

Autoantibodies in Idiopathic Inflammatory Myopathies.

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Abstract

Autoimmune response to nuclear and cytoplasmic autoantigens is detected in about 60-80% of patients affected with idiopathic inflammatory myositis such as polymyositis (PM), dermatomyositis (DM) and inclusion body myositis (IBM). Some of the serum autoantibodies are shared with other autoimmune diseases (myositis-associated antibodies MAA) and some of them are unique to myositis (myositis-specific antibodies MSA). The MSA are found approximately in the 40% of PM and DM patients, whereas the MAA in the 20-50% of the patients. Myositis-specific or myositis-associated antibody detection in patients' sera is useful in diagnosis and classification of these diseases, which are sometimes difficult to early recognize and correctly classify. Late diagnosis and mistaken classification of myositis postpone treatments leading to worse prognosis. Here we summarize major biochemical and clinical characteristics of known MAA and MSA, discussing also their possible role in the pathogenesis of myositis.

Key words: Polymyositis; dermatomyositis; myositis-specific autoantibodies; myositis-associated autoantibodies; anti-RNA synthetase.

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Myositis or idiopathic inflammatory myopathies (IIM) are a heterogeneous group of systemic diseases classified within the group of connective tissue diseases (CTDs). The three major categories of IIM are: polymyositis (PM), dermatomyositis (DM) and inclusion body myositis (IBM). They are a diverse group of diseases with a variety of clinical manifestations, immunologic abnormalities and disease course. CTD-myositis overlap syndromes or myositis in association with cancer are also found. The prominent features of myositis are progressive proximal muscle weakness, raised serum muscle enzymes (creatinine kinase, aldolase and lactate dehydrogenase) and characteristic electromyographic abnormalities. Muscle biopsy shows inflammatory infiltrates, necrotic areas, fibrosis and atrophy. Regenerating fibres are also found [10, 18]. Besides muscle abnormalities, patients with myositis may have other clinical features: cutaneous rash, mainly in DM, arthralgia or arthritis, pulmonary, gastrointestinal, cardiac and renal disorders.

Autoimmune response to nuclear and cytoplasmic autoantigens is found in about 60-80% of these patients. Some of the serum autoantibodies are shared with other

autoimmune diseases (myositis-associated antibodies MAAs) and some of them are unique to myositis (myositis-specific antibodies MSAs). It is thought that the humoral response plays an important role in the development of these diseases because of the high serum levels of autoantibodies in PM and DM; whereas in IBM the immune response is secondary to degenerative changes in skeletal muscle tissue [5, 6].

Myositis is a relatively rare but increasingly recognized disease. The diagnosis is performed according to the Bohan and Peter's 1975 criteria [6]. Sometimes it is difficult to early recognize and correctly classify the different subsets of myositis because in the beginning the disease could be clinically undefined. Late diagnosis and mistaken classification of myositis postpone the start of treatments leading to worse prognosis. MSA or MAA detection in patients' sera is therefore useful in the diagnosis and classification of these diseases. However, due to the difficulties associated with their detection, these antibodies are often not tested for. It is worthy to note that they can distinguish between the primary or the CTD-myositis overlap from the paraneoplastic subset. This aspect is certainly essential in the early recognition of cancer.

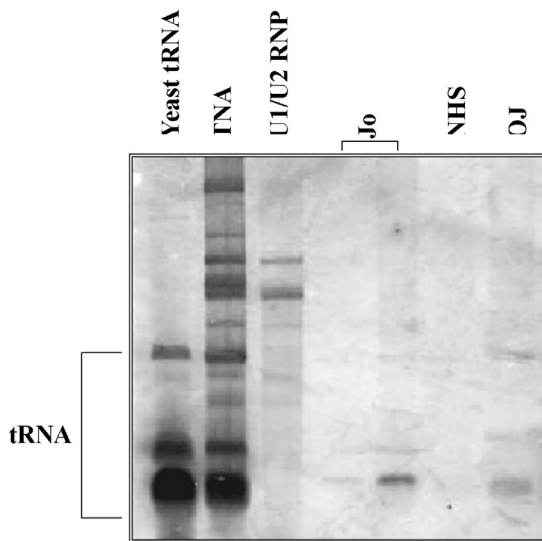


Figure 1: 8M urea-7% polyacrilamide gel electrophoresis (15x15 cm wide gel) of phenol-extracted immunoprecipitates from Jurkat total cell extract using Jo-1, OJ and U1/U1 RNP positive serum samples developed with Silver Stain. TNA=total nucleic acid NHS= normal human serum.

Myositis-Specific Antibodies (Msa)

The MSA are serological markers of disease. By means of standard techniques (for example immunofluorescence on HEp-2 cells) MSA can be found in about 40% of PM and DM patients. The identified MSA targets include three distinct groups of proteins: the aminoacyl-tRNA synthetases, the nuclear Mi-2 protein and components of the Signal Recognition Particle (SRP).

Anti-tRNA synthetase

The most common MSA that are found in approximately one third of the myositis patients, target the aminoacyl tRNA synthetases. These enzymes catalyse the ATP-dependent binding of one amino acid to its cognate tRNA during protein synthesis.

Most of the anti-tRNA synthetase antibodies are directed towards functional and highly conserved domains of the enzyme [19]. Up to now 6 out of 20 aminoacyl-tRNA synthetases have been described, but the most commonly detected one (20-30%) is the anti-histidyl-tRNA synthetase (Jo-1). Autoantibodies directed towards the other synthetases specific for alanine (anti-PL12), glycine (anti-EJ), isoleucine (anti-OJ), threonine (anti-PL7) and asparagine (anti-KS), have been reported in only a few patients (about 1%). Each synthetase is antigenically distinct from the others and antibodies directed against the different tRNA synthetases do not cross-react with the others. Moreover, individual patients have antibodies to only one tRNA synthetase; antibodies to more than one anti-

tRNA synthetase are very rare [12]. An important property of the anti-synthetase autoantibodies is that they inhibit the function of their target autoantigen in vitro [26].

The anti-tRNA synthetase antibodies are characteristically detected by indirect immunoprecipitation (IP) of the tRNA associated with the target of the antibodies, that is the antigenic tRNA synthetase. The immunoprecipitated tRNA forms a characteristic pattern after electrophoresis and subsequent Silver Staining of the gel (Fig. 1). Although the IP is a very specific technique, it has the sensitivity limit of the Silver Staining procedure ($>10\text{ng/mm}^2$ of RNA separated on gel). The metabolic labelling of the cell extract with radioisotopes prior to IP increases the sensitivity, but has also some difficulties.

The immunoblotting (IB) technique is specific and sensitive for the detection of the anti-Jo-1 antibodies (Fig. 2), but the other anti-tRNA synthetase antibodies are not detectable by IB. The Jo-1 antibody recognition is obviously mediated by conformational and linear epitopes, which are not damaged by the denaturation steps of the IB procedure.

The Jo-1 autoimmune response has been largely characterised both biologically and clinically. Anti-Jo-1 antibodies are found predominantly in PM patients and it has been observed that anti-Jo-1 positivity and titre values change during disease, and are related to therapy. The association of antibodies directed to the other tRNA synthetases with disease is still unknown, probably because they appear to be rare and because the IP technique necessary for their detection is not used in many laboratories.

Patients with anti-tRNA synthetase antibodies, including Jo-1, are affected by the so called "anti-synthetase syndrome" which is characterised by myositis, interstitial lung disease, arthritis, Raynaud's phenomenon and hand skin lesions (mechanic's hand).

Anti-Mi-2 antibodies

The anti-Mi-2 antibodies recognise a major protein of a nuclear complex formed by at least 7 proteins that is

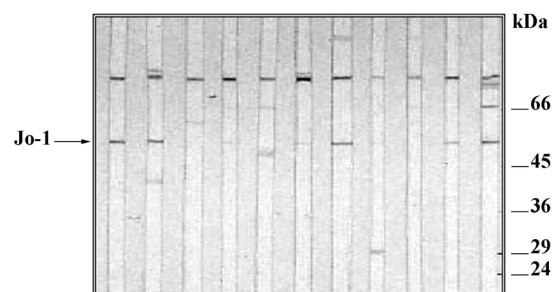


Figure 2: Western blot of 12.5% SDS-PAGE cytoplasmic Raji cell extract of different Jo-1 positive serum samples. Molecular weight markers are indicated in kilodaltons on the right.

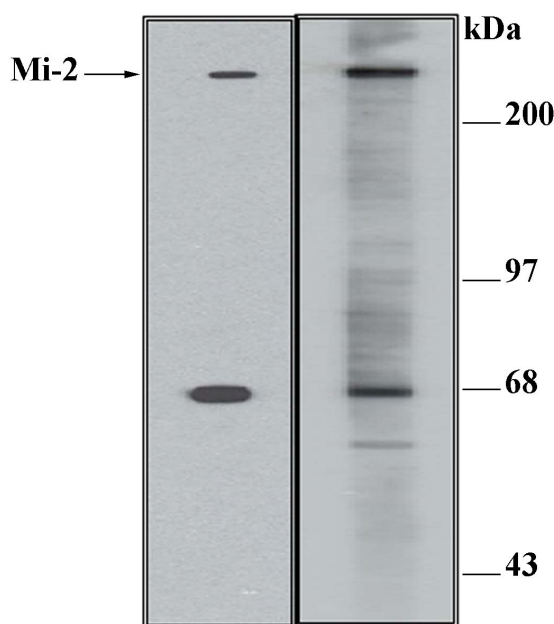


Figure 3: Western blot of 7% SDS-PAGE nuclear Raji cell extract of two Mi-2 positive serum samples. Molecular weight markers are indicated in kilodaltons on the right.

involved in the transcription process. The immunodominant antigen is a 240 kDa polypeptide [22]. Anti-Mi-2 antibodies can be detected either by IB (Fig. 3) or IP, since they recognise both conformational and linear epitopes. Autoantibodies recognising Mi-2 are considered specific serological markers of DM. They are detected in about 20% of the myositis patients and are associated with relatively acute onset, good prognosis and good response to therapy.

Anti-SRP antibodies

The anti-SRP antibodies are directed towards an RNA-protein complex that consists of 6 proteins and a 300 nucleotides RNA molecule (7SL RNA). This complex functions in the co-translational translocation of secretory and membrane proteins at the membrane of the endoplasmic reticulum [27]. The anti-SRP antibodies are detectable by both IB and IP which demonstrates that they recognise epitopes that are present on the native and denatured form of the proteins [25]. The anti-SRP antibodies precipitate the 7SL RNA that is specific for the antigen but does not bind it directly [18].

Patients with anti-SRP antibodies have very acute polymyositis with cardiac involvement, severe prognosis and poor response to therapy.

Myositis-Associated Antibodies (Maa)

The MAA are found in the 20-50% of the patients' sera; they are commonly encountered in other CTDs. The most important antigenic targets of the MAA are the PM/Scl nucleolar antigen, the nuclear Ku

antigen (p70/p80), the small nuclear ribonucleoproteins (snRNP) and the cytoplasmic ribonucleoproteins (RoRNP). The anti-PM/Scl autoantibodies are generally found in patients affected by myositis, scleroderma or polymyositis overlap with scleroderma [17], anti-Ku antibodies are found in patients with myositis overlap with other CTDs [11], antibodies directed against snRNP are frequently found in myositis patients and in patients with CTD overlap syndrome whereas antibodies towards Ro/SSA 60kDa, Ro/SSA52kDa and La/SSB proteins components of the RoRNP complex are almost exclusively found in patients affected by Sjögren syndrome and Systemic Lupus Erythematosus (SLE).

Role of the Autoantibodies in Pathogenesis of Myositis

The pathogenesis of myositis is still not known although it has been hypothesised (as in other CTDs), that in genetically susceptible individuals environmental factors (i.e. viral or bacterial infectious agents) may trigger the abnormal autoimmune response. It is believed that the humoral autoimmunity co-participates in the pathogenesis of these diseases given that autoantibodies are found in patients with PM and DM and in some cases seem to be associated with specific clinical features (anti-synthetase syndrome).

It is still not clear how autoimmune response arises as well as whether and by which effector mechanism these antibodies may induce the muscle tissue injury.

Several studies have been performed on modifications of autoantigens during apoptosis giving an alternative explanation for the generation of autoantibodies in autoimmune diseases [8, 9, 20, 28]. It has been shown that also some myositis-related autoantigens (i.e. Mi-2, Jo-1, OJ, PL12, Ku, PM/Scl) are specifically cleaved by Granzyme B during the cytotoxic T lymphocytes induced apoptosis [20].

Mice immunized with xenogeneic muscle homogenates or with rabbit myosin B (MB) fractions develop several symptoms similar to human PM and DM, such as inflammatory cell infiltration, extensive muscle cell necrosis and anti-MB antibody production. Mouse experimental auto-immune myositis (EAM) has been widely used for the study of PM and DM [16, 21]. The importance of developing a good animal model of an autoimmune disorder to define an effector mechanism of damage has been clearly demonstrated for several autoimmune diseases. Until now no EAM mice have been produced via immunisation, neither with nuclear and cytoplasmic antigens such as those recognised by the MSA, nor by muscle tissue injury induced by the injection of mouse monoclonal myositis specific autoantibodies.

Among the pathogenic aspects of myositis, the role of the humoral immunity is the less investigated field, but studies on autoantibody-mediated pathogenesis of the different CTDs are developing. For example, it has been

demonstrated that some marker autoantibodies of systemic connective tissue diseases, i.e. anti-dsDNA, anti-U1RNP and anti-ribosomal phosphoproteins (P proteins) [1-3, 13, 15], are able to bind and penetrate different cell lines in culture. The antibodies reach their antigenic targets with significant effects on the biochemical functions and viability of the cells. The antibody penetration can cause apoptosis [24] with the consequent releasing of intracellular materials. Such release could further induce the antigen-driven production of these antibodies leading to the epitope spreading autoimmune phenomenon. Alternatively, the apoptosis of regulatory T cells could amplify autoimmune response [3, 24].

These experimental findings suggest that some autoantibodies play an important role in the development and/or pathogenesis of the autoimmune disease, of which they are a specific serologic marker.

Perspectives

Up to now numerous studies have been published improving the knowledge of inflammatory muscle diseases, but some questions are still pending on the diagnostic and prognostic value of the myositis-specific and myositis-associated autoantibodies. Follow-up studies are needed to correlate these serological markers to main clinical manifestations, to activity and course of disease and to therapy. Moreover their potential pathogenic role in the induction of muscle tissue injury characteristic of myositis has not been yet investigated.

These studies will provide important contributions to understanding etiology and pathogenesis of idiopathic inflammatory myositis, suggesting new trends in the development of therapeutical approaches.

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