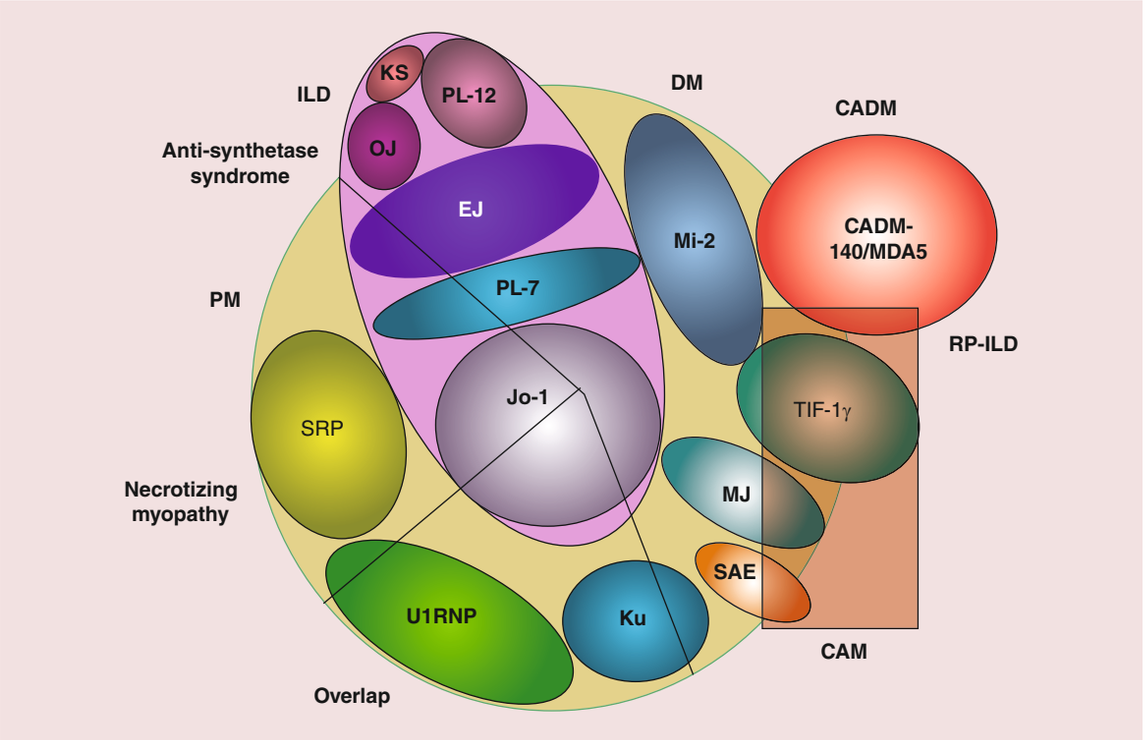

Figure 2. Myositis-specific autoantibodies and disease subsets. MSAs have been proven to correlate with specific clinical phenotypes and useful for the classification of DM as well as help for diagnosis, selection of treatment and prediction of outcome.
CADM: Clinically amyopathic dermatomyositis; CAM: Cancer-associated myositis; DM: Dermatomyositis; ILD: Interstitial lung disease; MSA: Myositis-specific autoantibody; PM: Polymyositis; RP-ILD: Rapidly progressive interstitial lung disease.

DM-specific antibodies will facilitate progress in the clinical management of DM in the future.

Future perspective

According to previous studies, up to approximately 70–80% of DM patients have one of the MSAs so far identified. Investigation of additional new MSAs would accelerate the understanding of this complicated disease by increasing the proportion of DM-specific autoantibody positivity in DM patients in the future. Moreover, DM patients might be classified into subgroups based on their serological and clinical characteristics to make diagnosis easier. This might lead to improvement in the long-term prognosis of DM patients. Furthermore, the elucidation of the associations between these autoantibodies and/or targeted autoantigens and disease pathogenesis might facilitate the development of new therapeutic approaches such as targeting specific molecular processes responsible for the progression or worsening of this condition.

Executive summary

Autoantibodies found in idiopathic inflammatory myopathy

- A spectrum of serum autoantibodies is present in patients with idiopathic inflammatory myopathy.
- These can be divided into two types: myositis-specific antibodies and myositis-associated antibodies.

Autoantibodies specific for dermatomyositis

- Six dermatomyositis (DM) or polymyositis/DM-specific antibodies (anti-aminoacyl transfer RNA synthetases [ARS], anti-Mi-2, anti-melanoma differentiation-associated gene 5 [MDA5], anti-transcription intermediary factor [TIF]-1γ, anti-nuclear matrix protein-2 [NXP-2] and anti-small ubiquitin-like modifier activating enzyme [SAE]) have been reported so far.
- DM patients who possess the same antibodies tend to have similar clinical phenotypes distinct from patients with other antibodies.
- Anti-ARS antibodies are specific for polymyositis/DM patients although some of these antibodies seem to have stronger associations with interstitial lung disease (ILD). These anti-ARS-positive patients commonly have fever, arthritis, Raynaud's phenomenon, mechanic's hands and chronic-type ILD.
- Anti-Mi-2 antibody is commonly found in patients with both adult and juvenile DM who have typical DM skin symptoms such as heliotrope rash or Gottron's sign and/or papules. Clinically, response to therapy for muscle symptoms is good and complications of ILD or neoplasia that negatively influence prognosis occur at low frequency.
- Anti-TIF-1γ antibody is also detected in both adult and juvenile DM including clinically amyopathic dermatomyositis (CADM). Skin lesions are usually severe and extend to broad areas with DM typical skin rash as well as V-sign and/or Shawl-sign. Adult patients with this anti-TIF-1γ antibody have a high frequency of malignancy whereas the frequency of ILD is low. On the other hand, both malignancy and ILD are rarely found in patients with juvenile onset DM with anti-TIF-1γ antibody.
- Anti-MDA5 antibody is present in DM including CADM. The ratio of CADM to classic DM and CADM is relatively high, especially in Japanese DM patients. Major characteristic skin manifestation is cutaneous ulcers. The most important feature of patients with this antibody is life-threatening RP-ILD. Therefore, determination of anti-MDA5 antibody could significantly benefit DM patients with the possible complication of RP-ILD.
- Anti-NXP-2 antibodies are detected in both adult DM and juvenile DM (JDM) patients although the frequency in adult patients is low. However, clinical characteristics of the two groups are distinctly different. JDM patients with anti-NXP-2 antibodies have a high likelihood of suffering from calcinosis and have a severe disease course. Adult DM patients with anti-NXP-2 antibodies have increased malignancies and therefore a possible poor prognosis.
- Finally anti-SAE antibody is identified in adult DM. The unique characteristic of DM patients with anti-SAE is precedence of cutaneous manifestations. These patients are at first diagnosed as CADM and later progress to muscle symptoms including dysphagia. No detailed review of these antibodies in patients with JDM is available so far.

Clinical applications of DM-specific antibodies

- It has been demonstrated that DM-specific antibodies are useful for diagnosis, therapeutic decision making, therapeutic effect and prediction of outcome, as well as subsetting into clinical phenotypes.

Involvement in the pathogenesis of DM

- Target autoantigens of DM-specific antibodies are either nuclear or cytoplasmic molecules that play an important role in vital phenomena such as gene transcription, protein translocation and antiviral defense. Recent studies have suggested possible involvement of target autoantigens in the pathogenesis of DM.

Conclusion

- Subsetting of DM patients using DM-specific autoantibodies would be beneficial for the diagnosis, treatment and prognosis of DM patients.
- Therefore, the development of assays for DM-specific antibodies that are applicable in daily routine clinical practice is crucial.
- The investigation of new autoantibodies specific for DM is important in terms of the improvement of diagnostic accuracy and treatment outcome in patients with DM, as well as for understanding the pathogenic mechanisms of DM.

Financial & competing interests disclosure

Authors are holding a patent on anti-MDA5 antibody-measuring kit. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

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