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REVIEW ARTICLE

Myositis-specific autoantibodies: detection and clinical associations

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Abstract In recent years, the detection and characterization of (novel) autoantibodies is becoming increasingly important for the early diagnosis of autoimmune diseases. The idiopathic inflammatory myopathies (IIM, also indicated with myositis) are a group of systemic autoimmune disorders that involve inflammation and weakness of skeletal muscles. One of the hallmarks is the infiltration of inflammatory cells in muscle tissues. A number of myositis-specific autoantibodies have been identified and these may be associated with distinct IIM subclasses and clinical symptoms. Here, we review all myositis-specific autoantibodies identified today as well as their target proteins, together with their clinical associations in IIM patients. Post-translational modifications that might be associated with the generation of autoantibodies and the development of the disease are discussed as well. In addition, we describe well established autoantibody detection techniques that are currently being used in diagnostic laboratories, as well as novel multiplexed methods. The latter techniques provide great opportunities for the simultaneous detection of distinct autoantibodies, but may also contribute to the identification of novel autoantibody profiles, which may have additional diagnostic and prognostic value. The ongoing characterization of novel autoantibody specificities emphasizes the complexity of processes involved in the development of such autoimmune diseases.

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Autoantibodies in idiopathic inflammatory myopathies

Autoantibodies directed against a variety of autoantigens are strongly associated with systemic autoimmune diseases. Within the heterogeneous group of connective tissue diseases (CTD), a wide range of nuclear and cytoplasmic autoantigenic targets have been described. However, it is still unclear why autoantibodies directed against these autoantigens are formed and whether they participate in pathological processes during disease development and/or progression. Post-translational modifications (PTMs) have been hypothesized to generate novel epitopes in antigenic proteins. The cross-presentation of these post-translationally modified epitopes might break immunological tolerance and initiate the development of an autoimmune response resulting in autoantibody formation.

The idiopathic inflammatory myopathies (IIM) are a group of disorders characterized by inflammation and weakness mainly of the muscles closest to the trunk of the body (proximal muscles). These disorders include polymyositis (PM), dermatomyositis (DM) and inclusion body myositis (IBM). IIM may be associated with inflammation in other organs, including the joints, heart, lungs, intestines and skin. In IIM, like in other systemic autoimmune diseases, distinct clinical phenotypes can be correlated with specific autoantibody targets in patients, which emphasizes the clinical relevance of these biomarkers [1]. Autoantibodies found in IIM patients can be divided into myositis-associated autoantibodies (MAA), which are not specific for the disease and are also found in other rheumatic

disorders, and myositis-specific autoantibodies (MSA), which are found primarily in patients with IIM. Tables 1 and 2 show a comprehensive overview of the autoantigens recognized by MSA and MAA together with their function. In this review, we will describe the most prevalent MSA in more detail, their detection and the clinical manifestations associated with them.

Autoantibody detection assays

The development of less invasive and more advanced imaging tools in order to discriminate between the clinical characteristics of various inflammatory myopathy subtypes, contributed greatly to the often difficult diagnosis of these diseases. The application of advanced techniques such as magnetic resonance imaging (MRI), X-ray computed tomography (CT), positron emission tomography (PET), and ultrasonography can also increase the sensitivity of detecting IIM prior to muscle biopsy [2]. In addition, several serological markers in IIM, which can be specific for certain IIM subtypes or be helpful in evaluating disease progression have been detected in the last decades. These biomarkers mostly are autoantibodies directed to one or more cellular components. The fact that only a limited number of proteins appear to be targets of autoantibodies suggests that distinct processes are involved in their generation.

A variety of techniques are available to detect the various autoantibodies for research or routine diagnostics, all with their distinct sensitivity and specificity traits. Conventional methods such as indirect immunofluorescence, immunoprecipitation, immunodiffusion and counter-immuno-electrophoresis, or immunoassays such as the enzyme-linked immunosorbent assay (ELISA) and

Table 1 Myositis-specific autoantibodies and their functional activities

Autoantibody	Autoantigen	Molecular mass (kDa)	Function of autoantigen
Anti-aaRS			
Anti-Jo-1/PL1	HisRS	54	Aminoacylation of tRNAs
Anti-PL7	ThrRS	80	
Anti-PL12	AlaRS	106	
Anti-EJ	GlyRS	75	
Anti-OJ	IleRS	150	
Anti-KS	AsnRS	65	
Anti-Ha	TyrRS	58	
Anti-Zo	PheRS	57/66	
Anti-tRNA			
	$tRNA^{His}$		Translation
	$tRNA^{Ala}$		
Anti-Mi-2			
	Mi-2α	220	Transcription regulation + Nucleosome remodeling
	Mi-2β	218	
Anti-SRP			
	SRP54	54	Protein translocation to the ER
	SRP68	68	
	SRP72	72	
Anti-p155/140			
	Tif1-γ	155	Ubiquitination
	Unknown	140	n.d.
Anti-CADM-140			
	MDA5	117	Innate immunity (RNA sensor)
Anti-SAE			
	SAE1	38	Sumoylation
	SAE2	72	
Anti-p140			
	NXP-2	107	RNA binding + nuclear transcription

n.d. not determined

Table 2 Myositis-associated autoantibodies and their functional activities

Autoantibody	Autoantigen	Molecular mass (kDa)	Function of autoantigen
Anti-PM/Scl			
	PM/Scl-75	75	RNA processing + degradation
	PM/Scl-100	100	
Anti-Mas			
	Serine-tRNA^{Sec}-protein complex	48	Selenocysteine incorporation
Anti-Ro/SS-A			
Anti-Ro52	Ro52	52	
Anti-SS-A	Ro60	60	RNA quality control
Anti-La/SS-B			
Anti-SS-B	La	48	RNA binding + Pol III transcription
Anti-RNP			
	U1A	34	Pre-mRNA splicing
	U1C	22	
	U1-70k	70	
Anti-Wa	Peptide	48	Unknown
Anti-PMS	a.o. PMS1, PMS2	n.d.	DNA binding protein complex involved in DNA-repair
Anti-Ku			
	Ku70	70	DNA dependent protein phosphorylation
	Ku80	80	
Anti-Fer			
	eEF1		Translation
Anti-KJ	Peptide	30/34	Translocation factor
Anti-56K	Nuclear RNP	56	Unknown

PM/Scl polymyositis, scleroderma overlap, *RNP* ribonucleoprotein, *PMS* postmeiotic segregation, *eEF1* eukaryotic elongation factor 1, *n.d.* not determined

immunoblotting are already in use for many years. In most cases, these techniques are suitable for the detection of a single autoantibody specificity. In contrast, a new generation of assays (so-called multiplexed analysis techniques) allows the simultaneous detection of several autoantibody specificities [3, 4]. The latter techniques probably will dominate serological profiling in diagnostic laboratories in the future provided that validation and reproducibility are ensured.

Immunofluorescence

Several antinuclear antibodies (ANA) are still being detected by indirect immunofluorescence on HEp-2 cells or tissue substrates. Some ANA-positive IIM sera may show a particular staining pattern that can be correlated with a distinct autoantigen. However, this technique is laborious and needs specific technical skills to perform. It is therefore less convenient for present day diagnostic analyses.

Immunoprecipitation, ELISA and immunoblotting

The most unambiguous way to determine the presence of autoantibodies against a particular autoantigen is the detection of an autoantibody by virtue of its binding to the antigen in its fully native state. Although the immunoprecipitation (IP) method is the most obvious choice, this technique is not widely applied because it is laborious, expensive and requires specific technical skills. Radiolabelled cell extracts are often used as substrates for immunoprecipitation assays and subsequent mass-spectrometry analysis can characterize the specific targets [5, 6] (Fig. 1). However, the antigen to be detected may be associated with other (non-antigenic) proteins and as a consequence the IP procedure often leads to a complex pattern of precipitated proteins of which only one or a few proteins are truly autoantigenic. Nevertheless, detection of autoantigenic biomarkers in IIM by immunoprecipitation techniques is still widely used.

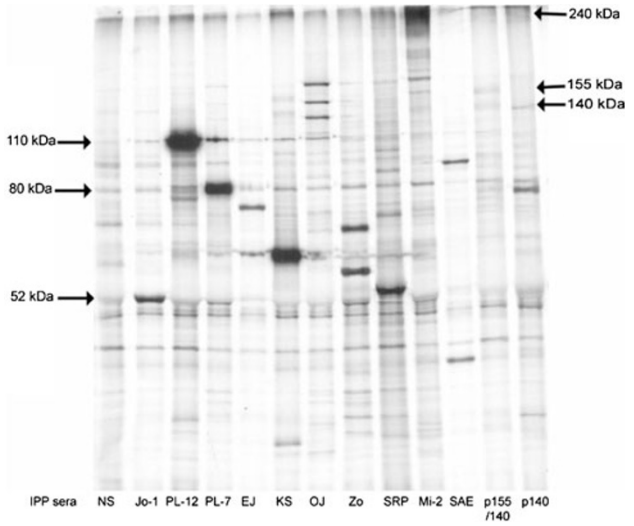

Fig. 1 Immunoprecipitation assay with myositis-specific autoantibodies. Polypeptides immunoprecipitated from ^{35}S-methionine-labelled K562 cell extracts by antibodies from patient sera were separated by SDS–polyacrylamide gelelectrophoresis and visualized by autoradiography. Sera used for immunoprecipitation included serum from a healthy individual (NS), anti-Jo-1, anti-PL-12, anti-PL-7, anti-EJ, anti-KS, anti-OJ, anti-Zo, anti-SRP, anti-Mi-2, anti-SAE, anti-p155/140, anti-p140 (reproduced from [6], by permission of The British Society for Rheumatology)

The first high-throughput assays such as ELISA increased the number of patient sera that could be analysed compared to the previously mentioned conventional assays. However, purification of the antigen and use of the correct reagents has to be monitored to warrant an optimal specificity [7, 8]. In addition, at least some conformational epitopes might be lost, because the antigen is bound to the ELISA plate. Immunoblotting (IB) analysis, in addition to ELISA, may increase the specificity of the signal detected in the immune assays. However, IB may allow the detection of autoantibodies only to linear epitopes, because the antigen is completely denatured during preparation of the blot. This might decrease sensitivity of the IB method as compared to IP and ELISA. As a consequence only, the ELISA method is widely used in routine laboratories where large numbers of sera have to be tested.

Multiplex assays

A number of multiplex-based assays have been developed during the years, with distinct methodological differences. In this review we will discuss the high-throughput-based micro-array format, described in this paragraph, and a macroscopic assay that has been developed for the simultaneous detection of multiple autoantibodies in IIM sera, the line-blot, which is described separately in the next paragraph. Multiplex assays are able to screen a single sample of blood or other biological fluid for an array of autoantibody specificities simultaneously. In this way a patient-specific autoantibody profile can be made. Future prospects suggest that antigen/patient-specific therapies might be developed from these patient-specific autoantibody profiles, which might prevent disease progression, remission or relapse over time. The basic principles of two main types of micro-array-based multiplex assays will be discussed below.

Solid surface-based autoantigen microarrays contain immobilized proteins or other biomolecules in predetermined positions on a solid surface. Interactions between the immobilized antigens and molecules in the serum sample such as (labelled) antibodies can be detected by fluorescence-based procedures [4, 9]. Such antigen arrays have already been used to screen the autoantibody profiles in autoimmune diseases, IgE reactivity in allergy, and the immune response to infections, vaccination, and cancer reviewed by [4]. However, this technique is based on commercialized or home-made microarrays that depend on specific technical expertise and equipment (Fig. 2). In order to simplify technical aspects as much as possible so-called addressable-bead autoantigen microarrays have been developed [10, 11]. Individual antigens of interest are chemically coupled to beads of different colours. Subsequently, sera or other biological fluids can be analysed in a microtiter well containing a bead mixture as described by Tozzoli [3], Fritzler 12]. One laser will measure the colour of the specific antigen-coupled bead, whereas a second laser determines the presence and quantity of a fluorochrome-coupled secondary antibody bound to the bead (Fig. 3). Both autoantigen microarray techniques have advantages over the conventional techniques in terms of reduced sample volumes, enhanced sensitivity, automation and increased numbers of samples that can be tested. The

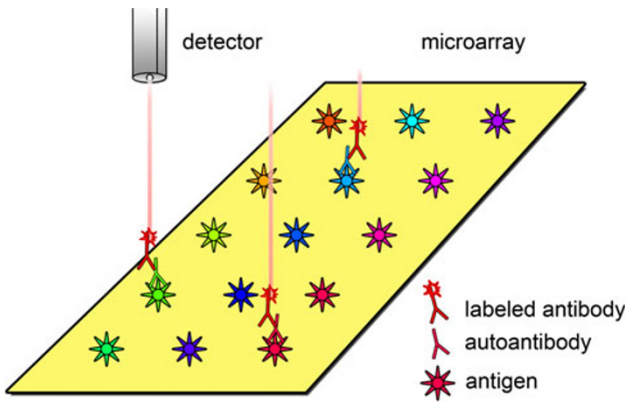

Fig. 2 Schematic representation of the solid surface-based autoantigen array. Autoantigenic molecules are immobilized at defined positions on a solid surface. Binding of autoantibodies in patient sera to the immobilized autoantigens can be detected via fluorescently labelled secondary antibodies

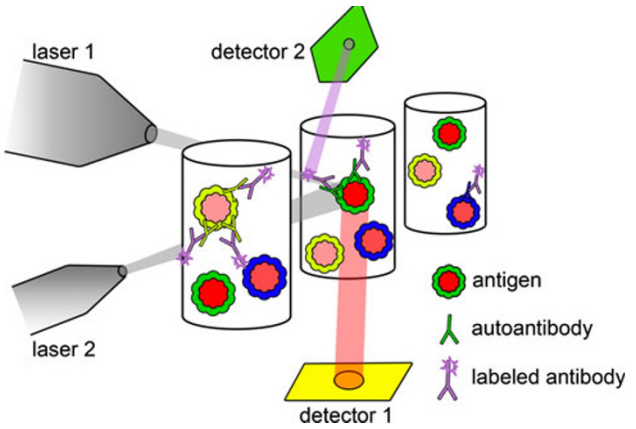

Fig. 3 Schematic representation of a addressable-bead autoantigen immunoassay. Purified autoantigens are coupled to differentially labelled microbeads that can be detected by illumination with a laser. Patient sera can be incubated with mixtures of beads each coated with a different autoantigen. One laser is used to identify the specific antigen coupled to each bead based upon the fluorescent properties of the bead, and a second laser is used to determine the binding of autoantibodies to the beads after incubation with secondary antibodies conjugated to a distinct fluorophore

sometimes imperfect specificity and reproducibility of these methods can be obstacles for their implementation in routine diagnostics.

Line-blot assay

The line-blot assays, or so-called line immunoassays (LIAs), are based on immunoblotting procedures that spot purified antigens on protein-binding membranes without the need of gel electrophoresis (Fig. 4). The laborious purification procedure of native antigens is being replaced by the more reproducible production of highly purified recombinant antigens or synthetic peptides. These developments contribute to the increased sensitivity and specificity of commercially available line-blots. Several LIAs of different manufacturers have recently been clinically validated in multicenter studies, and the results indicate that LIAs are becoming a suitable alternative to the more costly and complex techniques sometimes used in diagnostic laboratories reviewed by [13, 14].

Myositis-specific autoantibodies

Aminoacyl-tRNA synthetases

Function

An efficient and reliable transcription and translation of the genetic code is essential for cell survival. Genes are transcribed into messenger RNA (mRNA), which contains a

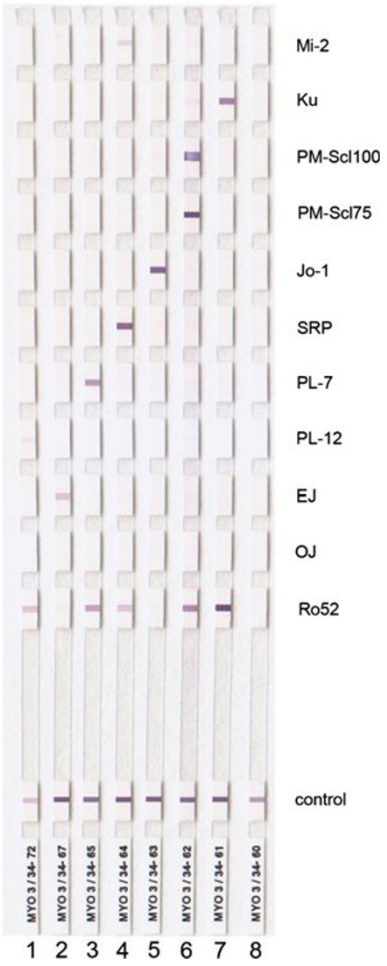

Fig. 4 Line-blot for autoantibody detection in IIM sera. Strips (*vertical*) containing a series of recombinant IIM-related autoantigens were incubated with seven IIM patient sera (1–7) and a control serum (8). Antibody binding was visualized using the protocol provided by the manufacturer. The strips were incubated with sera containing the following autoantibodies (1) anti-PL12, (2) anti-EJ, (3) anti-PL7, (4) anti-SRP and anti-Mi-2, (5) anti-Jo-1, (6) anti-PM-Scl75 and anti-PM-Scl100, (7) anti-Ku

string of nucleotide triplets, called codons. During translation every codon is translated into one particular amino acid (aa) with the help of transfer RNAs (tRNAs) (Fig. 5). The mRNA sequence is thus converted into a chain of amino acids that makes up a functional polypeptide. The tRNAs involved are specific for both the amino acid and the mRNA codon. The amino acids are coupled to their cognate tRNA via an esterification reaction that is catalyzed by specific aminoacyl tRNA synthetases (aaRSs) in a two-step reaction:

$$\text{aaRS} + \text{aa} + \text{ATP}\ \text{aaRS} \leftrightarrow \text{aa} \sim \text{AMP} + \text{PPi}$$

$$\text{aaRS} * \text{aa} \sim \text{AMP} + \text{tRNA} \leftrightarrow \text{aaRS} + \text{aa-tRNA} + \text{AMP}$$

Most of the 20 canonical amino acids are recognized by a single aaRS, with the exception of GluRS and ProRS in

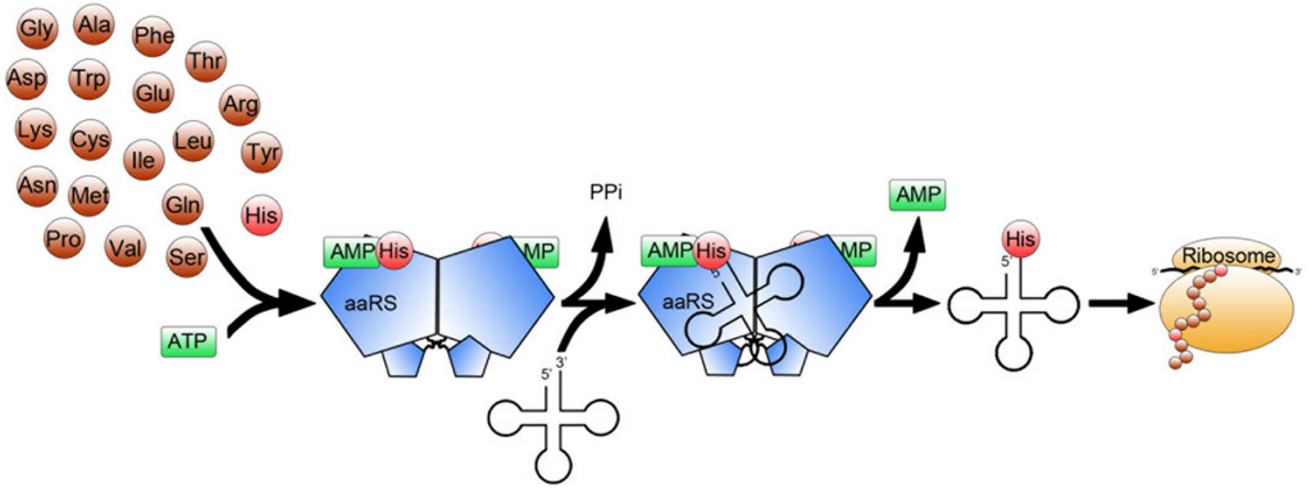

Fig. 5 Aminoacylation of tRNA by histidyl-tRNA synthetase. The HisRS, as well as other aaRSs, catalyze the ATP-dependent esterification reaction that is needed to couple an amino acid to its cognate tRNA. Subsequently, the aminoacylated-tRNA can be used in translation. *AMP* adenosine monophosphate, *ATP* adenosine triphosphate, *PPi* inorganic phosphate

higher eukaryotes [15, 16]. The aminoacylated-tRNA, or so-called charged tRNA, is used by the ribosome to incorporate the appropriate amino acid into the growing peptide chain.

In addition to the wellknown function of aaRSs described above, a diverse array of other biological functions has been described as well. These include amino acid biosynthesis, DNA replication, RNA splicing, cell cycle control, and apoptosis (reviewed by [17, 18]).

Depending on highly conserved sequence motifs and aminoacylation acitivities, the aaRSs can be divided into two classes, Class I and Class II, and three sub-classes a, b, and c (Fig. 6). The catalytic domain of Class I aaRSs consists of a typical Rossmann fold (nucleotide-binding domain) and two highly conserved sequence motifs whereas the Class II aaRSs are characterized by an anti-parallel β-strand surrounded by α-helices, and three conserved sequence motifs [19]. Additionally, Class I aaRSs mainly exist as monomeric or dimeric complexes and aminoacylate the 2′-hydroxyl group of the terminal ribose of tRNA. Class II aaRSs usually are dimeric or tetrameric structures and aminoacylate the 3′-hydroxyl group of the terminal ribose. The only exception is the PheRS that belongs to the Class II aaRSs but nevertheless aminoacylates the 2′-OH of the ribose. Higher eukaryotic cells have been shown to contain a high molecular mass multi-synthetase complex (MSC), which contains nine aaRSs and three accessory proteins (Fig. 6). The MSC is thought to promote protein synthesis, proofreading activity, and serve as a reservoir of regulatory molecules that are involved in functions other than aminoacylation [20, 21].

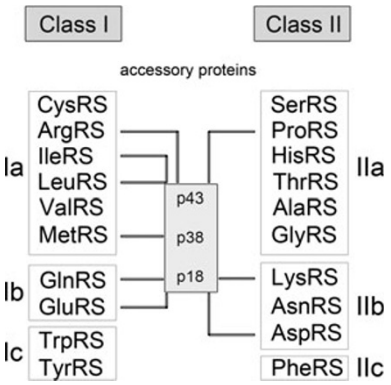

Fig. 6 Classification of aminoacyl-tRNA synthetases. AaRSs are (sub)classified according to the chemical specificity of the reaction they catalyze and on the presence of conserved domains in their amino acid sequences (Class I and II, and subclasses a, b, and c). The aaRS that are associated with the multisynthetase complex (MSC) are connected to the three MSC accessory proteins (p43, p38, and p18) via *bold lines*

Potentially disease-related PTMs

Three aaRSs (HisRS, AlaRS, and IleRS) have been described as substrates for the serine protease Granzyme B (GrB), which is present in granules of cytotoxic T lymphocytes (CTLs) or natural killer (NK) cells and plays an important role in granule exocytosis-induced cytotoxicity [22, 23]. The exposure to GrB leads to unique fragments of autoantigens, which are not detected during other forms of cell death [24]. Indeed, almost all IIM autoantigens (except SSA/Ro52) can be cleaved by GrB, in contrast to non-autoantigenic proteins, which are either not cleaved or cleaved at sites also used by caspases [24]. The fragments

generated by GrB, which are believed to contain new and unique epitopes, may help in breaking tolerance against the autoantigens when they are encountered by the immune system. The cleavage products as well as the full-length autoantigenic HisRS and AsnRS have been described to exert chemotactic properties through their interaction with the chemokine receptors CCR3 or CCR5, in contrast to the non-autoantigenic AspRS and LysRS [25, 26]. Levine et al. [27] found a proteolytically sensitive HisRS conformation in normal lungs compared to muscle tissue and proposed the lung as a target site where initiation and propagation against HisRS may take place. However, the GrB-induced aaRS fragmentation and the subsequent dendritic cell-driven autoreactive $CD4^+$ T cell activation have not been demonstrated in IIM patients yet.

An additional cleavage product of HisRS has been described by Ramirez-Sandoval et al. [28] upon the induction of apoptosis in HEp-2 cells by camptothecin. Also TyrRS, and its close homologue TrpRS, are both described to stimulate chemotactic activities as well as angiogenesis in vitro upon cleavage [29, 30]. The exposure to reactive oxygen and/or nitrogen species (ROS/RNS) may also lead to post-translationally modified biomolecules and result in the loss of immunological tolerance. Interestingly, increased levels of ROS/RNS have been reported to be present in inflammatory autoimmune diseases [31, 32]. We have observed selective oxidative modifications in HisRS, but the significance of these for IIM remains to be established (Van Dooren et al., manuscript submitted for publication)

Antibodies to aaRSs and their clinical associations

Autoantibodies directed to aminoacyl-tRNA synthetases are the most commonly detected MSA in adult IIM patients. They can be found in about 40% of individuals with IIM and in lower frequencies in juvenile IIM patients [33]. In addition, autoantibodies against the cognate tRNAs have been found [34, 35]. Most anti-aminoacyl-tRNA synthetase antibodies (anti-aaRS) are directed against the Class II aaRSs, including PL1/Jo-1 (HisRS), PL7 (ThrRS), PL12 (AlaRS), EJ (GlyRS), KS (AsnRS), and Zo (PheRS). Only two Class I aaRS autoantibody targets, namely OJ (IleRS) and Ha (TyrRS) have been described so far [6]. Most IIM patients with anti-aaRS produce autoantibodies against a single aaRS, although in rare cases antibodies may occur directed to two aaRSs [36].

The anti-Jo-1 (or anti-HisRS) is the most frequently occurring autoantibody in PM/DM patients, its presence nearly excludes one of the three IIM, being inclusion-body myositis [37–39]. Different clinical and immunological features are associated with the presence of anti-Jo-1 antibodies, but whether the autoantibodies are a cause or a consequence of the development of the disease remains unknown, despite the fact that a correlation between the titer of anti-Jo-1 antibody and disease activity has been observed [40].

The major epitope of the Jo-1 molecule has been defined as a coiled-coil structure within the protein [41], although antibodies directed to other parts of the Jo-1 protein can be detected as well [42, 43]. Class switching, affinity maturation, species-specificity, and spectrotype broadening during the Jo-1 antibody response have been detected, which suggests a T cell-dependent and antigen-driven response in IIM patients [41, 43–45]. These processes also suggest that epitope spreading during the course of the disease may occur. Most studies that investigate the autoepitopes on the Jo-1 protein report activities that recognize multiple, both linear and conformation-dependent Jo-1 epitopes.

Myositis-specific autoantibody profiles are often associated with distinct clinical features [46]. Indeed, anti-aaRS antibodies are associated with a unique clinical syndrome, the so-called anti-synthetase syndrome. Characteristics of the anti-synthetase syndrome include myositis, Raynaud's phenomenon, arthralgia, fever, skin changes and interstitial lung disease, although clinical features may differ between patients with the anti-synthetase syndrome [47, 48]. The interstitial lung disease is important as it may negatively affect prognosis [49].

A number of studies investigated the genetic background of IIM patients and tried to correlate phenotypic characteristics with genetic predispositions. Besides associations with the human leukocyte antigen (HLA) 8.1 ancestral haplotype (HLA-DRB1*03-DQA1*05-DQB1*02), significant correlations were found between the presence of anti-Jo-1 antibody and HLA-DRB1*0301 and/or HLA-DPB1*0101 (reviewed by [50]). There are also data supporting the idea that candidate IIM autoantigens can be involved in the induction and propagation of the autoimmune response. The expression of the Jo-1 antigen was found to be elevated in regenerating muscle of IIM patients compared to normal muscle [51, 52]. However, clear pathologic relevance of these antibodies in IIM has not been found yet.

How to assay for anti-aaRS antibodies

Commercially based LIAs can be used to test for several anti-aaRS antibodies, including anti-Jo-1, anti-PL7, and anti-PL12 (see Fig. 4; [13, 14, 53]). However, reaction conditions (e.g. temperature) have to be standardized, because they can have profound effects on the experimental outcome [13]. Recently, LIA strips have been developed that also detect anti-EJ and anti-OJ reactivities. Other less frequently found anti-aaRS reactivities such as

anti-KS, anti-Ha, and anti-Zo are not commercially exploited yet. The latter anti-aaRSs are still being detected by the more conventional immunoblot- and immunoprecipitation-based techniques.

Mi-2

Function

The chromatin in the nucleus of eukaryotic cells consists of a densely packed complex of DNA and proteins. The basic structure is the nucleosome, each of which contains an octamer of four core histones (H2A, H2B, H3, and H4) and a piece of approximately 150 basepairs DNA tightly wrapped around it. To allow gene transcription and chromosome replication, the chromatin is dynamically and orderly unfolded and, after replication or transcription repression, reformed.

Chromatin structure remodeling is mainly regulated via two mechanisms reviewed by [54, 55]. The first involves covalent modification of nucleosomes such as methylation of DNA and (de-)acetylation of lysine residues in the core histones. The second mechanism involves dynamic changes of the histone–DNA interactions within the nucleosome in an ATP-dependent manner, which results in an increased or decreased accessibility of nucleosomal DNA (Fig. 7). The major remodeling enzymes involved can be categorized based on their ATPase subunits. These include the ISWI/SNF2L-type ATPases and the chromodomain helicase DNA-binding (CHD) protein family.

The Mi-2 protein was first identified as a DM-specific autoantigen and later shown to be a subunit of the nucleosome remodeling and deacetylation (NuRD) complex [56, 57]. The core subunits of the Mi-2/NuRD complexes are classified into the CHD protein family and are found to occur in different subunit compositions, depending on their functional activity reviewed by [58]). The components include histone de-acetylases (HDAC1 and HDAC2), retinoblastoma associated proteins (RbAp), metastasis-associated protein (MTA), a methyl-CpG-binding domain containing protein (MBD), and either one of the highly related Mi-2α (CHD3) and Mi-2β (CHD4) proteins [58–60]. The NuRD complexes are unique transcriptional regulators since they combine three enzymatic activities, namely histone deacetylation, histone demethylation, and ATP-dependent chromatin remodeling activities.

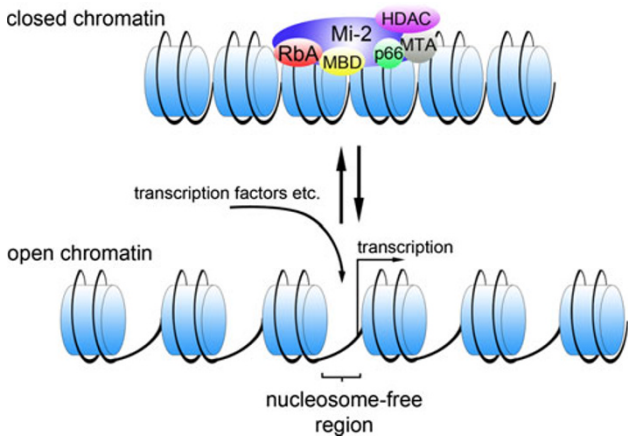

Fig. 7 Predicted model of chromatin remodeling by the Mi-2/NuRD complex. The core subunits of the Mi-2/NuRD complex are suggested to be involved in modifying the chromatin structure, which can result in the initiation and/or maintenance of gene repression

Potentially disease-related PTMs

The Mi-2/NuRD complex or Mi-2 protein complex has been described to be a substrate for modifying enzymes such as enzymes conjugating the small ubiquitin-related modifier (SUMO) and GrB [24, 61]. However, a direct relation between modified protein and IIM pathogenesis has not been demonstrated yet.

Antibodies to Mi-2 and their clinical associations

Reichlin and co-workers were the first to describe anti-Mi-2 antibodies in IIM patients and to date the anti-Mi-2 antibody can be detected in up to 20% of DM patients [62, 63]. The anti-Mi-2-positive patient sera are able to immunoprecipitate a major protein of approximately 240 kDa, together with up to eight other components of the Mi-2 complex (200, 150, 72, 65, 63, 50, and 34 kDa) [56, 64] (Fig. 1). Subsequently, two highly related Mi-2 proteins, Mi-2α and Mi-2β (calculated molecular masses are 220 and 218 kDa, respectively), which both contain epitopes that are recognized by anti-Mi-2 antibodies, have been identified as antigens in patients [57, 65]. Although anti-Mi-2 antibodies seem to be more tightly associated with DM, in several studies anti-Mi-2-positive PM and IBM patients have been detected as well [8, 66]. Therefore, cutaneous characteristics such as Gottron's papules, heliotrope rash, cuticular overgrowth and so-called V- and shawl sign, in combination with autoantibody profiles are considered to be the most reliable diagnostic features for DM [1]. Anti-Mi-2-positive patients tend to respond well to steroid therapy and thus have a relatively good prognostic profile [67].

Recent studies have found elevated Mi-2 expression levels in (regenerating) DM muscle biopsies and in certain tumors associated with autoimmune IIM [51, 68]. Additionally, Kashiwagi et al. [69] described a crucial role of Mi-2 in the development and repair of the basal epidermis, which may support a pathophysiological role for Mi-2 in the development of DM. Moreover, latitudinal differences have been demonstrated to affect the occurrence of DM.

These studies suggest an important role of environmental factors such as UV-light in the development of DM and anti-Mi-2 formation [70–73]. Anti-Mi-2 antibodies were detected in juvenile DM as well as adult DM, although autoantibody frequency may vary between the different DM subtypes [74–76]. Genetic predispositions in different HLA genes of (non-)Caucasians, but also protein tyrosine phosphatase and pro-inflammatory cytokine genes have been shown to be involved in the development of IIM reviewed by [50]). The HLA-DRB1*0302 and HLA-DRB1*0701 alleles have been described as risk factors for the generation of anti-Mi-2 antibodies in African-American and Caucasian individuals [50, 77].

How to assay for anti-Mi-2 antibodies

Several diagnostic assays have been used over the years to detect anti-Mi-2 antibody reactivities in patient sera. To date, home-made tests are still more accurate, however, due to an enhanced reproducibility, sensitivity, and specificity, line-blot techniques will probably be preferred over the more conventional ELISA and IP assays in the future [53].

Signal recognition particle

Function

Proteins that are involved in intercellular signaling are either being secreted or transported to the plasma membrane. The intracellular translocation of such proteins, from translational site to functional site, is regulated by a specialized secretory pathway. First, newly synthesized proteins have to be directed to the endoplasmic reticulum (ER), which is a signal peptide sequence driven process. This sequence is located at the N-terminus of the protein, and protein translocation can occur post-translationally or co-translationally. Co-translational translocation involves the so-called signal recognition particle (SRP), which is a ribonucleoprotein (RNP) complex that consists of six proteins (9, 14, 19, 54, 68, and 72 kDa) and a RNA molecule of approximately 300 nucleotides, termed 7SL RNA [78]. In the SRP complex two distinct RNP domains can be distinguished (Fig. 8) [79–81]. The S-domain consists of SRP19, SRP54, SRP68, SRP72, and a forked 7SL RNA moiety. It recognizes the signal peptide on the growing peptide chain and targets the complex to the ER membrane. The Alu-domain is formed by SRP9, SRP14 at the opposite end of the 7SL RNA and probably causes a transient translational arrest until the ribosome/nascent chain complex has docked to the ER. The SRP particle has a high affinity for ribosomes containing a protruding signal peptide. After binding, the SRP-ribosome complex binds the SRP receptor (SR) on the ER membrane via GTP-dependent interactions. Subsequently, the ribosome can dock onto the translocon and the binding between signal sequence and SRP complex is broken, allowing translation to resume. Finally, after GTP hydrolysis, SRP and the SR dissociate and the SRP complex is recycled for a following round of translocation reviewed by [82]).

Potentially disease-related PTMs

Casciola-Rosen et al. [24] described SRP72 as a substrate for GrB and suggested a pathogenic role for the unique fragments generated in this way.

Antibodies to SRP and their clinical associations

Reeves et al. [83] were the first to describe the SRP as an autoantigen in IIM patients. Autoantibodies directed to SRP54 are most frequently found, although anti-SRP72, anti-SRP68, and anti-7SL RNA reactivities have been reported as well [84–86]. Patients that generate anti-SRP antibodies (up to 5% of patients with IIM) appear to form a distinct clinical and histopathological subset within the IIM [86, 87]. The so-called necrotizing myopathy seen in anti-SRP-positive patients, include necrotic and regenerating myofibers. Muscle enzyme elevations as well as lower numbers of infiltrating lymphocytes are found in muscle biopsies compared to PM. In addition, patients with anti-SRP antibodies frequently reveal an unusually severe weakness, acute onset, rapid disease progression, and resistance to treatment [84, 87, 88]. These distinct characteristics emphasize that anti-SRP antibodies can be used as a biomarker for a distinct subgroup of IIM patients. Anti-SRP antibodies are rarely detected in juvenile IIM [6].

How to assay for anti-SRP antibodies

Anti-SRP antibodies, similar to anti-aaRS, anti-Mi-2, and several anti-MAA, can be detected via commercial LIAs. However, Ronnelid et al. [13] found discrepancies in the autoantibody reactivities when commercial LIAs were used at different temperatures. This temperature sensitivity is most pronounced for weakly positive and negative samples, which may result in false- or false-negative diagnoses. This emphasizes the importance of further evaluation and development of commercial LIAs, in order to ensure that specificity and sensitivity are warranted.

CADM-140

Function

The innate immune system utilizes a specific group of receptors, the so-called pattern recognition receptors

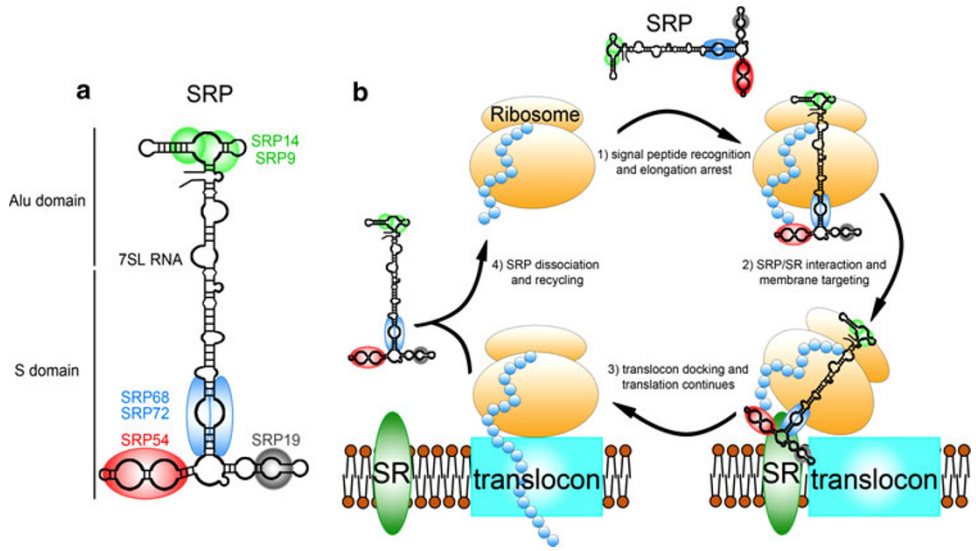

Fig. 8 SRP mediates translocation of nascent, signal peptide-containing proteins: **a** schematic structure of SRP, **b** mechanism of protein translocation mediated by SRP. First, SRP binds to the N-terminal signal peptide of the growing peptide chain which results in elongation arrest (*1*). Subsequently, the ribosome/SRP complex is targeted to the SRP receptor (SR) at the ER membrane by specific sequences in the SRP proteins (*2*). Docking of the complex to the membrane bound translocon releases the signal peptide from the SRP complex and enables translation to continue (*3*). The SRP dissociates and is recycled for additional translocation events (*4*)

(PRRs), to detect specific pathogenic components [89]. Three major classes of PRRs have been identified, including Toll-like receptors (TLRs), retinoic acid-inducible gene I (RIG-I)-like helicases (RLHs) and nucleotide-oligomerization domain (NOD)-like receptors (NLRs). The melanoma differentiation-associated gene 5 (MDA5), or CADM-140 autoantigen, together with RIG-I and LGP2 (laboratory of genetics and physiology 2) are members of the RLH helicase family. Both RIG-I and MDA5 contain N-terminal caspase-recruitment domains (CARDs) followed by a DExD/H box RNA helicase domain, whereas LGP2 has a similar helicase domain but lacks a CARD domain. LGP2 has been suggested to function as a negative regulator of RIG-I/MDA5 signaling [90–92]. The TLRs and RLHs are able to sense viral RNA and DNA components and downstream signaling results in the activation of cells to produce type I interferons (IFNs) and pro-inflammatory cytokines. These factors stimulate the immune system by activating natural killer (NK) and T cells, and promote the induction of apoptosis of virus-infected cells and the cellular anti-viral response that interferes with cellular and viral processes.

Potentially disease-related PTMs

Barral et al. [93] describe MDA5/CADM-140 degradation products, both in puromycin-induced apoptotic HeLa cells and poliovirus-infected cells, which is suggested to antagonize the type I IFN-induced anti-viral response. Disease-associated MDA5/CADM-140 modifications have not been described yet.

Antibodies to CADM-140 and their clinical associations

The IIM classification criteria enable clinicians to categorize several distinct IIM populations. Some individuals, the so-called amyopathic DM patients, exert typical skin manifestations of DM but little or no evidence of clinical IIM. When patients do not develop clinical symptoms of IIM for at least 2 years after the onset of skin manifestations they will be classified as clinically amyopathic DM (CADM) [94]. Japanese patients with CADM develop a rapidly progressive interstitial lung disease (ILD), which frequently appears to be resistant to treatment and which may lead to early death [95, 96]. Sato et al. [97] were the first to describe a novel antibody (anti-CADM-140) that immunoprecipitated a 140-kDa protein in 53% of the CADM patients, whereas no such antibodies were detected in any of the PM or DM patients (Fig. 1). Recently, it was shown that this 140-kDa protein is identical to MDA5 [98]. It is intriguing that anti-CADM-140 antibodies are only reported to occur in Japanese patients so far. Nakashima et al. [99] confirmed the anti-CADM-140 antibody to be specific for CADM, although a few positive patients showed symptoms of IIM (DM according to criteria). A recent report from Hoshino et al. [100], suggested that anti-CADM140/MDA5 autoantibodies could be used as a novel serological marker to distinguish between different DM subsets and associated clinical complications.

How to assay for anti-CADM-140 antibodies

No commercial assay is available yet for the detection of the anti-CADM-140 antibody. Sato et al. [97] used immunoprecipitation, immunoblotting, and indirect immunofluorescence techniques to detect this specificity. In addition, an ELISA, containing the recombinant CADM-140 protein as antigen, was used to test for anti-CADM-140 antibodies with a fairly high sensitivity and specificity [98]. The recombinant CADM-140 antigen might be suitable for the development of a commercial test.

p155/140

Function

The Tif1-γ, or p155/140 protein has been characterized as a member of the transcriptional intermediary factor 1 (Tif1) gene family [101–103]. Similar to the other Tif1 members, Tif1-α and Tif1-β, Tif1-γ contains several specific protein domains, including a RING finger, B-boxes, a coiled-coil domain, a PHD/TTC domain, and a bromodomain involved in protein–protein interactions. Several possible functions of the Tif1-γ protein have been described, including regulation of transcription and maintenance of tissue homeostasis [104, 105].

While the antigenic p155 protein (one of the two antigenic proteins) has been identified as Tif1-γ, the identity of the p140 protein remains elusive [103].

Potentially disease-related PTMs

No modifications have been associated with disease.

Antibodies to p155/140 and their clinical associations

Several studies indicated that anti-p155/140 antibodies appear to be associated with adult and juvenile DM (JDM) patients [106]. Up to 23% of DM patients seem to contain autoantibodies reactive with a 155/140-kDa protein doublet (Fig. 1). The positive adult DM patients often have more severe cutaneous involvement and an increased risk for malignancies (particularly adenocarcinomas) [106–109]. Follow-up studies confirmed the cancer-associated DM (C-ADM) subset specificity of the anti-p155/140 antibody [100]. The anti-p155/140 antibody is detected in JDM sera with a similar frequency as in adult DM, although the association with malignancy in JDM was not observed [110].

How to assay for anti-p155/140 antibodies

Anti-p155/140 was first identified using an immunoprecipitation procedure with C-ADM patient sera and radiolabelled K562 cell extracts and immunoblotting [106]. Recently, the immunoprecipitation of biotinylated recombinant Tif1-γ was used to evaluate the clinical features associated with anti-p155/140 in DM patients [100]. The availability of such recombinant proteins might encourage the development of a commercial diagnostic test.

Small ubiquitin-like modifier activating enzyme (SAE)

Function

Many enzymes are involved in post-translational protein modifications, and thereby regulate protein activity, subcellular localization, stability, and protein–protein interactions. These protein modifications can be a response to environmental triggers or depend on the cellular state. The SUMO protein family is structurally related to ubiquitin, and, like ubiquitin, can be post-translationally conjugated to other proteins by a similar mechanism. SUMO needs to be activated before post-translational conjugation can occur. This process is regulated by the SUMO-activating enzyme (E1) and involves the small ubiquitin-like modifier activating enzyme (SAE), which consists of a heterodimer of SAE1 and SAE2. Sumoylation is found to be involved in a variety of processes such as nuclear protein transport, DNA replication and repair, and cell division [111]. In addition, the number of substrates is increasing and emphasizes the diverse roles of SUMO modification in many cellular processes [112]. Although several SUMO family members have been shown to play role in the sumoylation of proteins, information about the consequences of sumoylation on the functions of these proteins is rather scarce.

Potentially disease-related PTMs

No disease-associated modifications have been documented.

Antibodies to SAE and their clinical associations

Betteridge et al. [113] reported a novel autoantibody in adult DM patients that immunoprecipitated two uncharacterized bands at 40 and 90 kDa. Further proteomic analysis identified the autoantigenic targets as small ubiquitin-like modifier activating enzyme (SAE) subunits. A large scale autoantibody screening by IP identified anti-SAE antibodies in 8.4% of DM patients. These results suggest that anti-SAE may identify a new serological IIM subset that present initially CADM and progress to develop IIM with a high frequency of systemic features except interstitial pneumonia.

How to assay for anti-SAE antibodies

Anti-SAE antibodies can be detected by immunoprecipitation and show a diffuse, coarse, speckled, nucleolar-sparing pattern in indirect immunofluorescence analysis [113, 114].

p140

Function

The NXP-2 protein is a recently identified nuclear matrix associated protein. Ectopically expressed NXP-2 mutants and structure prediction programs allowed the identification of several functional domains for NXP-2, including RNA-binding, nuclear matrix-binding, and coiled-coil domains [115]. Northern blot analysis showed NXP-2 to be expressed in specific tissues and at different levels. Moreover, RNA-binding and nuclear-binding capacities of NXP-2 suggest a similar role in the control of post-transcriptional processes as are described for other nuclear matrix proteins that contain similar domains [116]. Rosendorff and co-workers [117] reported a transcriptional repressor activity for the NXP-2 in vitro, which may be mediated by SUMO modification. These results suggest that NXP-2 may be involved in tissue-specific RNA metabolism and maintenance of nuclear structure rather than being a constitutive factor in nuclear functions.

Potentially disease-related PTMs

NXP-2 was recently described to be a candidate substrate for SUMO modification, which might result in the down-regulation of transcription [112, 117]. However, whether this results in the initiation of pathogenic mechanisms leading to immunological dysregulation is not known yet.

Antibodies to p140 and their clinical associations

Oddis et al. [118] described autoantibodies directed against a 140-kDa target (anti-MJ) in a cohort of juvenile IIM patients. Later, Gunawardena et al. [119] identified antibodies to a 140-kDa protein in 23% of juvenile IIM sera that form a distinct JDM subset. The p140 autoantigen targeted by these antibodies is different from the p155/140 antigen described above. Immunodepletion experiments with reference anti-p140-positive sera and anti-NXP-2 monoclonal antibodies suggested that the p140 protein is identical to the MJ autoantigen. Recent studies of Targoff's group [120] characterized the p140/MJ antigen and described the p140 protein as the nuclear matrix protein (NXP-2).

The presence of this anti-p140 antibody is associated with calcinosis, which defines a different clinical phenotypic JDM subset.

How to assay for anti-p140 antibodies

Radiolabelled immunoprecipitation assays have been used to identify the presence of this antibody activity [119].

Novel MSAs

Christopher-Stine et al. [121] recently described a novel autoantibody in a subset of individuals suffering from necrotizing myopathy, which previously were considered to be autoantibody negative. They studied 255 patients with myopathies and found 38 muscle biopsies with necrotizing myopathies. Twenty-six patients were diagnosed as autoantibody negative, whereas in the remaining 12 sera anti-SRP or anti-aaRS activities were detected. Screening of the 26 negative sera for putative novel autoantibody reactivities by immunoprecipitation resulted in the detection of two antigenic proteins with molecular weights of approximately 200 and 100 kDa in 62% of these patients. The evaluation of clinical data suggested that patients with anti-200/100 antibodies form a separate group of necrotizing myopathies that are associated with the exposure to statin medication and are responsive to immunosuppression. Further characterization of the anti-200/100 target might contribute to the understanding how this particular subset can develop.

Concluding remarks and perspectives

The characterization of MSA and the development of methods to detect these autoantibodies in patient sera for diagnostic purposes represent an active field of research. A series of novel MSA have been identified during the last decade and new technologies have been developed for their identification and detection, especially using multiplex assays. The application of multiplex assays will not only facilitate the simultaneous detection of multiple autoantibody reactivities, MSA as well as MAA, but may also allow the identification of autoantibody profiles that might have additional diagnostic or prognostic value.

Although the prevalence of MSAs in IIM patients is generally relatively low (<20%), for a number of them clinical associations have indeed been identified. Several reports indicate that MSAs are able to discriminate between distinct disease entities and/or can be correlated to disease severity, which suggests that MSAs might help define IIM subtypes and predict disease progression, and/or treatment. The number of MSA is steadily growing and the

diverse functions and subcellular localizations of the associated autoantigenic targets suggest selective mechanisms in the development of these autoantibodies. Whether autoantibodies directly contribute to the development and/or progression of IIM, however, remains elusive. All MSA that have been characterized up to now are associated with PM and/or DM. An intriguing question is whether MSA are also produced by patients with the third major type of IIM, IBM.

The detection of novel autoantibody targets in the different immune-mediated myopathies emphasizes the complexity of the mechanisms that define the development of these autoimmune diseases. Further studies that elucidate structure and function of autoantigenic targets will be essential in understanding the underlying pathological mechanisms in IIM.

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Conflict of interest None.

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