Clinical and Biochemical Characteristics of Autoantibody Systems in Polymyositis and Dermatomyositis

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POLYMYOSITIS (PM) and dermatomyositis (DM) are inflammatory muscle diseases of unknown etiology. There is strong evidence for cellular immune mechanisms of muscle damage in these disorders. In recent years, numerous autoantibodies have been identified in patients with myositis, but their role in producing this clinical syndrome is unknown. In this report, we summarize the current state of knowledge about myositis-related autoantibodies, including characterization of the antigens, epidemiology, clinical significance, and the clues these autoantibodies may provide concerning the etiology and pathogenesis of the disease.

ANTIBODIES TO MUSCLE PROTEINS

The search for autoantibodies in patients with PM started with attempts to detect antibodies against muscle fibers and individual muscle proteins. The initial studies, using indirect immunofluorescence with muscle as the substrate, found no differences among sera of patients with PM, other muscle diseases, and normals.[1] This may have been a consequence of the strong nonspecific affinity of immunoglobulins for skeletal muscle fibers.

Further investigations of antibodies to specific muscle components, particularly human myosin and myoglobin, have detected such antibodies in sera of patients with PM. Depending on the assay used, there is varying specificity for the disease. Antibodies to myosin were initially reported to be present in almost 50% of sera from patients with PM, but were also present in a similar percentage of sera from patients with other neuromuscular diseases and healthy controls.[2] Using more highly purified myosin, Wada et al have recently developed a radioimmunoassay for antibodies to myosin and have found such antibodies in 18 of 20 patients (90%) with PM. A high degree of specificity for this assay was claimed as antibodies were present in only six of 33 patients (18%) with other muscle diseases, and then in much lower titer. Antibodies to myosin were not detected in 43 patients with other neurologic or autoimmune diseases.[3]

Antibodies to myoglobin were reported by Nishikai and Homma to be present in seven of eleven patients with PM using a double immunodiffusion assay. None was detected in a total of 64 patients with rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), scleroderma, and in normals. However, patients with other myopathies were not studied as controls.[4] In a subsequent study, the investigators used a passive hemagglutination technique and detected antibodies to myoglobin in 71% of patients with PM. The antibody was not specific for PM as antibodies to myoglobin were also detected in patients with myasthenia gravis (31%), muscular dystrophy (10%), and in low titer in some patients with RA, SLE, and scleroderma.[5]

Antibodies to muscle proteins such as myosin and myoglobin are probably nonspecific reactions to muscle injury and subsequent release of these antigens. It is difficult to understand how antibodies to such internal muscle proteins could play a role in the initiation of muscle damage. The assays described above are not generally used for the diagnosis or observation of patients with PM.

ANTIBODIES TO NUCLEAR AND CYTOPLASMIC ANTIGENS

In recent years, the focus of study of autoantibodies in PM has shifted from muscle to subcellular particles. Some of the autoantibodies are detected by immunofluorescence testing and the

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Table 1. Biochemical Characteristics of Antigens Recognized by Myositis-Related Autoantibodies

Antigen	Location	Predominant ANA Pattern	Properties or Function	References
Ku	Nucleus	Reticular nuclear*	Protein, binds free ends of dsDNA	6, 7
Jo-1	Cytoplasm	Variable	Histidyl tRNA synthetase	30, 31
Mi-1	Nucleus	Weak rim† Particulate‡	Protein, shares antigenic determinants with bovine IgG	10
Mi-2	Nucleus	Homogeneous	Protein, unknown function	11
Pm-Scl	Nucleolus	Nucleolar	Protein, unknown function	12-15
nRNP	Nucleus	Speckled	Ribonucleoprotein containing U1 RNA	16

*On human liver substrate; negative on rat liver substrate.
†On mouse liver substrate.
‡On mouse spleen substrate.

remainder are identified as precipitin lines in double immunodiffusion assays against specified tissue extracts (Tables 1 and 2).

The introduction of human epithelial cells as a substrate for antinuclear antibody (ANA) assays has greatly enhanced the usefulness of this procedure to detect autoantibodies in PM. When rodent liver or kidney was used as the tissue substrate for indirect immunofluorescence, antinuclear antibodies were detected in about one third of patients with PM.[25,26] However, using HEp-2 cells (a human-derived cell line) as the substrate, 89 of 114 (78%) sera from patients with PM were positive. Both nuclear and cytoplasmic staining were observed. The most common pattern was fine speckled staining of the nucleus (61%), followed by homogeneous cytoplasmic staining (19%), and a nucleolar pattern (10%). Other patterns identified were centromere, small bright speckled, nuclear and cytoplasmic, nucleolar and centromere, and filamentous cytoplasmic. No single pattern on HEp-2 cells predominated among the sera that were negative on mouse kidney substrate.[25] There are no known correlations between the pattern of HEp-2 staining and the clinical syndrome.

Using a complement fixation assay, Reichlin and Mattioli in 1976 first detected an antibody to calf-thymus extract in the serum of a patient with DM.[27] Subsequent study of this prototype serum, Mi, using agar gel diffusion found at least two different precipitating antigens. One, designated Mi-1, was a protein with a molecular weight (mol wt) of 150,000 daltons that appeared to bear some antigenic relationship to bovine IgG although it was not an immunoglobulin. Antibodies to Mi-1 were detected in two of 19 patients with DM but in 0 of 39 patients with PM, 60 patients with other connective tissue or muscle diseases, or 18 healthy controls. Later studies of anti-Mi-1 found this antibody to be present in about 5% of sera from patients with SLE. These sera also contained other precipitating antibodies, predominantly Sm or nRNP. Anti-Mi-1 was not found in sera from patients with SLE which did not also contain other precipitating antibodies.[24] Anti-Mi-1 sera produced varying ANA patterns depending on the substrate used.[10]

The second antibody in Mi serum, Mi-2, is the complement fixing antibody originally described by Reichlin and Mattioli. Of the known autoantibodies, anti-Mi-2 is the most specific for DM, present by ELISA in 11 of 52 patients with DM,

Table 2. Clinical Characteristics of Myositis-Related Autoantibodies

Autoantibody	Prevalence in Myositis	Other Disease Associations	References
Ku	50% of PM-scleroderma overlap in Japanese patients	Present in 5% of American patients with SLE	6, 7, 17
Jo-1	30% of PM, <10% of DM, 50% to 100% of PM with interstitial lung disease	Rarely detected in interstitial lung disease without myositis	8, 18, 21-23, 29
Mi-1	3% of DM, not detected in PM	Present in 5% of SLE	10, 24
Mi-2	20% of DM, rare in PM	None known	11
Pm-Scl	8% of PM (half have PM-scleroderma overlap)	Rarely detected in scleroderma without myositis	12-14, 25
nRNP	4% to 15% of PM and DM	Common in SLE and overlap syndromes	18, 25

but in only two of 87 patients with PM. None of 93 patients with other connective tissue diseases or 35 healthy controls had significant titers of anti–Mi-2. ELISA proved to be more sensitive than immunodiffusion as immunodiffusion detected anti–Mi-2 only in patients with DM, and only in those patients with a strongly positive ELISA for Mi-2 antibodies. Studies using partially purified Mi-2 antigen showed it to be protein with major bands of 53,000 and 61,000 daltons by SDS–polyacrylamide gel electrophoresis. Anti–Mi-2 sera yields a homogeneous nuclear ANA pattern with either HEp-2 or mouse liver substrate.[11]

Antibodies to a different cellular antigen were identified in 1977 by Wolfe et al, who noted a line of precipitation in double immunodiffusion between calf thymus nuclear extract and sera of patients with myositis.[12] This antigen–antibody system, originally designated PM-1, was subsequently found to give multiple lines of precipitation. The predominant antigen has been named PM-Scl. Fifty percent to 66% of the patients with antibody to PM-Scl have an overlap syndrome of scleroderma and myositis; the remainder have only PM or DM. The antibody has also been rarely found in patients with scleroderma without myositis.[13,14] Antibodies to PM-Scl were identified in 8% of 168 myositis patients.[25] Longitudinal studies have shown that antibody status at time of diagnosis remains constant and does not change with disease activity.[12]

Attempts to characterize the PM-Scl antigen have shown it to be primarily nucleolar in location when examined by immunofluorescent techniques.[13] Further evidence for the nucleolar origin of PM-Scl is provided by a study that showed nucleolar extracts to be 10- to 50-fold enriched for PM-Scl antigen over whole calf thymus extract, whereas nucleoli-poor fractions did not demonstrate the antigen.[15] The PM-Scl antigen has been shown to be a heat sensitive, trypsin sensitive acidic protein and to be DNAse and RNAse resistant. It has not been completely purified and its function remains unknown.[12,14]

A second autoantibody found in patients with PM-scleroderma overlap is anti–Ku. Antibodies to Ku were originally described in nine of a series of 330 Japanese patients with connective tissue disease. Seven had a PM overlap syndrome (six PM-PSS, one PM-PSS-SLE), one had PSS, and one had SLE. Three of the nine patients also had anti–PM–Scl antibodies.[6] Anti–Ku has also been found in American patients, present in three of 60 with SLE.[17] Biochemical studies have shown Ku to be a protein that binds specifically to the free ends of double-stranded DNA.[7] By indirect immunofluorescent technqiues, the antigen has been localized to the nucleus but spares the nucleolus.[6]

At this time, antibodies to Mi-1, Mi-2, PM-Scl, and Ku are all laboratory findings without any known or hypothesized relationship to the etiology or pathogenesis of myositis. The low frequency of these autoantibodies in patients with PM and DM makes it unlikely that they play a significant role in the disease process, though their function in any individual patient is unknown. Further understanding of the significance of these autoantibodies may not be achieved until the target antigens are identified.

In distinction to these autoantibodies, another class that has been extensively investigated are those directed against nRNP. Such antibodies are usually associated with connective tissue diseases other than pure PM, including various overlap syndromes with features of SLE, scleroderma, and myositis. However, anti–nRNP has also been found in 4% to 15% of sera from patients with isolated PM and DM.[18,25] Antibodies to nRNP have been shown to react with the protein components of ribonucleoproteins containing U1 RNA.[16] Ribonucleoproteins containing U1 RNA appear to regulate the posttranscriptional processing of messenger RNA by ensuring the precise excision of unneeded segments from newly transcribed hetereogeneous nuclear RNA.[28] Anti–RNP antibodies have been shown to block this processing activity in cell free systems.[19] However, a relationship between the cellular defect and the clinical syndromes in which antibodies to nRNP are found has not yet been established. A different antibody which reacts with ribonucleoproteins of U2 RNA has recently been described in one patient with PM-scleroderma overlap.[20] The function of this ribonucleoprotein is currently unknown.

Antibody to Jo-1 was the first autoantibody identified with reactivity against a specific enzyme. Using double immunodiffusion with calf thymus extract, Nishikai and Reichlin demonstrated a strong precipitin line of common speci-

ficity in eight of 26 sera (30%) from patients with PM and in one of 22 sera (4%) from patients with DM. Of 102 patients with other connective tissue and muscle diseases, only one, a patient with PM-PSS overlap, had antibodies to Jo-1. The other ten patients with PM-PSS overlap were negative.[21] Subsequent studies have confirmed the prevalence of anti–Jo-1 in patients with PM and DM as 30% and <10%, respectively, and the absence of anti–Jo-1 in patients with other connective tissue diseases.[18,22] In addition to this relationship with the clinical presentation, the production of antibody to Jo-1 may be under genetic influence. Arnett et al reported HLA-DR3 present in seven of 11 patients with PM who were anti–Jo-1 positive (64%) but present in only eight of 36 patients with PM who were anti–Jo-1 negative (22%). No significant difference was found in the prevalence of HLA-DRw6 between these two groups, but all patients with Jo-1 antibodies had DR3, DRw6, or both.[18]

Examination of the clinical significance of antibody to Jo-1 has shown a strong association between the presence of this antibody and interstitial lung disease. This association was initially recognized by Yoshida et al, who reported finding antibodies to Jo-1 in 11 of 324 Japanese patients with connective tissue disease. Ten of the patients had PM (including two with overlap syndromes) and one had DM. All 11 patients had interstitial lung disease by chest x-ray. In contrast, interstitial lung disease was present in only 22% of the patients with PM or DM who had no detectable antibody to Jo-1. There was no relationship between the titer of antibody to Jo-1 and the severity of the interstitial lung disease, assessed either by chest x-ray or diffusion capacity. The presence of interstitial lung disease was the only difference detected between patients positive and those negative for antibody to Jo-1. There were no significant differences between these groups in age at diagnosis, sex, creatine kinase elevation, or response to corticosteroids.[22] In other studies, Bernstein et al reported finding 20 English patients with antibodies to Jo-1 after screening 1,000 sera. Thirteen of the patients had PM with interstitial lung disease. In two of the patients with PM, antibody to Jo-1 was present when the patients had lung disease alone, before myositis was diagnosed. Of the 20 patients, two had interstitial lung disease and sclerodactyly in the absence of myositis.[23] This association between antibody to Jo-1 and PM with interstitial lung disease had also been confirmed in American patients.[29] After the association was identified, it was reported that the American patient who provided the prototype serum did have PM with interstitial lung disease.[8]

Attempts to localize the Jo-1 antigen within the cell have failed to produce consistent results. About 33% of sera containing antibody to Jo-1 are ANA-positive using mouse kidney substrate, a prevalence similar to that found in sera from patients with PM lacking antibody to Jo-1. However, using HEp-2 cells as substrate, up to 66% of the sera with antibody to Jo-1 could be shown to have ANA activity. Staining was predominantly nuclear, although cytoplasmic and nucleolar patterns were also seen.[25] The reason for the nuclear localization is unknown as Jo-1 is believed to be a cytoplasmic enzyme. However, a preliminary investigation showed that antibody partially purified from two sera originally demonstrating nuclear fluorescence did give cytoplasmic fluorescence. The suggestion has been made that there may be a factor in whole serum that interferes with detection of Jo-1 antigen by indirect immunofluoresence.[9]

Extensive biochemical characterization of the Jo-1 antigen has been accomplished, yielding interesting and provocative results. Initial investigations identified both protein and transfer RNA (tRNA) in the immunoprecipitate formed between HeLa cell extracts and serum containing antibody to Jo-1. Further studies by Matthews and Bernstein showed that sera containing antibody to Jo-1 could block the charging of histidine to its tRNA. They therefore postulated that Jo-1 could be a histidyl-tRNA synthetase.[30] This was confirmed by Yang et al, who purified histidyl-tRNA synthetase from rat liver and showed it to be identical to the Jo-1 antigen.[31]

Since these studies, other tRNA-related autoantibodies have been detected in a small number of sera from patients with myositis. An autoantibody originally called PL-7 and directed against threonyl-tRNA[32] was found in sera of five patients, four of whom had myositis. A similar autoantibody, which precipitated a ribonucleoprotein complex containing threonyl-tRNA and an unknown small RNA molecule,

was reported in the sera of one patient with PM,[33] and yet a third antibody, directed against both alanyl-tRNA synthetase and deproteinized tRNA, has been recently described.[34]

As with the previously described autoantibodies, no evidence directly linking these antibodies to the pathogenesis of muscle disease has been presented. Rather, the antibodies may serve as a clue to the etiology of PM. Identification of Jo-1 and other tRNA-synthetase related antibodies has furthered speculation that PM is initiated by a viral infection. This association is based on two related facts. First, some of the RNA viruses of the enterovirus group have been shown to mimic small tRNAs in their ability to be charged with specific amino acids.[35,36] Secondly, at least some cases of PM have been thought to have a viral etiology, and enteroviruses have been implicated in a range of muscle disorders. Coxsackieviruses, in particular, are known to produce both acute (Bornholm disease) as well as chronic (myocarditis) muscle disease.

On the basis of these data, Matthews and Bernstein have hypothesized that antibodies to tRNA synthetase seen in patients with PM may result from the binding of the charging enzyme complex to viral RNA, rendering the host enzyme immunogenic.[30] Thus, once a chronic inflammatory muscle disease has been established, the antibodies that have developed to the patient's own charging enzymes may be the only evidence remaining of a prior viral infection.

It is not known if these or any of the other autoantibodies found in patients with PM play a role in perpetuating the muscle inflammation or are merely epiphenomena. The variety of autoantibodies found in PM and DM serve to emphasize the heterogeneity of these syndromes. In the case of antibody to Jo-1, it is likely that its presence reflects the occurrence of a prior viral infection that may have acted to initiate the immunopathologic process observed in patients with PM, and thus may serve as a way of defining a particular clinical subset. Study of this more homogeneous group of patients may then lead to further understanding of both the etiology and pathogenesis of the inflammatory muscle diseases.

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