MINIREVIEW

Laboratory Evaluation of the Inflammatory Myopathies

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INTRODUCTION

The idiopathic inflammatory myopathies (IIM) are a heterogeneous group of disorders characterized by muscle weakness resulting from chronic muscle inflammation of unknown cause. The etiology and pathogenesis of these diseases are poorly understood, but current data suggest that they may develop in genetically susceptible individuals after exposure to environmental agents that induce immune activation and chronic inflammation (70, 113, 116, 142). Patients with these disorders often develop systemic manifestations, including cutaneous, cardiac, pulmonary, and gastrointestinal involvement, which adversely affect the prognosis (69, 116). Treatments for myositis have been poorly studied, but corticosteroids and other immunosuppressive agents, such as methotrexate and azathioprine, are the mainstays of therapy (98, 100). The IIM are a rare group of disorders, with an incidence of about 5 to 10 cases per 10⁶ people per year and a bimodal age distribution, with the first peak occurring in childhood between the ages of 10 and 14 years and a larger peak occurring in adulthood between 45 and 64 years of age (75).

The IIM include the distinct clinicopathologic entities of polymyositis (PM), dermatomyositis (DM), cancer-associated myositis, juvenile DM (JDM), and myositis overlap syndromes (myositis in association with other connective tissue diseases) (13, 14); other rare forms of myositis, such as inclusion body, granulomatous, eosinophilic, ocular, and focal myositis, have also been described (77). The IIM can usually be distinguished from dystrophic, metabolic, toxic, and endocrine myopathies by the diagnostic criteria proposed by Bohan and Peter (13, 14). These criteria include proximal, symmetric muscle weakness; elevation in the serum of the levels of enzymes derived from skeletal muscle; myopathic changes on electromyography, including increased amplitude and duration of fibrillation potentials; muscle biopsy showing myofiber regeneration and degeneration accompanied by perivascular or endomysial lymphocytic infiltrates or perifascicular atrophy; and in the case of DM, the presence of the heliotrope rash or Gottron's papules (for a review, see reference 117). Because the IIM frequently begin with nonspecific symptoms, such as fatigue, arthralgias, and myalgias which may mimic other disorders, the establishment of a specific diagnosis and the initiation of treatment can be delayed, resulting in a poorer prognosis (33, 54). In the evaluation of these patients, the laboratory plays an important role in establishing a diagnosis, in providing a basis for dividing patients into relatively homogeneous subsets, and in contrib-

uting objective measures to supplement clinical information. This information provided by laboratory analysis, in combination with clinical assessment of disease activity, is used as the basis for therapeutic decisions over the disease course.

In this regard, over the past decade, several research techniques have emerged as promising tools for evaluating disease activity or defining subsets of patients with IIM. Magnetic resonance imaging of proximal muscles, using T2-fat suppression or short tau inversion recovery sequences, provides an anatomic view of the involved muscles and demonstrates inflammation which correlates with other measures of disease activity (35, 46, 111). Magnetic resonance imaging may be more sensitive than measurement of the levels of creatine kinase (CK) in serum in assessing disease activity in patients with myositis. Magnetic resonance spectroscopy can estimate the levels of P_i, phosphocreatine, and ATP in tissues. Data suggest that the levels of these compounds are abnormal in inflamed muscles and return toward normal with therapy (90, 110, 111). Finally, certain polymorphic genetic loci governing immune responses, particularly human leukocyte antigens (HLAs), appear to be risk factors for the development of myositis. These alleles include HLA-DRB1 *0301 and HLA-DQA1 *0501, but they may differ in the clinical and serologic IIM groups discussed below (3, 36, 69, 76, 122). The clinical utilities of these research and immunogenetic studies in assessing disease activity or in defining more homogeneous subsets of patients are unknown and will require further study. This minireview is therefore limited to the clinical chemistry and immunology laboratory findings that can be used to enhance the diagnosis and evaluation of patients with myositis.

LABORATORY ABNORMALITIES IN THE IIM

A number of clinical laboratory abnormalities may be encountered in the evaluation of patients with myositis, and some of these can lead to misdiagnoses (115, 133). Table 1 summarizes the common clinical laboratory abnormalities found in the routine evaluation of patients with IIM. The enzymatic activity of circulating CK is the laboratory parameter most widely used to follow patients with DM or PM because of its high degree of sensitivity, relative muscle specificity, and good correlation with disease activity and muscle strength (139, 140, 154). Nevertheless, serum CK levels alone can never substitute for a thorough evaluation of the patient, which includes muscle strength and functional assessments, as part of the measurements of myositis disease activity. At the onset of illness, serum CK activity may be elevated as much as 10 to 100 times above the upper limit of normal. Most investigators agree that CK levels are elevated in over 95% of patients with DM or PM at presentation with the illness or during the course of disease, making it the most frequently abnormal sarcoplasmic enzyme in the sera of patients with myositis (15, 49). Although only a

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TABLE 1. Frequent laboratory abnormalities in patients with myositis

Laboratory test	Comments
Muscle-associated enzymes (CK, AST, ALT, LD, aldolase)	A nonspecific guide to the degree of myositis. Generally useful for assessing the degree of myositis, but may not always correlate with disease activity. May be normal in 5 to 10% of patients with IIM at the onset of myositis, and may become normal later, despite active disease, because of loss of muscle mass or circulating inhibitors of enzyme activity. Rises may predate clinical flares of disease by weeks to months, and conversely, decreases may precede clinical responses to therapy by a similar time period. Levels also relate to muscle mass, so are higher in men than in women. Elevation of transaminases often result in the misdiagnosis of non-A, non-B hepatitis in patients with myositis.
CK MB fraction	A correlate of disease activity and a common source of misdiagnosis of myocardial infarction. Probably arises from myoblasts rather than from cardiac muscle in most patients with myositis.
Creatinuria	Increased urinary excretion of creatine indicates muscle damage. Because of variability in the daily excretion of creatine, a minimum of two 24-h urine collections are required.
Creatinine	Decreased levels in serum often reflect long-standing myositis and muscle atrophy.
Myoglobin in serum or urine	May correlate with muscle inflammation better than muscle enzymes and may predict clinical relapse. Because of diurnal variation, myoglobin levels should be measured at the same time of day.
Heme-positive urine without erythrocytes	Usually indicates myoglobinuria in patients with myositis.
Leukocyte and platelet counts	Elevated values sometimes seen with active myositis.
Immunoglobulins	Hypergammaglobulinemia seen in about 10% of patients with IIM in which the level correlates with myositis activity. Rarely hypo- or agammaglobulinemia is seen with a myositis-like illness and echoviral infection. Elevated IgE levels seen in some juvenile dermatomyositis patients.
Erythrocyte sedimentation rate	Elevated in 50% of patients with IIM. Does not correlate with disease activity.
Factor VIII-related antigen	Elevated in patients with active JDM and correlates with disease activity, but elevation occurs in only a subset of patients.
Neopterin	Correlates with disease activity in patients with JDM.
ANA	Seen in 60 to 90% of patients with IIM and a good discriminator of IIM from other forms of myopathy, in which ANAs are much less frequent. Cytoplasmic staining in a diffuse pattern suggests the presence of another autoantibody.
Rheumatoid factor	Seen in $\leq 8\%$ of patients with IIM. Causes a misdiagnosis of rheumatoid arthritis.
Extractable nuclear antibodies SSA/Ro, SSB/La, and Sm	Seen in $\leq 20\%$ of patients with IIM.

small percentage of patients have normal CK levels at the time of presentation, before the initiation of treatment, our experience in evaluating more than 300 adult patients with IIM suggests that a higher proportion of patients with DM and inclusion body myositis than those with PM and overlap myositis have normal CK levels.

The levels of CK and other muscle-derived enzymes in serum are generally useful in following myositis activity and responses to therapy, although the magnitude of the elevation does not always correlate with global disease activity (139). This is partly because the enzyme levels usually decrease 3 to 8 weeks before improvement in muscle strength and rise 5 to 6 weeks before clinical relapse (99) and because enzyme levels do not reflect the degree of cutaneous, pulmonary, gastrointestinal, articular, and other nonmuscular involvement in the IIM. The presence of normal CK levels in the face of active disease, demonstrated by muscle weakness and accompanied by inflammation on muscle biopsy or magnetic resonance imaging, may be related to the suppression of CK release by corticosteroids (47), the presence of inhibitors of CK enzyme activity in serum (58), or the presence of extensive muscle atrophy because of chronic disease (140). Reports of a normal serum CK level as an indicator of a poor prognosis in patients with DM (37) have been not been substantiated (34, 42) or found to be the case in our experience. Because muscle mass determines resting CK values, men tend to have higher baseline levels than women. Black individuals also have higher CK levels than Caucasian or Hispanic individuals (12, 162). In patients with systemic lupus erythematosus (SLE), rheumatoid arthritis, and other connective tissue diseases, the CK level is

frequently depressed below normal levels (132, 157), and therefore, a normal CK level in these patients may indicate active myositis. Laboratories also differ in their CK assays, normal ranges, and assay reproducibilities. Thus, the serum CK level should always be interpreted in the context of prior levels in an individual patient, which have been measured by a single laboratory.

The elevation in serum CK levels in patients with myositis is usually due to increases in the MM isoenzyme fraction which is released from skeletal muscle, but an elevation in the MB isoenzyme, normally found in myocardium, may occur as an indicator of skeletal muscle regeneration (68, 130). MB isoenzyme elevation may also be seen with the myocarditis associated with IIM, although several reports have found no correlation between MB fraction increases and myocardial involvement (49, 63, 120). Elevation in the ratio of CK-MB to total CK, which is higher in patients with myocardial infarction than in patients with myositis (30), may be helpful in distinguishing these two entities. Persistent elevation of MB levels is commonly seen in patients with skeletal muscle damage but not in patients with myocardial infarction (59, 153). The levels of macro-CK type 1, a complex of a CK isoenzyme and immunoglobulin, are elevated in patients with myositis in comparison with the levels in patients with heart disease, infections, malignancies, and other disorders (64), although the correlation of macro-CK levels with disease activity remains to be determined.

The levels of aldolase, lactate dehydrogenase (LD), and transaminases (both serum glutamic oxalacetic transaminase [SGOT]/aspartate aminotransferase [AST] and serum glu-

tamic pyruvic transaminase [SGPT]/alanine aminotransferase [ALT]) in serum are also increased in patients with IIM during active muscle disease, but elevations in these enzyme activities occur less frequently and to a smaller extent than CK level elevation (15, 49). LD isoenzyme 5 is associated with skeletal muscle (130), but elevations in the levels of LD isoenzymes 1, 2, and 3 may occur with chronic myositis (88, 120). Carbonic anhydrase III, located in type I muscle fibers, is also frequently elevated in patients with IIM and other diseases associated with skeletal muscle damage (106, 137).

Most clinicians agree that the serial evaluation of muscle enzymes is valuable in following the disease activity in patients with IIM, although studies have not validated this point (99). Our experience suggests that the serial enzyme levels follow a pattern, but not a time course, similar to that seen with myocardial infarction (112). At disease onset, the degree of elevation of serum CK activity is greater than the magnitude of elevation of the transaminases, and transaminase levels elevate to a greater extent than those of LD or aldolase do. In patients who respond to therapy, serum CK levels decline over a 2- to 8-week period (99); this is followed by a decline in the levels of the transaminases and a subsequent decline in LD and aldolase levels. Therefore, the serial evaluation of serum aldolase activity is frequently useful in following the disease activity in patients later in their illness (108).

Twenty-four-hour urinary creatine excretion, which reflects muscle mass and damage, is elevated in adult patients with myositis and other muscle diseases (113, 131, 155). In pediatric patients, creatine excretion in urine varies inversely with age (22). Some studies suggest that creatinuria is a more sensitive indicator of active myositis than CK levels (131, 155). Creatinuria may be used to monitor disease activity in patients with chronic disease when serum muscle enzyme levels are normal, although muscle atrophy will result in a persistently elevated level of urine creatine (113). Serum creatinine may also be decreased in the presence of chronic active myositis because of the loss of muscle mass (78).

In patients with active IIM, myoglobinemia is present more frequently than an elevation in CK levels (57, 71, 94). Serum myoglobin levels may correlate with muscle strength better than serum muscle enzyme levels, and myoglobin levels may return to normal before enzyme levels do (6, 61). Myoglobinemia can be predictive of clinical relapse in some patients before rises in serum muscle enzyme values or the loss of muscle strength (17, 56). Because of the diurnal variation in myoglobin levels, with a peak concentration occurring between 3 a.m. and 9 a.m., serial levels should be obtained at a constant time of day (16). Heme-positive urine or isolated proteinuria by dipstick in the absence of microscopic erythrocytes is usually indicative of myoglobinuria (55, 57, 66). Rhabdomyolysis leading to acute renal failure is extremely rare in patients with IIM (38), probably because the rate of development and degree of myolysis are lower in patients with IIM than in patients with myotoxic exposures.

An elevation in serum muscle enzyme levels and an alteration in related clinical chemistry laboratories may be due to other conditions which should be considered in the differential diagnosis of the IIM. Some 60 to 80% of patients with hypothyroidism develop mild elevations in serum muscle enzyme levels, although CK levels may be elevated as much as 160 times above normal (41, 89). Urinary creatine excretion, however, is normal in patients with hypothyroidism (118, 130). Excessive exercise may induce an elevation in CK and other muscle enzymes as much as 18 times above normal as well as an increase in the CK-MB fraction and myoglobinuria (91, 95, 160). The degree of CK elevation is related to the duration and

severity of exercise; maximal CK elevation occurs 8 to 16 h after exercise, and CK levels return to normal after 48 h (130). Serum CK level elevations may also occur in patients with myocardial infarction, patients who have received intramuscular injections or other trauma, patients with central nervous system diseases or congenital, metabolic, and other myopathies, as well as after exposure to certain drugs and toxins (68, 70, 130). Serum aldolase levels may be abnormally high in patients with hepatic disease or erythrocyte hemolysis (39, 72). In patients with myositis, elevated serum transaminase levels in the absence of a CK determination may be mistakenly attributed to hepatic disease rather than underlying muscle inflammation (45). Steroid myopathy, in which the proximal muscle weakness occurs in the pelvic girdle muscles more than the shoulder girdle or neck flexor muscles, may be differentiated from the IIM by the presence of an elevated urinary creatine excretion with simultaneously normal serum muscle enzyme levels (5).

Laboratory tests other than those relating to muscle damage can be useful in assessing myositis disease activity. In our experience, peripheral leukocyte counts rise during active myositis in many patients who have the antisynthetase antibodies (discussed below), and platelet counts also correlate with disease activity in many adult patients with DM or PM. Patients with active myositis also frequently develop the euthyroid sick syndrome, as characterized by elevated levels of reverse triiodothyronine (T3) in serum and decreased free T3 levels in serum, which can be useful in following patients with chronic disease who have no elevation in serum muscle enzyme levels. Care must be taken, however, to measure thyroid functions consistently in relation to steroid dosing, because glucocorticoids decrease the synthesis of thyroid-binding globulin, inhibit thyroid-stimulating hormone (TSH) secretion, and reduce the conversion of T4 to T3, leading to a decreased free T3 level (20). Serum osteocalcin levels have also been shown to correlate with disease activity in patients with JDM (121).

IMMUNOLOGIC ABNORMALITIES IN THE IIM

The immunologic and inflammatory indices available in the clinical laboratory are of variable utility in following the disease course of patients with IIM. The Westergren sedimentation rate has been reported to be elevated in approximately 50% of patients with DM or PM (28, 29), although in our experience a smaller proportion of patients develops an elevated sedimentation rate. The degree of elevation also does not correlate with disease activity or response to therapy (15). C-reactive protein levels are normal in most patients with IIM, including those with active disease (44), and thus an elevated level may be more indicative of an underlying infection or other inflammatory condition.

Immunopathologic studies have demonstrated the deposition of C3, immunoglobulin (Ig), and the membrane attack complex in the microvasculature of muscle tissue from patients with DM and JDM (60, 158). Evidence of complement activation, with elevated levels of C3d, has been reported in patients with active JDM (134). Serum IgG levels are elevated in some adult patients with DM or PM and patients with JDM with active disease (67, 96). A dermatomyositis-like illness has also been observed in children with congenital hypo- or agammaglobulinemia and usually accompanies disseminated or central nervous system echovirus infections (8, 159). Elevated serum IgE levels have been observed in some patients with JDM, but not in adult patients with DM, particularly in those with intractable skin disease, atopy, or calcinosis (52, 87, 105).

Circulating immune complexes have been reported (9, 107) but are of unknown clinical utility.

Markers of endothelial cell damage and mononuclear cell activation appear to correlate with disease activity and may reflect the underlying vasculopathy and cellular immune activation which contribute to the pathogenesis of some forms of myositis (25, 31, 32). Levels in plasma of von Willebrand factor VIII-related antigen, an indicator of vascular endothelial injury, are elevated in patients with JDM and clinically active disease (18, 134). The levels of factor VIII-related antigen are most commonly elevated at disease onset, but the levels are not increased in all patients (43, 108). The levels of thrombomodulin, another marker of endothelial damage in plasma, have been reported to be increased in adult patients with active DM or PM (103). The levels of neopterin, a marker of activated mononuclear cell responses in serum, correlate with disease activity in patients with JDM (27).

Additional markers of immune system activation, most of which are available only in the research immunology laboratory, have been studied for their utility in monitoring disease activity in patients with IIM. Immunohistochemical and molecular studies of the types of mononuclear cells present in the muscles of patients with IIM suggest that DM may be primarily humorally mediated, whereas PM is more cellularly mediated (2, 32, 97). Consistent with these findings, patients with DM and active disease have significantly higher proportions of circulating CD20⁺ DR⁺ (activated B) lymphocytes, whereas patients with PM have higher levels of peripheral CD3⁺ DR⁺ (activated T) lymphocytes (51, 79, 96). Additionally, patients with DM or PM and active disease have abnormally elevated proportions of peripheral blood mononuclear cells expressing interleukin-2 (IL-2) receptors as well as the late activation markers CD26 and TLIISA-1 (79). Adult patients with active disease also develop higher levels of soluble CD8 and soluble IL-2 receptors in serum than patients with inactive disease and healthy controls (80, 151, 152). The levels of cytokines IL-1 α and IL-2 are elevated in patients with active PM (161). Elevation of these activation markers precedes CK level increases and clinical flares of disease activity by 1 to 2 months, and they decline rapidly with therapy.

Antinuclear autoantibodies (ANAs) are seen in 60 to 90% of patients with IIM, depending on the group of patients studied, and can be helpful in distinguishing the inflammatory myopathies from some other forms of muscle disease (86, 109, 116, 126). The frequency of positive ANAs rises with age in healthy individuals, however, and some patients without myositis who have malignancies, infections, or other autoimmune processes have a positive ANA reaction. Indirect immunofluorescence on HEp-2 cells is also useful for detecting the autoantibodies specifically found in patients with myositis, known as the myositis-specific autoantibodies (MSAs); MSAs often show diffuse cytoplasmic staining and occasionally demonstrate nuclear staining (141). Autoantibodies to extractable nuclear antigens (ENAs), detected by Ouchterlony double immunodiffusion, including Ro/SSA, La/SSB, Sm, and U1-RNP, may be present in as many as 20% of patients with IIM, and the frequencies of these differ depending upon the IIM clinical group (69). Rheumatoid factor is seen at a high titer in 5 to 10% of patients with IIM and may lead to a misdiagnosis of rheumatoid arthritis (69). Antithyroglobulin and antimicrosomal antibodies are present in <10% of patients with IIM and are not specifically present in a particular subset (69). Other autoantibodies, including antibodies to myosin and myoglobin (93, 156), endothelial cells (21, 138), and a 56-kDa antigen (1, 85), do not appear to define distinct groups of patients, are not

specific for myositis, and may only be epiphenomena secondary to muscle damage from a variety of causes.

AUTOANTIBODIES ASSOCIATED WITH IIM

As mentioned above, patients with IIM have a variety of autoantibodies with various clinical utilities. One group of these autoantibodies, which are found only in patients with myositis and are known as the MSAs, are primarily directed against proteins or the RNAs involved in the process of protein synthesis, referred to as translation. MSAs have proven to be particularly useful for research and clinical purposes, partly because a given patient has only one MSA and each MSA appears to define a relatively homogeneous group of patients which share similar epidemiologic aspects, clinical features, immunogenetics, and responses to therapy (Table 2) (54, 65, 69, 76, 142). MSAs are present in 25 to 40% of adult patients with IIM, depending on the series, and are beginning to be recognized in children with IIM as well (73, 128). Circulating levels of some MSAs appear to correlate with myositis disease activity, but there is no direct evidence that MSAs have any pathogenic role (81). MSAs can be grouped on the basis of the target autoantigen: autoantibodies to aminoacyl-tRNA synthetases (antisynthetases), antibodies to other cytoplasmic autoantigens (for example, anti-signal recognition particle), and autoantibodies to nuclear antigens (anti-Mi-2).

Antisynthetase autoantibodies are directed toward cytoplasmic enzymes that catalyze the esterification of a particular amino acid to its cognate tRNA. In some patients, best documented in those with anti-alanyl-tRNA synthetase autoantibodies (19), autoantibodies that are also directed against the tRNA as well as present in serum. Anti-histidyl-tRNA synthetase autoantibodies (anti-Jo-1) are the most common MSA, but autoantibodies reacting with synthetases for alanine (PL-12) (143), threonine (PL-7) (145), glycine (EJ) (150), and isoleucine (OJ) (149) also exist. Patients with IIM and antisynthetase autoantibodies share common clinical features, including interstitial lung disease and pulmonary fibrosis (in 50 to 100% of patients, depending on the series) (10, 50, 69, 73); deforming, nonerosive symmetric small joint arthritis superficially resembling rheumatoid arthritis (60 to 100%) (101), Raynaud's phenomenon (60 to 93%) (69, 73), fever (87%), and a roughening and cracking of the lateral and palmar aspects of the fingers known as mechanic's hands (71%) (69). Rarely, antisynthetase autoantibodies may occur in patients without clinical myositis who have other clinical features of an antisynthetase syndrome, such as interstitial lung disease or arthritis (19, 143). Anti-Jo-1 autoantibodies are more common in patients with PM, but the other antisynthetases are more frequent in patients with DM (69). Patients with antisynthetase autoantibodies tend to develop increased disease activity as therapy for myositis is withdrawn and have a relatively poor prognosis because of complications related to their pulmonary disease (54, 69).

Autoantibodies to the signal recognition particle (SRP) are directed against one or more of the core proteins of the SRP, a ribonucleoprotein (RNP) complex that mediates the targeting of newly synthesized polypeptides to the endoplasmic reticulum (104, 124, 147). Patients with anti-SRP autoantibodies all have PM, in which the myositis tends to be severe and acute in onset, cardiac involvement is common, the disease is usually resistant to steroid therapy, requiring the addition of cytotoxic agents, and mortality is relatively high (69, 147).

Anti-Mi-2 autoantibodies immunoprecipitate a complex of seven nuclear proteins, including a 240-kDa protein that may

TABLE 2. Autoantibodies in patients with myositis^a

Autoantibody and name	Antigen	Means of detection	Frequency (%)	HLA associations	Clinical associations
MSAs					
Antisynthetases					
Jo-1	Histidyl-tRNA synthetase	IIF (cytopl) ID, IPP, WB, EIA, AAI	18–20	DR3, DRw52, DQA1 *0501	PM > DM; antisynthetase syndrome: interstitial lung disease, Raynaud's, fevers, arthritis, mechanic's hands
PL-7	Threonyl-tRNA synthetase	IIF (cytopl) ID, IPP, AAI	≤3	DRw52	Antisynthetase syndrome
PL-12	Alanyl-tRNA synthetase + tRNA ^{Ala}	IIF (cytopl), IPP, AAI	≤3	DRw52	Antisynthetase syndrome
EJ	Glycyl-tRNA synthetase	IIF (cytopl), IPP, WB, AAI	≤2	DRw52	DM > PM; antisynthetase syndrome
OJ	Isoleucyl-tRNA synthetase	IIF (cytopl), IPP, AAI	≤2	DRw52	Antisynthetase syndrome
Other MSAs					
SRP	Signal recognition particle proteins	IIF (cytopl), IPP, WB	≤5	DR5, DRw52	PM; severe myositis, acute onset, frequent cardiac involvement, resistant to treatment
Mi-2	Unidentified nuclear protein	ANA (nuclear), ID, IPP	≤8	DR7, DRw53	DM; V- and shawl-sign rashes, cuticular overgrowth, good prognosis
MAAs					
PM-Scl	Nucleolar protein complex	ANA (nuclear, nucleolar), ID, IPP, WB	≤8	DR3	IIM; scleroderma and overlap syndromes
Ku	DNA-binding proteins	ANA (nuclear); ID, IPP, WB	1	?	DM; SLE, Sjogren's syndrome, pulmonary hypertension, overlap syndromes
U1-RNP	U1 small nuclear RNP	ANA (nuclear speckled), ID, IPP, WB, EIA	12	?	IIM; scleroderma, SLE, and overlap syndromes
U2-RNP	U2 small nuclear RNP	ANA (nuclear speckled), IPP, WB	<3	?	IIM; scleroderma
Autoantibodies with uncertain clinical specificity					
KJ	Unidentified translation factor	IIF (cytopl) ID, WB	<1	?	PM; interstitial lung disease, Raynaud's
Fer	Elongation factor 1 α	IIF (cytopl), IPP	<1	?	?
Mas	tRNA ^{Ser} and ? related protein	IIF (cytopl), IPP	1	?	Alcoholic rhabdomyolysis, chronic hepatitis

^a Abbreviations: IIF, indirect immunofluorescence on HEP-2 cells; ID, double immunodiffusion; ANA, antinuclear autoantibody; IPP, immunoprecipitation; WB, Western blot (immunoblot); EIA, enzyme immunoassay; AAI, aminoacylation inhibition test for antisynthetase enzyme activity; cytopl, cytoplasmic. Adapted from reference 142 with permission from the publisher.

be the target autoantigen, whose functions are unknown (92). Anti-Mi-2 autoantibodies have been observed exclusively in patients with DM, and as many as 15 to 20% of adults with DM have anti-Mi-2 autoantibodies. These patients typically have "V"-sign (rash occurring over the anterior neck) and "shawl"-sign (rash occurring over the upper back and arms) rashes, as well as cuticular overgrowth, and tend to respond well to treatment (69).

Additional autoantibodies, known as myositis-associated autoantibodies (MAAs) are found frequently, but not exclusively, in patients with myositis and may also have a role in identifying groups of patients who share common clinical and genetic features. Included in this group are autoantibodies to U1-RNP, U2-RNP (23), PM/Scl (127), and Ku (123, 125). Patients with anti-PM-Scl or anti-Ku autoantibodies do not have any of the known MSAs, whereas patients with anti-U1-

RNP autoantibodies may additionally have an MSA (139a). Anti-U1-RNP autoantibodies react with the 70-kDa RNP, A and C polypeptides, as well as U1-RNA (23), whereas anti-U2-RNP reacts with U2-RNP, six low-molecular-weight proteins, and U2-RNA (24, 84). Anti-U1-RNP autoantibodies are found in 30 to 40% of patients with SLE who also have antibodies to Sm (4), whereas high-titer antibodies to U1-RNP in the absence of anti-Sm are found in patients who have mixed connective tissue disease (135, 136), an overlap syndrome that shares features of SLE, PM and DM, and systemic sclerosis.

Anti-PM-Scl autoantibodies immunoprecipitate a complex of at least 11 proteins. The major antigen is a 100-kDa protein recognized by 95% of patients who have this autoantibody (7). These antibodies are most commonly associated with a myositis-scleroderma overlap syndrome (53, 74, 102). However, patients with anti-PM-Scl autoantibodies who have pure myo-

sitis or scleroderma often develop some clinical features of the other.

Anti-Ku autoantibodies, directed toward characteristic 70- and 80-kDa proteins which bind DNA, were originally described for patients with myositis-scleroderma overlap syndromes (83). More common in Japanese than U.S. patients (48, 125), anti-Ku autoantibodies have also been observed in patients with SLE, DM, scleroderma, thyroid disease, and Sjogren's syndrome (125, 163).

Other autoantibodies have been rarely observed in patients with IIM. These include autoantibodies to Mas, FER, and KJ (69, 144, 146). All are cytoplasmic antigens whose function is not known, and further study is needed to confirm if they are MSAs or MAAs.

Among the routine clinical immunology laboratory tests, cytoplasmic staining of HEP-2 cells by indirect immunofluorescence is the single most important finding suggesting the presence of MSAs. MSAs producing a cytoplasmic staining pattern on HEP-2 cells are those involved in protein translation, including antisynthetase autoantibodies, anti-SRP, anti-Mas, anti-Fer, and anti-KJ (62). Many MSAs and MAAs are routinely detected in sera by their capacity to produce an immunoprecipitation line of identity with prototype sera in a double-immunodiffusion Ouchterlony assay with calf thymus or liver extracts as sources of antigens. The autoantibodies detected by this technique include anti-Jo-1, -PL-7, -KJ, -Mi-2, and -PM-Scl. No precipitin lines have been identified with sera from patients with only anti-PL-12, -EJ, -OJ, or -SRP autoantibodies. Anti-Ku is occasionally detected by double immunodiffusion, particularly against calf thymus extract as the substrate (114, 139a). Although Ouchterlony immunodiffusion is highly specific for precipitating autoantibodies when performed properly, false-positive and false-negative results can result from (i) inaccurate reading of the plates, (ii) low levels of the autoantibodies in serum, or (iii) low levels of unstable and rare autoantigens in the antigen extracts.

The most sensitive method for detecting MSAs and MAAs is immunoprecipitation of protein and RNA antigens from HeLa cell extracts by using patients' immunoglobulins bound to protein A (148). This technique, however, is cumbersome and expensive, and therefore remains a research tool. Enzyme-linked immunosorbent assay, which uses purified autoantigens as the target, is a sensitive method for both detecting and quantitating myositis autoantibodies (11, 81, 82, 148), but its specificity depends on the purity of the autoantigen. Since all of the MSAs inhibit the known functions of their targets, assays that assess the capacity of sera with MSAs to inhibit aminoacylation or translation have been developed as research tools to confirm the presence of some of these autoantibodies (143, 148). The genes for several MSA and MAA target autoantigens have now been cloned (26, 40, 98, 119, 129, 164, 165). Cloning and expression of the targets of the remaining MSAs and MAAs should enable the development of purified antigens, which will greatly enhance the ease, specificity, and sensitivity of detection of these autoantibodies.

SUMMARY

The laboratory plays an important role in the diagnosis, evaluation, and classification of the heterogeneous group of diseases known as the IIM, which are characterized by chronic muscle inflammation. Serial measurements of the levels of muscle-derived enzymes in serum are the traditional laboratory studies used to follow the clinical course of patients with IIM, although other laboratory tests can also be useful in assessing myositis disease activity. Several markers of immune

system activation, including cytokines and lymphocyte markers, show promise as possibly more sensitive measures of myositis disease activity. Discovery of a unique group of MSAs over the past decade has provided an immunologic basis for defining relatively homogeneous subsets of patients who share similar clinical features, disease courses, and responses to therapy. Future investigations of novel immunologic activation markers, as well as the cloning and expression of target autoantigens of the MSAs, should allow better diagnostic assays, enhanced prognosis, and a better understanding of the pathogenesis of these disorders.

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REFERENCES

1. Arad-Dann, H., D. Isenberg, E. Ovadia, Y. Shoenfeld, J. Sperling, and R. Sperling. 1989. Autoantibodies against a nuclear 56 kDa protein: a marker for inflammatory muscle disease. *J. Autoimmun.* 2:877-888.
2. Arahata, K., and A. G. Engel. 1984. Monoclonal antibody analysis of mononuclear cells in myopathies. I. Quantitation of subsets according to diagnosis and sites of accumulation and demonstration and counts of muscle fibers invaded by T cells. *Ann. Neurol.* 16:193-208.
3. Arnett, F. C., R. Goldstein, M. Duvic, and J. D. Reveille. 1988. Major histocompatibility complex genes in systemic lupus erythematosus, Sjogren's syndrome, and polymyositis. *Am. J. Med.* 85:38-41.
4. Arnett, F. C., R. G. Hamilton, M. G. Roebber, J. B. Harley, and M. Reichlin. 1988. Increased frequencies of Sm and nRNP autoantibodies in American blacks compared to whites with systemic lupus erythematosus. *J. Rheumatol.* 15:1773-1776.
5. Askari, A., P. J. Vignos, Jr., and R. W. Moskowitz. 1976. Steroid myopathy in connective tissue disease. *Am. J. Med.* 61:485-492.
6. Askmark, H., P. O. Osterman, L. E. Roxin, and P. Venge. 1981. Radioimmunoassay of serum myoglobin in neuromuscular diseases. *J. Neurol. Neurosurg. Psych.* 44:68-72.
7. Baker, J. R., Jr., F. W. Miller, A. D. Steinberg, and K. D. Burman. 1991. Thyroid stimulating and thyrotrophin binding-inhibitory immunoglobulin activity in patients with systemic lupus erythematosus having thyroid function abnormalities. *Thyroid* 1:229-234.
8. Bardelas, J. A., J. A. Winkelstein, D. S. Y. Seto, T. Tsai, and A. D. Rogol. 1977. Fatal ECHO 24 infection in a patient with hypogammaglobulinemia: relationship to dermatomyositis-like syndrome. *J. Pediatr.* 90:396-399.
9. Behan, W. M., and P. O. Behan. 1977. Complement abnormalities in polymyositis. *J. Neurol. Sci.* 34:241-246.
10. Bernstein, R. M., S. H. Morgan, and J. Chapman. 1984. Anti-Jo-1 antibody: a marker for myositis with interstitial lung disease. *Br. Med. J.* 289:151-152.
11. Biswas, T., F. W. Miller, Y. Takagaki, and P. H. Plotz. 1987. An enzyme-linked immunosorbent assay for the detection and quantitation of anti-Jo-1 antibody in human serum. *J. Immunol. Methods* 98:243-248.
12. Black, H. R., H. Quallich, and C. B. Garelick. 1986. Racial differences in serum creatine kinase levels. *Am. J. Med.* 81:479-487.
13. Bohan, A., and J. B. Peter. 1975. Polymyositis and dermatomyositis. *N. Engl. J. Med.* 292:403-407.
14. Bohan, A., and J. B. Peter. 1975. Polymyositis and dermatomyositis. *N. Engl. J. Med.* 292:344-347.
15. Bohan, A., J. B. Peter, R. L. Bowman, and C. M. Pearson. 1977. A computer-assisted analysis of 153 patients with polymyositis and dermatomyositis. *Medicine (Baltimore)* 56:255-286.
16. Bombardieri, S., A. Clerico, L. Riente, M. G. Del Chicca, and C. Vitali. 1982. Circadian variations of serum myoglobin levels in normal subjects and patients with polymyositis. *Arthritis Rheum.* 25:1419-1424.
17. Borleffs, J. C. C., R. H. Derksen, and D. P. Bar. 1987. Serum myoglobin and creatine kinase concentrations in patients with polymyositis or dermatomyositis. *Ann. Rheum. Dis.* 46:173-175.
18. Bowyer, S. L., C. G. Ragsdale, and D. B. Sullivan. 1989. Factor VIII related antigen and childhood rheumatic diseases. *J. Rheumatol.* 16:1093-1097.
19. Bunn, C. C., R. M. Bernstein, and M. B. Mathews. 1986. Autoantibodies against alanyl-tRNA synthetase and tRNA^{Ala} coexist and are associated with myositis. *J. Exp. Med.* 163:1281-1291.
20. Cavalieri, R. R. 1991. The effects of nonthyroid disease and drugs on thyroid function tests. *Med. Clin. N. Am.* 75:27-39.
21. Cervera, R., G. Ramirez, J. Fernandez-Sola, D. D'Cruz, J. Casademont, J. M. Grau, R. A. Asherson, M. A. Khamashta, A. Urbano-Marquez, and G. R. Hughes. 1991. Antibodies to endothelial cells in dermatomyositis: association with interstitial lung disease. *Br. Med. J.* 302:880-881.

22. Clark, L. C., H. L. Thompson, E. I. Beck, and W. Jacobson. 1951. Excretion of creatine and creatinine by children. *Am. J. Dis. Child.* **81**:774-783.
23. Craft, J. 1992. Antibodies to snRNPs in systemic lupus erythematosus. *Rheum. Dis. Clin. N. Am.* **18**:311-335.
24. Craft, J., T. Mimori, T. L. Olsen, and J. A. Hardin. 1988. The U2 small nuclear ribonucleoprotein particle as an autoantigen: analysis with sera from patients with overlap syndromes. *J. Clin. Invest.* **81**:1716-1724.
25. Crowe, W. E., K. E. Bove, J. E. Levinson, and P. K. Hilton. 1982. Clinical and pathogenetic implications of histopathology in childhood polydermatomyositis. *Arthritis Rheum.* **25**:126-139.
26. Cruzen, M. E., and S. M. Arfin. 1991. Nucleotide and deduced amino acid sequence of human threonyl-tRNA synthetase reveals extensive homology to the *Escherichia coli* and yeast enzymes. *J. Biol. Chem.* **266**:9919-9923.
27. DeBenedetti, F., M. DeAmici, L. Aramini, N. Ruperto, and A. Martini. 1993. Correlations of serum neopterin concentrations with disease activity in juvenile dermatomyositis. *Arch. Dis. Child.* **69**:232-235.
28. DeVere, R., and W. G. Bradley. 1975. Polymyositis: its presentation, morbidity and mortality. *Brain* **98**:637-666.
29. Edwards, R. H. T., D. A. Isenberg, C. M. Wiles, A. Young, and M. L. Snaith. 1981. The investigation of inflammatory myopathy. *J. R. Coll. Phys. London* **15**:19-24.
30. El Allaf, M., J. P. Chapelle, D. El Allaf, A. Adam, M. E. Faymonville, P. Laurent, and C. Heugthem. 1986. Differentiating muscle damage from myocardial injury by means of the serum creatine kinase isoenzyme MB mass measurement/total CK activity ratio. *Clin. Chem.* **32**:291-295.
31. Emslie-Smith, A. M., and A. G. Engel. 1990. Microvascular changes in early and advanced dermatomyositis: a quantitative study. *Ann. Neurol.* **27**:343-356.
32. Engel, A. G., K. Arahata, and A. Emslie-Smith. 1990. Immune effector mechanisms in inflammatory myopathies, p. 141-157. *In* B. H. Waksman (ed.), *Immunologic mechanisms in neurologic and psychiatric disease*. Raven Press, New York.
33. Fafalakis, R. G., M. G. E. Peterson, and C. J. Kagen. 1994. Strength in polymyositis and dermatomyositis: best outcome in patients early. *J. Rheumatol.* **21**:643-648.
34. Fernandez-Sola, J., J. C. Pou, J. M. G. Junyent, and A. Urbano-Manquez. 1991. Normal creatine kinase activity in idiopathic inflammatory myopathy: does it have prognostic implications? *J. Rheumatol.* **18**:1640-1641.
35. Fraser, D. D., J. A. Frank, M. Dalakas, F. W. Miller, J. E. Hicks, and P. Plotz. 1991. Magnetic resonance imaging in the idiopathic inflammatory myopathies. *J. Rheumatol.* **18**:1693-1700.
36. Friedman, J. M., L. M. Pachman, M. L. Maryjowski, R. M. Radvany, W. E. Crowe, H. Hanson, J. E. Levinson, and C. H. Spencer. 1983. Immunogenetic studies of juvenile dermatomyositis, HLA-DR antigen frequencies. *Arthritis Rheum.* **26**:210-216.
37. Fudman, E. J., and T. J. Schnitzer. 1986. Dermatomyositis without creatine kinase elevation: a poor prognostic sign. *Am. J. Med.* **80**:329-332.
38. Ghosh, A. K., V. Sakhuja, K. Joshi, K. L. Gupta, and K. S. Chugh. 1993. Acute myositis complicated by myoglobinuric acute renal failure. *J. Assoc. Physicians India* **41**:453-454.
39. Giesker, D., and G. N. Bowers. 1979. The comparative utility of serum creatine kinase versus serum aldolase in the evaluation of muscle disorders. *Conn. Med.* **43**:699-704.
40. Gluthner, M., and P. A. Bautz. 1992. Cloning and characterization of the cDNA coding for a polymyositis-scleroderma overlap syndrome-related nucleolar 100-kD protein. *J. Exp. Med.* **176**:973-980.
41. Goldman, J., R. Matz, R. Mortimer, and R. Freeman. 1977. High elevations of creatine phosphokinase in hypothyroidism: an isoenzyme analysis. *JAMA* **238**:325-326.
42. Gran, J. T., G. Myklebust, and S. Johansen. 1993. Adult idiopathic polymyositis without elevation of creatine kinase: case report and review of the literature. *Scand. J. Rheumatol.* **22**:94-96.
43. Guzman, J., R. E. Petty, and P. N. Malleon. 1994. Monitoring disease activity in juvenile dermatomyositis: the role of von Willebrand factor and muscle enzymes. *J. Rheumatol.* **21**:739-743.
44. Haas, R. H., R. F. Dyck, V. Dubowitz, and M. B. Pepys. 1982. C-reactive protein in childhood dermatomyositis. *Ann. Rheum. Dis.* **41**:483-485.
45. Helfgott, S. M., E. Karlson, and E. Beckman. 1993. Misinterpretation of serum transaminase elevation in occult myositis. *Am. J. Med.* **95**:447-449.
46. Hernandez, R. J., D. B. Sullivan, T. L. Chenevert, and D. R. Keim. 1993. MR imaging in children with dermatomyositis: musculoskeletal findings and correlation with clinical and laboratory findings. *Am. J. Roentgenol.* **161**:359-366.
47. Hinderks, G. J., and J. Frohlich. 1979. Low serum creatine kinase values associated with administration of steroids. *Clin. Chem.* **25**:2050-2051.
48. Hirakata, M., T. Mimori, M. Akizuki, J. Craft, J. A. Hardin, and M. Homma. 1992. Autoantibodies to small nuclear and cytoplasmic ribonucleoproteins in Japanese patients with inflammatory muscle disease. *Arthritis Rheum.* **35**:449-456.
49. Hochberg, M. C., D. Feldman, and M. B. Stevens. 1986. Adult onset polymyositis/dermatomyositis: an analysis of clinical and laboratory features and survival in 76 patients with a review of the literature. *Semin. Arthritis Rheum.* **15**:168-178.
50. Hochberg, M. C., D. Feldman, M. B. Stevens, F. C. Arnett, and M. Reichlin. 1984. Antibody to Jo-1 in polymyositis/dermatomyositis: association with interstitial pulmonary disease. *J. Rheumatol.* **11**:663-665.
51. Ishida, T., Y. Matsumoto, M. Ohashi, and R. Sasaki. 1993. Analysis of lymphocyte subpopulations in peripheral blood in adult and juvenile cases of dermatomyositis. *J. Dermatol.* **20**:30-34.
52. Ishida, T., M. Ohashi, Y. Matsumoto, J. Morikawa, and R. Sasaki. 1993. Connection of atopic disease in Japanese patients with juvenile dermatomyositis based on serum IgE levels. *Clin. Rheumatol.* **12**:41-48.
53. Jablonska, S., T. P. Chorzelski, M. Blaszczyk, M. Jarzabek-Chorzelska, V. Kumar, and E. H. Beutner. 1993. Scleroderma/polymyositis overlap syndromes and their immunologic markers. *Clin. Dermatol.* **10**:457-472.
54. Joffe, M. M., L. A. Love, R. L. Leff, D. D. Fraser, I. N. Targoff, J. E. Hicks, P. H. Plotz, and F. W. Miller. 1993. Drug therapy of the idiopathic inflammatory myopathies: predictors of response to prednisone, azathioprine, and methotrexate and a comparison of their efficacy. *Am. J. Med.* **94**:379-387.
55. Jones, B. F., D. M. Howarth, C. R. Levi, and J. S. Glass. 1990. Proteinuria in polymyositis. *J. Rheumatol.* **17**:859-860.
56. Kagen, L. J. 1977. Myoglobinemia in inflammatory myopathies. *JAMA* **237**:1448-1452.
57. Kagen, L. J. 1978. Myoglobin: methods and diagnostic uses. *Crit. Rev. Clin. Lab. Sci.* **9**:273-302.
58. Kagen, L. J., and S. Aram. 1987. Creatine kinase activity inhibitor in sera from patients with muscle disease. *Arthritis Rheum.* **30**:213-217.
59. Keshgegian, A. A., and N. V. Feinberg. 1984. Serum creatine kinase MB isoenzyme in chronic muscle disease. *Clin. Chem.* **30**:575-578.
60. Kissel, J. T., J. R. Mendell, and K. W. Rammohan. 1986. Microvasculature deposition of complement membrane attack complex in dermatomyositis. *N. Engl. J. Med.* **314**:329-334.
61. Kroll, M., J. Otis, and L. Kagen. 1986. Serum enzyme, myoglobin and muscle strength relationships in polymyositis and dermatomyositis. *J. Rheumatol.* **13**:349-355.
62. Lang, B. A., R. M. Laxer, G. Murphy, E. D. Silverman, and C. M. Roifman. 1991. Treatment of dermatomyositis with intravenous gammaglobulin. *Am. J. Med.* **91**:169-172.
63. Larca, L. J., J. T. Coppola, and S. Honig. 1981. Creatine kinase MB isoenzyme in dermatomyositis: a noncardiac source. *Ann. Intern. Med.* **94**:341-343.
64. Lee, K. N., G. Csako, P. Bernhardt, and R. J. Elin. 1994. Relevance of macro creatine kinase type 1 and type 2 isoenzymes to laboratory and clinical data. *Clin. Chem.* **40**:1278-1283.
65. Leff, R. L., S. H. Burgess, F. W. Miller, L. A. Love, I. N. Targoff, M. C. Dalakas, M. M. Joffe, and P. H. Plotz. 1991. Distinct seasonal patterns in the onset of adult idiopathic inflammatory myopathy in patients with anti-Jo-1 and anti-signal recognition particle autoantibodies. *Arthritis Rheum.* **34**:1391-1396.
66. Lim, R., and J. L. W. Parker. 1988. Apparent haematuria: an early sign in polymyositis. *Br. Med. J.* **296**:1725-1726.
67. Lisak, R. P., and B. Zweiman. 1976. Serum immunoglobulin levels in myasthenia gravis, polymyositis, and dermatomyositis. *J. Neurol. Neurosurg. Psych.* **39**:34-37.
68. Lott, J. A., and L. B. Abbott. 1986. Creatine kinase isoenzymes. *Clin. Lab. Med.* **6**:547-576.
69. Love, L. A., R. L. Leff, D. D. Fraser, I. N. Targoff, M. Dalakas, P. H. Plotz, and F. W. Miller. 1991. A new approach to the classification of idiopathic inflammatory myopathy: myositis-specific autoantibodies define useful homogeneous patient groups. *Medicine (Baltimore)* **70**:360-374.
70. Love, L. A., and F. W. Miller. 1993. Noninfectious environmental agents associated with myopathies. *Curr. Opin. Rheumatol.* **5**:712-718.
71. Lovece, S., and L. J. Kagen. 1993. Sensitive rapid detection of myoglobin in serum of patients with myopathy by immunoturbidimetric assay. *J. Rheumatol.* **20**:1331-1334.
72. Mandell, B. F. 1991. Aldolase in the diagnosis of myositis. *Am. J. Med.* **90**:662.
73. Marguerie, C., C. C. Bunn, H. L. Beynon, R. M. Bernstein, J. M. Hughes, A. K. So, and M. J. Walport. 1990. Polymyositis, pulmonary fibrosis and autoantibodies to aminoacyl-tRNA synthetase enzymes. *Q. J. Med.* **77**:1019-1038.
74. Marguerie, C., C. C. Bunn, J. Copier, R. M. Bernstein, J. M. Gilroy, C. M. Black, A. K. So, and M. J. Walport. 1992. The clinical and immunogenetic features of patients with autoantibodies to the nucleolar antigen PM-Scl. *Medicine (Baltimore)* **71**:327-336.
75. Medsger, T. A., W. N. Dawson, and A. T. Masi. 1970. The epidemiology of polymyositis. *Am. J. Med.* **48**:715.
76. Miller, F. W. 1993. Myositis-specific autoantibodies. Touchstones for understanding the inflammatory myopathies. *JAMA* **270**:1846-1849.
77. Miller, F. W. 1994. Classification and prognosis of inflammatory muscle disease. *Rheum. Dis. Clin. N. Am.* **20**:811-826.
78. Miller, F. W., et al. Unpublished data.
79. Miller, F. W., L. A. Love, S. A. Barbieri, J. E. Balow, and P. H. Plotz. 1990. Lymphocyte activation markers in idiopathic myositis: changes with disease

- activity and differences among clinical and autoantibody subgroups. *Clin. Exp. Immunol.* **81**:373-379.
80. Miller, F. W., L. A. Love, S. A. Twitty, and P. H. Plotz. 1989. Soluble CD8 and interleukin-2 receptor levels are measures of disease activity in the idiopathic inflammatory myopathies. *Arthritis Rheum.* **32**(Suppl.):S33.
 81. Miller, F. W., S. A. Twitty, T. Biswas, and P. H. Plotz. 1990. Origin and regulation of a disease-specific autoantibody response. Antigenic epitopes, spectrotypic stability, and isotype restriction of anti-Jo-1 autoantibodies. *J. Clin. Invest.* **85**:468-475.
 82. Miller, F. W., K. A. Waite, T. Biswas, and P. H. Plotz. 1990. The role of an autoantigen, histidyl-tRNA synthetase, in the induction and maintenance of autoimmunity. *Proc. Natl. Acad. Sci. USA* **87**:9933-9937.
 83. Mimori, T., J. A. Hardin, and J. A. Steitz. 1986. Characterization of the DNA-binding protein antigen Ku recognized by autoantibodies from patients with rheumatic disorders. *J. Biol. Chem.* **261**:2274-2278.
 84. Mimori, T., M. Hinterberger, I. Pettersson, and J. A. Steitz. 1984. Autoantibodies to the U2 small nuclear ribonucleoprotein in a patient with scleroderma-polymyositis overlap syndrome. *J. Biol. Chem.* **259**:560-565.
 85. Misaki, Y., G. J. M. Pruijn, W. C. M. van der Kemp, and W. J. van Venrooij. 1994. The 56K autoantigen is identical to human annexin XI. *J. Biol. Chem.* **269**:4240-4246.
 86. Montecucco, C., A. Ravelli, R. Caporali, S. Viola, F. DeGennaro, S. Albani, and A. Martini. 1990. Autoantibodies in juvenile dermatomyositis. *Clin. Exp. Rheumatol.* **8**:193-196.
 87. Moore, E. C., F. Cohen, S. D. Douglas, and V. Gutta. 1992. Staphylococcal infections in childhood dermatomyositis—association with the development of calcinosis, raised IgE concentrations and granulocyte chemotactic defect. *Ann. Rheum. Dis.* **51**:378-383.
 88. Morton, B. D., and B. E. Stadland. 1980. Serum enzyme alterations in polymyositis: possible pitfalls in diagnosis. *Am. J. Clin. Pathol.* **73**:556-557.
 89. Newman, A. J., and C. Lee. 1980. Hypothyroidism simulating dermatomyositis. *J. Pediatr.* **97**:772-774.
 90. Newman, E. D., and R. J. Kurland. 1992. P-31 magnetic resonance spectroscopy in polymyositis and dermatomyositis. Altered energy utilization during exercise. *Arthritis Rheum.* **35**:199-203.
 91. Nicholson, G. A., G. J. Morgan, M. Meerkin, E. R. Strauss, and J. G. McLeod. 1986. The effect of aerobic exercise on serum creatine kinase activities. *Muscle Nerve* **9**:820-824.
 92. Nilasena, D. S., Q. Ge, M. B. Frank, and I. N. Targoff. 1990. Molecular cloning of the dermatomyositis-associated Mi-2 antigen. *Arthritis Rheum.* **33**(Suppl.):S22.
 93. Nishikai, M., and M. Homma. 1977. Circulating autoantibody against human myoglobin in polymyositis. *JAMA* **237**:1842-1844.
 94. Nishikai, M., and M. Reichlin. 1977. Radioimmunoassay of serum myoglobin in polymyositis and other conditions. *Arthritis Rheum.* **20**:1514-1518.
 95. Nuviala, R. J., L. Roda, M. G. Lapieza, B. Boned, and A. Giner. 1992. Serum enzymes activities at rest and after a marathon race. *J. Sports Med. Phys. Fit.* **180**:186.
 96. O'Gorman, M. R. G., V. Corrochano, J. Roleck, and L. M. Pachman. 1994. Abnormalities in the relative percentages of lymphocyte subsets in the peripheral blood on untreated children with juvenile dermatomyositis. *Arthritis Rheum.* **37**(Suppl):R15.
 97. O'Hanlon, T. P., M. C. Dalakas, P. H. Plotz, and F. W. Miller. 1994. Predominant TCR-alpha beta variable and joining gene expression by muscle-infiltrating lymphocytes in the idiopathic inflammatory myopathies. *J. Immunol.* **152**:2569-2576.
 98. Oddis, C. V. 1993. Therapy for myositis. *Curr. Opin. Rheum.* **5**:742-748.
 99. Oddis, C. V., and T. A. Medsger, Jr. 1988. Relationship between serum creatine kinase level and corticosteroid therapy in polymyositis-dermatomyositis. *J. Rheumatol.* **15**:807-811.
 100. Oddis, C. V., and T. A. Medsger, Jr. 1989. Current management of polymyositis and dermatomyositis. *Drugs* **37**:382-390.
 101. Oddis, C. V., T. A. Medsger, Jr., and L. A. Cooperstein. 1990. A subluxing arthropathy associated with the anti-Jo-1 antibody in polymyositis/dermatomyositis. *Arthritis Rheum.* **33**:1640-1645.
 102. Oddis, C. V., Y. Okano, W. A. Rudert, M. Trucco, R. J. Duquesnoy, and T. A. Medsger, Jr. 1992. Serum autoantibody to the nucleolar antigen PM-Scl: clinical and immunogenetic associations. *Arthritis Rheum.* **35**:1211-1217.
 103. Ohdama, S., S. Takano, S. Miyake, T. Kubota, K. Sato, and N. Aoki. 1994. Plasma thrombomodulin as a marker of vascular injuries in collagen vascular diseases. *Am. J. Clin. Pathol.* **101**:109-113.
 104. Okada, N., T. Mimori, R. Mukai, H. Kashiwagi, and J. A. Hardin. 1987. Characterization of human autoantibodies that selectively precipitate the 7SL RNA component of the signal recognition particle. *J. Immunol.* **15**:3219-3223.
 105. Olson, N. Y., and C. B. Lindsley. 1989. Immunoglobulin E levels and disease manifestations in childhood dermatomyositis. *Arthritis Rheum.* **32**(Suppl.):S151.
 106. Osterman, P. O., H. Askmark, and P. J. Wistrand. 1985. Serum carbonic anhydrase III in neuromuscular disorders and in healthy persons after a long-distance run. *J. Neurol. Sci.* **70**:347-357.
 107. Pachman, L. M., and N. Cooke. 1980. Juvenile dermatomyositis: a clinical and immunologic study. *J. Pediatr.* **96**:226-234.
 108. Pachman, L. M., D. Dilling, D. Litt, M. L. Miller, and J. S. Sinacore. 1993. Sequential studies of neopterin, von Willebrand factor antigen, creatine kinase and aldolase in juvenile dermatomyositis: correlates of disease activity? *Arthritis Rheum.* **36**(Suppl.):S257.
 109. Pachman, L. M., J. M. Friedman, M. L. Maryjowski-Sweeney, O. Jonnason, R. M. Radvany, G. C. Sharp, M. A. Cobb, N. D. Battles, W. E. Crowe, and C. W. Fink. 1985. Immunogenetic studies of juvenile dermatomyositis. III. Study of antibody to organ-specific and nuclear antigens. *Arthritis Rheum.* **28**:151-157.
 110. Park, J. H., J. P. Vansant, N. G. Kumar, and S. J. Gibbs. 1990. Dermatomyositis: correlative MR imaging and P-31 spectroscopy for quantitative characterization of inflammatory disease. *Radiology* **177**:473-479.
 111. Park, J. H., T. L. Vital, N. M. Ryder, M. Hernanz-Schulman, C. L. Partain, R. R. Price, and N. J. Olsen. 1994. Magnetic resonance imaging and P-31 magnetic resonance spectroscopy provide unique quantitative data useful in the longitudinal management of patients with dermatomyositis. *Arthritis Rheum.* **37**:736-746.
 112. Pasternak, R. C., E. Braunwald, and J. S. Alpert. 1987. Acute myocardial infarction, p. 982-985. *In* E. Braunwald, K. J. Isselbacher, R. G. Petersdorf, J. D. Wilson, J. B. Martin, and A. S. Fauci (ed.), *Harrison's principles of internal medicine*. McGraw-Hill Book Co., New York.
 113. Pennington, R. J. T. 1981. Biochemical aspects of muscle disease, p. 417-447. *In* J. Walton (ed.), *Disorders of voluntary muscle*. Churchill Livingstone, Edinburgh.
 114. Plotz, P., and I. Targoff. Myositis-associated antigens: aminoacyl-tRNA synthetases Jo-1, PL-7, PL-12, EJ, and OJ. *In* Van Venrooij (ed.), *Autoantigens and clinical significance of autoantibodies*, in press.
 115. Plotz, P. H. 1992. Not myositis. A series of chance encounters. *JAMA* **268**:2074-2077.
 116. Plotz, P. H., M. Dalakas, R. L. Leff, L. A. Love, F. W. Miller, and M. E. Cronin. 1989. Current concepts in the idiopathic inflammatory myopathies: polymyositis, dermatomyositis, and related disorders. *Ann. Intern. Med.* **111**:143-157.
 117. Plotz, P. H., and F. W. Miller. 1994. Inflammatory muscle disease: etiology and pathogenesis, p. 13.1-13.10. *In* J. H. Klippel and P. A. Dieppe (ed.), *Rheumatology*. The C. V. Mosby Co., St. Louis.
 118. Poncher, H. G., M. B. Visscher, and H. W. Woodward. 1934. Creatine metabolism in children with hypothyroidism. *JAMA* **102**:1132-1135.
 119. Raben, N., F. Borriello, J. Amin, R. Horwitz, D. Fraser, and P. Plotz. 1992. Human histidyl-tRNA synthetase: recognition of amino acid signature regions in class 2a aminoacyl-tRNA synthetases. *Nucleic Acids Res.* **20**:1075-1081.
 120. Rasmussen, L. H., H. N. Madsen, and S. D. Ladefoged. 1985. Creatine phosphokinase MB and lactate dehydrogenase isoenzyme 1 in polymyositis. *Scand. J. Rheumatol.* **14**:427-430.
 121. Reed, A., M. Haugen, L. M. Pachman, and C. B. Langman. 1990. Abnormalities in serum osteocalcin values in children with chronic rheumatic diseases. *J. Pediatr.* **116**:574-580.
 122. Reed, A. M., L. Pachman, and C. Ober. 1991. Molecular genetic studies of major histocompatibility complex genes in children with juvenile dermatomyositis: increased risk associated with HLA-DQA1 *0501. *Hum. Immunol.* **32**:235-240.
 123. Reeves, W. H. 1992. Antibodies to the p70/p80 (Ku) antigens in systemic lupus erythematosus. *Rheum. Dis. Clin. N. Am.* **18**:391-414.
 124. Reeves, W. H., S. K. Nigam, and G. Blobel. 1986. Human autoantibodies reactive with the signal-recognition particle. *Proc. Natl. Acad. Sci. USA* **83**:9507-9511.
 125. Reeves, W. H., M. Satoh, J. Wang, C. H. Chou, and A. K. Ajmani. 1994. Antibodies to DNA, DNA-binding proteins, and histones. *Rheum. Dis. Clin. N. Am.* **20**:1-28.
 126. Reichlin, M., and F. C. Arnett. 1984. Multiplicity of antibodies in myositis sera. *Arthritis Rheum.* **27**:1150-1156.
 127. Reichlin, M., P. J. Maddison, I. Targoff, T. Bunch, F. Arnett, G. Sharp, E. Treadwell, and E. M. Tan. 1984. Antibodies to a nuclear/nucleolar antigen in patients with polymyositis overlap syndromes. *J. Clin. Immunol.* **4**:40-44.
 128. Rider, L. G., F. W. Miller, I. N. Targoff, D. D. Sherry, E. Samayoa, M. Lindahl, D. M. H. Wener, L. M. Pachman, and P. H. Plotz. 1994. A broadened spectrum of juvenile myositis: myositis-specific autoantibodies in children. *Arthritis Rheum.* **37**:1534-1538.
 129. Romisch, K., J. Webb, J. Herz, S. Prehn, R. Frank, M. Vingron, and B. Dobberstein. 1989. Homology of 54K protein of signal-recognition particle, docking protein and two E. coli proteins with putative GTP-binding domains. *Nature (London)* **340**:478-482.
 130. Rosalki, S. B. 1989. Serum enzymes in disease of skeletal muscle. *Clin. Lab. Med.* **9**:767-781.
 131. Rowell, N. R., and G. M. Fairris. 1986. Biochemical markers of myositis in dermatomyositis. *Clin. Exp. Dermatol.* **11**:69-72.

132. **Sanmarti, R., A. Collado, J. Gratacos, and J. L. Bedini.** 1994. Reduced activity of serum creatine kinase in rheumatoid arthritis: a phenomenon linked to the inflammatory response. *Br. J. Rheumatol.* **33**:231–234.
133. **Schwieterman, W. D., M. S. Wand, P. H. Plotz, and F. W. Miller.** 1993. Misdiagnosis of idiopathic inflammatory myopathy. *Arthritis Rheum.* **36**(Suppl.):S118.
134. **Scott, J. P., and C. Arroyave.** 1987. Activation of complement and coagulation in juvenile dermatomyositis. *Arthritis Rheum.* **30**:572–576.
135. **Sharp, G. C., W. S. Irvin, C. M. May, H. R. Holman, F. C. McDuffie, E. V. Hess, and F. R. Schmid.** 1976. Association of antibodies to ribonucleoprotein and other rheumatic diseases. *N. Engl. J. Med.* **295**:1149–1154.
136. **Sharp, G. C., W. S. Irvin, E. M. Tan, R. G. Gould, and H. R. Holman.** 1972. Mixed connective tissue disease—an apparently distinct rheumatic disease syndrome associated with a specific autoantibody to an extractable nuclear antigen (ENA). *Am. J. Med.* **52**:148–159.
137. **Shima, K.** 1984. Human muscle carbonic anhydrase III (CA-III). *Hokkaido Igaku Zasshi* **59**:98–116.
138. **Stein, D. P., S. C. Jordan, M. Toyoda, O. Galler, and M. C. Dalakas.** 1993. Anti-endothelial cell antibodies in dermatomyositis. *Neurology* **43**:A356.
139. **Straus, K. W., H. Gonzalez-Buritica, M. A. Khamashta, and G. R. V. Hughes.** 1989. Polymyositis-dermatomyositis: a clinical review. *Postgrad. Med.* **65**:437–443.
- 139a. **Targoff, I. N.** Personal communication.
140. **Targoff, I. N.** 1988. Laboratory manifestations of polymyositis/dermatomyositis. *Clin. Dermatol.* **6**:76–92.
141. **Targoff, I. N.** 1993. Humoral immunity in polymyositis/dermatomyositis. *J. Invest. Dermatol.* **100**:116S–123S.
142. **Targoff, I. N.** 1994. Immune manifestations of inflammatory muscle disease. *Rheum. Dis. Clin. N. Am.* **20**:857–880.
143. **Targoff, I. N., and F. C. Arnett.** 1990. Clinical manifestations in patients with antibody to PL-12 antigen (alanyl-tRNA synthetase). *Am. J. Med.* **88**:241–251.
144. **Targoff, I. N., F. C. Arnett, L. Berman, C. O'Brien, and M. Reichlin.** 1989. Anti-KJ: a new antibody associated with the syndrome of polymyositis and interstitial lung disease. *J. Clin. Invest.* **84**:162–172.
145. **Targoff, I. N., F. C. Arnett, and M. Reichlin.** 1988. Antibody to threonyl-transfer RNA synthetase in myositis sera. *Arthritis Rheum.* **31**:515–524.
146. **Targoff, I. N., and J. Hanas.** 1989. The polymyositis-associated Fer antigen is elongation factor 1-alpha. *Arthritis Rheum.* **32**:S81.
147. **Targoff, I. N., A. E. Johnson, and F. W. Miller.** 1990. Antibody to signal recognition particle in polymyositis. *Arthritis Rheum.* **33**:1361–1370.
148. **Targoff, I. N., and M. Reichlin.** 1987. Measurement of antibody to Jo-1 by ELISA and comparison to enzyme inhibitory activity. *J. Immunol.* **138**:2874–2882.
149. **Targoff, I. N., E. P. Trieu, and F. W. Miller.** 1993. Reaction of anti-OJ autoantibodies with components of the multi-enzyme complex of aminoacyl-tRNA synthetases in addition to isoleucyl-tRNA synthetase. *J. Clin. Invest.* **91**:2556–2564.
150. **Targoff, I. N., E. P. Trieu, P. H. Plotz, and F. W. Miller.** 1992. Antibodies to glycyl-transfer RNA synthetase in patients with myositis and interstitial lung disease. *Arthritis Rheum.* **35**:821–830.
151. **Tokano, Y., Y. Kanai, H. Hashimoto, K. Okumura, and S. Hirose.** 1992. Soluble interleukin 2 receptors in patients with polymyositis/dermatomyositis. *Ann. Rheum. Dis.* **51**:781–782.
152. **Tokano, Y., T. Obara, H. Hashimoto, K. Okumura, and S. Hirose.** 1993. Soluble CD4, CD8 in patients with polymyositis/dermatomyositis. *Clin. Rheumatol.* **12**:368–374.
153. **Tsung, S. H., T. Y. Huang, and J. I. Lin.** 1982. CK-MB isoenzyme in patients with polymyositis. *Am. J. Med. Sci.* **283**:174–177.
154. **Tymms, K. E., E. M. Beller, J. Webb, L. Schrieber, and W. W. Buchanan.** 1990. Correlation between tests of muscle involvement and clinical muscle weakness in polymyositis and dermatomyositis. *Clin. Rheumatol.* **9**:523–529.
155. **Vignos, P. J., and J. Goldwyn.** 1972. Evaluation of laboratory tests in diagnosis and management of polymyositis. *Am. J. Med. Sci.* **263**:291–308.
156. **Wada, K., S. Ueno, T. Hazama, S. Ogasahara, J. Kang, M. Takahashi, and S. Tarui.** 1983. Radioimmunoassay for antibodies to human skeletal muscle myosin in serum from patients with polymyositis. *Clin. Exp. Immunol.* **52**:297–304.
157. **Wei, N., N. Pavlidis, G. Tsokos, R. Elin, and P. H. Plotz.** 1981. Clinical significance of low creatine phosphokinase values in patients with connective tissue diseases. *JAMA* **246**:1921–1923.
158. **Whitaker, J. N., and W. K. Engel.** 1972. Vascular deposits of immunoglobulin and complement in idiopathic inflammatory myopathy. *N. Engl. J. Med.* **286**:333–338.
159. **Wilfert, C. M., R. H. Buckley, T. Mohanakumar, J. F. Griffith, S. C. Katz, J. K. Whisnant, P. A. Eggleston, M. Moore, E. Treadwell, M. N. Oxman, and F. S. Rosen.** 1977. Persistent and fatal central-nervous-system ECHO-virus infections in patients with agammaglobulinemia. *N. Engl. J. Med.* **296**:1485.
160. **Wolf, P. L., J. A. Lott, G. J. Nitti, and R. Bookstein.** 1987. Changes in serum enzymes, lactate, and haptoglobin following acute physical stress in international-class athletes. *Clin. Biochem.* **20**:73–77.
161. **Wolf, R. E., and B. A. Baethge.** 1990. Interleukin-1 alpha, interleukin-2, and soluble interleukin-2 receptors in polymyositis. *Arthritis Rheum.* **33**:1007–1014.
162. **Worrall, J. G., V. Phongsathorn, R. J. L. Hooper, and E. W. Paice.** 1990. Racial variation in serum creatine kinase unrelated to lean body mass. *Br. J. Rheumatol.* **29**:371–373.
163. **Yaneva, M., and F. C. Arnett.** 1989. Antibodies against Ku protein in sera from patients with autoimmune diseases. *Clin. Exp. Immunol.* **76**:366–372.
164. **Yaneva, M., J. Wen, A. Ayala, and R. Cook.** 1989. cDNA-derived amino acid sequence of the 86-kDa subunit of the Ku antigen. *J. Biol. Chem.* **264**:13407–13411.
165. **Zhang, M., P. D. Zamore, M. Carmo-Fonseca, A. I. Lamond, and M. R. Green.** 1992. Cloning and intracellular localization of the U2 small nuclear ribonucleoprotein auxiliary factor small subunit. *Proc. Natl. Acad. Sci. USA* **89**:8769–8773.