Myositis-specific autoantibodies or myositis-associated autoantibodies can often be found in serum of patients with polymyositis and dermatomyositis. The presence of these autoantibodies can be significant in patient diagnosis and classification. Recent studies have provided new information about many of these specific autoantibodies. Among the more important developments were identification of a new antisynthetase, reacting with asparaginyl-tRNA synthetase; the detection of antibodies to the tRNA\textsuperscript{is} in over a third of anti-Jo-1 sera; and the description of distinctive features of the histopathology of patients with anti-Jo-1. New information about the cellular role of the antigens was discovered, including a role for Mi-2 antigen in chromosomally-mediated regulation of transcription as part of a nucleosome remodeling complex, and a potential role for PM-Scl antigen in ribosomal RNA processing as part of an exosome. The reason for the production of the autoantibodies, and the reason particular antigens are targeted, are key questions. Recent studies have suggested that antigen cleavage during apoptosis, particularly by granzyme B, may be an important factor. Whether the antibodies play a role in tissue injury remains unknown.

The most important questions about the myositis AuAbs remain the reason for production of these antibodies, the reason the particular antigens are targeted, and the role of the antibodies in tissue injury. Several recent studies have addressed these issues. Progress has been made in understanding the cellular role of certain antigens, developing hypotheses to explain the multiple AuAbs produced, identification of new antibodies, and further clarification of the clinical associations of myositis antibodies.

Myositis autoantibodies
AuAbs in myositis may be grouped into three categories: myositis-specific autoantibodies (MSAs), myositis-associated autoantibodies (MAAs), and tissue-specific AuAbs. MSAs, such as anti-Jo-1, have high specificity for myositis in that most patients with the AuAb have myositis. MAAs, such as anti-U1RNP, have an association with myositis, but also occur in other conditions that may be more closely associated. They are not considered specific for the disease. Tissue-specific antibodies include AuAbs to muscle antigens, endothelial antigens, or other specific antigens not found generally in most cells. They are not involved in fundamental cellular processes, and are usually not detected by general screening tests such as those for antinuclear antibodies by indirect immunofluorescence, or for antibodies to extractable nuclear antigens by immunodiffusion. Tissue-specific antibodies with high specificity for PM or DM have not been identified.
Tissue-specific antibodies that can be helpful in the evaluation of patients with myositis were recently reviewed [5]. MSAs are more useful clinically for diagnosis of myositis than MAAs, as discussed recently [6], although there may be diagnostic utility in detection of MAAs. This does not necessarily mean MAAs are less significant for the disease, and does not exclude a pathogenetic role.

**Antisynthetases**

The most common group of MSAs is directed at the aminoacyl-tRNA synthetases (aaRS), a group of enzymes that attach tRNAs to the corresponding amino acid (catalyze formation of aminoacyl-tRNA). Anti-Jo-1 is the most common anti-aaRS and the most common established MSA. Other anti-aaRS antibodies are quite uncommon and are less common than other MSAs (Mi-2, SRP) and several MAAs.

Five antisynthetases had been identified by 1990, but recently a sixth anti-aaRS antibody was described. As discussed by Hirakata and Nagai in this issue, antibodies to asparaginyl-tRNA synthetase (anti-KS) were originally found in patients with interstitial lung disease [7•], and the majority of patients identified to date with anti-KS antibodies have had interstitial lung disease alone, rather than the full antisynthetase syndrome that includes myositis. Anti-KS with myositis has been seen, however. Some antisynthetases may not be myositis-specific; however, although the relative frequency of the components of the syndrome may vary for different antisynthetases, all antisynthetases appear to be specific for the antisynthetase syndrome.

The reason aaRSs as a class are targeted is not yet known. Because there is a general similarity in structure between synthetases, and analogy in function, which can lead to similarities in how they act in the cell, it is possible that a common structural factor is involved, but the antibodies to different synthetases do not crossreact. Hypotheses related to function of the enzymes have been proposed, as discussed by Hirakata and Nagai.

Synthetases have been divided into two classes based on overall structure, characteristic motifs, site of tRNA attachment, and other features. Most of the aaRSs that are antigens in myositis are members of Class 2, and have the characteristic motifs of Class 2 enzymes. The newly described anti-AsnRS fits this general category, further supporting the impression that this is an important feature contributing to the targeting of these enzymes. However, one of the synthetase antigens, isoleucyl-tRNA synthetase (OJ) is a Class 1 enzyme that is part of the multienzyme complex of synthetases [8]. Patients with this antibody also have a form of the antisynthetase syndrome [8–10], although it appears to have less myositis specificity than anti-Jo-1. This exception may have significance in understanding the factors involved.

Generally, previous studies have found that antisynthetases target the synthetase enzyme itself, rather than the associated tRNA. An exception is anti-PL-12, the antibody to alanyl-tRNA synthetase [11]. Sera with this antibody almost always also have antibodies that react directly with tRNAAla, generally in addition to antibodies that react with the synthetase [11,12]. This had been the basis of hypotheses for the possible generation of these antibodies. Previously, only rarely had sera with other antisynthetases been found to have antibodies to their cognate tRNAs; they were considered to react exclusively with the synthetase protein. Recently, however, Brouwer et al. [13] found that anti-Jo-1 sera are actually much more likely to react with tRNAAla than had previously been appreciated, occurring in 14 of 37 anti-Jo-1 sera tested. The reason for the difference in results is not certain. It was suggested that this may be due to use of a more sensitive technique for detection, or that more active sera were tested. The significance of the findings is also uncertain. They might reflect mechanisms for induction of anti-aaRSs, but may instead represent epitope spreading to another component of a macromolecular complex, because these high-affinity enzymes can exist in complex with the substrate tRNAs. As with anti-PL-12, generally the antibodies to the tRNA are found only in sera with antibodies to the synthetase enzyme. This tends to support the epitope spreading concept for development of the antibodies. This is analogous to this group’s similar findings with sera containing anti-U1RNP antibodies, in which antibodies to the U1RNA were also found [14–16]. In that case, antibodies to the RNA varied with disease activity. It will be interesting to study this aspect in the future, because better disease activity markers for myositis are needed.

In a preliminary report, Nagaraju et al. [17] described a mouse model in which myositis was induced by creating a transgenic mouse expressing HLA Class I molecules. These mice developed anti-Jo-1 antibodies, as demonstrated by a specific ELISA. Of great interest was the fact that the antibodies developed after induction of the myositis, and that they developed in response to perturbations specific to muscle. It has previously been observed that anti-Jo-1 occurred before the onset of clinically detectable myositis [18]. This has also been observed for anti-EJ [19]. It has been the general observation that the antibodies are almost always found in the first available sample from myositis and lupus patients. However, because our ability to detect clinical myositis is undoubtedly less sensitive than our ability to detect the antibody, our impressions of events may be misleading. These findings support the hypothesis that the anti-
bodies are related to fundamental etiologic factors in the disease, if not directly involved in pathogenesis themselves. This model holds great promise for understanding the relation of anti-Jo-1 to myositis.

The epitopes that anti-tRNA antibodies in antisynthetase sera react with have also been studied. Previous studies demonstrated that the anticodon loop was the target of anti-tRNAα in anti-PL-12 sera. A recent study [20] demonstrated that modified bases in this region played an important role. The study of Brouwer et al. [13] also addressed the issue of epitopes of the tRNAβs. Using ribonuclease protection and other techniques, it was concluded that the D and T loops contained a major conformational epitope. In contrast to the previous findings with anti-tRNAα, which Brouwer et al. confirmed, the anticodon loop was not involved. Thus, anti-tRNAs associated with antisynthetases do not necessarily target analogous regions. This is, however, consistent with the epitope spreading hypothesis, as they are exposed in the tRNA-synthetase complex.

In addition, preliminary characterization of the epitope on the alanyl-tRNA synthetase protein recognized by anti-PL-12 sera was reported [21]. The major reactive region for 3 sera tested was in the region of amino acids 730 to 951, which is toward the C-terminal end of the molecule, separated from the Class 2 motifs and catalytic region. This is not analogous to the region of the major epitope for histidyl-tRNA synthetase (the Jo-1 protein), which is in the N-terminal portion.

Generally, the myositis associated with antisynthetases was felt to be similar to the myositis seen in the absence of AuAbs. A recent report challenged this assumption, further supporting the concept that these AuAbs are defining a distinct subset of disease [22••]. The histologic pattern of perifascicular atrophy on the muscle biopsy has previously been associated with DM, and felt to represent effects of capillary loss and ischemic damage. This is generally not seen in PM or IBM, which instead show cell-mediated attack on non-necrotic fibers. Mozaffer and Pestronk [22••] found that perifascicular atrophy and perimysial inflammation (another characteristic feature of DM) were seen in all of their anti-Jo-1-positive myositis patients, most of which did not have the DM rash. In contrast to findings associated with their DM patients, however, they did not find capillary loss, with a capillary index of 0.88 compared with 0.55 in DM. Also, they noted fragmentation of perimysial connective tissue, which was not found in most DM patients, but was characteristic of fasciitis but not PM/DM (although fasciitis did not have myofiber abnormalities). Cell-mediated attack on non-necrotic fibers was uncommon in anti-Jo-1 biopsies. This suggests that there are significant distinctive features in anti-Jo-1-associated myositis, possibly reflecting unique pathogenetic mechanisms.

All described antisynthetases have generally similar clinical associations, including the well-known and well-established association with a high frequency of interstitial lung disease in myositis patients. The clinical associations of anti-Jo-1 have been examined in several series, as discussed previously [23], but the clinical associations of non-Jo-1 antisynthetases generally have been based on very small series and analogy to anti-Jo-1. Certainly, the similarities of the non-Jo-1 antisynthetase patients thus far observed to those with anti-Jo-1 is striking. However, additional information is needed. A recent case report by Wasko et al. [24] demonstrated that inflammatory arthritis may occur in anti-PL-7 patients, as it may in those with anti-Jo-1. Additional patients with anti-OJ have been reported, with similar anti-synthetase syndrome to those previously identified [9,10]. One report suggested that AuAbs, occasionally including anti-Jo-1, could occur in inclusion body myositis [25], but the techniques used to detect the antibody were not discussed. Others have found that anti-Jo-1 did not occur in IBM [26]. The latter is consistent with our unpublished findings in screening tests, and in previous studies [27]. Although the frequency of ANA may be higher in IBM than the 5% found in the general population [27], we have not seen MSAs or MAAs in IBM patients.

**Anti-Mi-2**

The role of aaRSs in cell biology and biochemistry had been recognized long before their identification as autoantigens. In contrast, two myositis antibodies, anti-Mi-2 and anti-PM-Scl, were identified early by double immunodiffusion, but little was known about the nature of the antigens until very recently. Their molecular structures were studied and characterized prior to any knowledge about their cellular function. Recent studies have begun to provide some information in this regard. Anti-Mi-2 is myositis-specific, and is usually associated with DM as opposed to PM [27,28], with rare exceptions [29]. The molecular structure of Mi-2 antigen was studied by two groups in 1995 [30,31], and both groups identified a protein that reacted with anti-Mi-2 sera. The proteins were different [32], but they showed a relatively high degree of overall similarity, with regions of near identity. They were labeled Mi-2α and Mi-2β. The Mi-2α sequence was later more fully characterized [33,34]. These proteins showed sequences related to zinc fingers (“PHD fingers”, for plant homeodomain), found in a number of proteins [32]. Several of these proteins are involved in transcriptional regulation at the chromosomal level, by unwinding chromatin and thus providing access to DNA for transcription to occur. The protein that Mi-2β was found to be most closely related
to, the human SNF2 protein [30], is involved in this process. Both forms of Mi-2 were also found to have a series of helicase motifs, including a DEAH region, suggesting that they function as helicases in the cell, which would be compatible with a role in chromatin modification. They have a “chromo” domain, “helicase” domain, and “DNA binding” domain, and have been formally named CHD3 (Mi-2α) and CHD4 (Mi-2β), as part of a larger family of proteins with these domains [33]. It was then found that Mi-2β occurs in a complex that also included two other proteins that were known to be involved in chromatin modification, the histone deacetylases [35]. Acetylation of core histones can lead to gene activation by modification of the nucleosome structure, resulting in access for transcription or other processes. Mi-2-related helicases are thought to act by an alternative, ATP-dependent mechanism to modify the nucleosome. The complex containing Mi-2β and histone deacetylases, labeled the nucleosome remodeling-deacetylase (NuRD) complex, thus has alternative mechanisms for affecting the nucleosome [35–38]. Other proteins in the complex, or transiently associated with the complex, may affect its function, and target the NuRD complex to particular genes [35,36,39–41]. One function of NuRD may involve the “silencing” of genes with methylated DNA [36,37]. It was found also that the human papillomavirus oncoprotein (E7) must bind to the Mi-2β protein (as well as Rb protein) in order to accomplish transformation of cells [42]. Although anti-Mi-2 immunoprecipitates this entire complex, including Mi-2 protein (migrating at 240 kd) [43], histone deacetylases, histone binding proteins, and others, the major antigen is the Mi-2 240 kd protein [31,43]. Thus far, Mi-2α has not been found in this complex, and the role of Mi-2α has not been as well characterized [35].

**Anti-PM-Scl**

The role of PM-Scl antigen is less clear. Although 75% of patients with this antibody have at least some evidence of myositis, anti-PM-Scl is considered a MAA rather than a MSA because some patients have scleroderma alone, or rarely other conditions [44]. For some, myositis is the main manifestation, and half of those with myositis have a DM rash. Thus, anti-PM-Scl is more closely associated with myositis than other MAAs. The antigen has been shown to be a multi-subunit complex of at least 11 proteins [44]. The major antigen is the 100 kd protein [45], because almost all anti-PM-Scl patient sera react with it. Many anti-PM-Scl patient sera also react with the “75kd” protein, which migrates in the 70–75kd region on PAGE but has a calculated molecular weight closer to 40 kd [46]. The remaining proteins are smaller and usually nonantigenic. Immunofluorescence and other studies localized the complex to the nucleolus and the nucleus [47,48], but little else was known about the antigen. Determining the sequence of the antigenic proteins in the early 1990s [45,46,49] provided few clues about its function initially. However, a later analysis and comparison of sequences found a relationship to RNase D of *Escherichia coli* [50]. A further important clue to its cellular function was revealed recently by identifying and characterizing a protein in yeast with homology to the 100kd PM-Scl protein [51]. This protein, Rrp6p, has a role in processing of the 3’ end of the 5.8S ribosomal RNA. A single large RNA precursor is processed to produce the 18S, 5.8S, and 25S ribosomal RNAs. Final maturation of the 3’ end of 5.8S requires Rrp6p and other proteins. Among these are Rrp4p and several other yeast exonucleases and proteins that are components of a multi-subunit complex called the “exosome.” The exosome performs a crucial cellular function, and is required for growth. Briggs *et al.* [51] found Rrp6p to be distinct from the exosome, although weak or transient association with it was not excluded. The possibility of an effect of Rrp6p on mRNA was not excluded, although it did not seem to affect tRNAs.

A subsequent study, however, suggested that the PM-Scl complex was the human homologue of the exosome. Allmang *et al.* [52••] found that the exosome, which has several components with 3’–5’ exonuclease activity, was involved in several forms of RNA processing. It had an overall structure and size similar to that of PM-Scl, and they found that at least 3 components of the yeast exosome had homologous counterparts in the PM-Scl complex, including the 100 kd protein. They also found a cytoplasmic form of the complex without the 100 kd protein, which is consistent with the finding that anti-PM-Scl sera do not usually show cytoplasmic staining by indirect immunofluorescence, because 100 kd is the major antigen.

The epitopes recognized by anti-PM-Scl have been among the most extensively studied of myositis antigens. The major reactivity was previously localized within a small stretch of the 100 kd protein, and a significant secondary epitope was also identified [49,53,54]. Now additional information is available suggesting a conformational epitope [55]. This is of interest, because the previously identified epitopes, which were preserved by blotting though not strictly linear, did not explain all activity of the antibodies.

**New antibodies**

Several recent reports have dealt with new AuAb associations with myositis. These include studies of AuAbs that were newly described but not specific for myositis, such as AuAbs to the proteasome [56,57], or studies of AuAbs reacting with muscle cells [58]. Also reported were studies of several autoantibodies in myositis that are primarily found in other conditions, such as anti-
endothelial cell antibodies [59], antihistone antibodies [60], and antiphospholipid antibodies [61]. It had previously been found that autoantibodies to 52 kd Ro/SSA (Ro52) were associated with PM and DM, and were particularly frequent (58%) in sera that had anti-Jo-1 [62]. Recently, Frank et al. [63] found that this association between anti-Ro52 and anti-Jo-1 can be extended to certain other MSAs and MAAs. Sera with other antisynthetase autoantibodies (anti-PL-7 or anti-PL-12) had a comparable frequency of anti-Ro52 (69% and 67%, respectively) to that of anti-Jo-1 (70% in this study). Also, 47% of anti-PM-Scl sera, and 43% of anti-SRP sera had anti-Ro52. As in the study of Rutjes et al. [62], a much higher proportion of anti-Ro52-positive sera with myositis antibodies were anti-Ro60 negative than is seen in lupus or Sjogren syndrome. Anti-Ro52 frequency was not elevated in rheumatoid arthritis or scleroderma, nor was it increased in myositis sera with anti-Mi-2. The significance of these findings remains to be determined.

Of particular note were AuAbs that had not previously been identified, but may be specific for myositis. Among these were the AuAbs to human PMS1 and PMS2, proteins involved in mismatch repair. These AuAbs were described recently by Casciola-Rosen et al. [64] in a preliminary report. It was also found that this antigen was cleaved by granzyme B [65••]. The targeting of both hPMS1 and hPMS2 proteins suggests crossreactivity or a targeting of the class, as with antisynthetases. Further studies of sensitivity and specificity will be needed, along with studies to characterize the subgroup associations. A second antibody of note, also described only in preliminary reports thus far, was anti-MJ, which was associated with juvenile DM [66,67]. The anti-MJ subgroup varied in severity and did not have distinctive characteristics, but the association with juvenile DM is of particular interest because such a small proportion of juvenile DM patients have the previously identified MSAs and MAAs, despite the fact that this is the form of myositis for which there is the best evidence for humoral mechanisms mediating tissue injury. It is detected by immunoprecipitation of a 140 kd protein, and was more common in two juvenile DM groups tested than other myositis AuAbs.

**Mechanisms of autoantibody generation**

These findings raise again the question of how the function of the antigens might relate to the observation that the antibodies are associated with myositis. It was suggested by Zhang et al. [36] that the finding that certain components of the NuRD complex played a role in malignancy might relate to the association of malignancy with DM. However, most patients with anti-Mi-2 do not have malignancy (with occasional exceptions), and most patients with malignancy-associated myositis do not have anti-Mi-2 or other myositis AuAbs [27]. There is no obvious link between the functions described for Mi-2 and those of the synthetases.

There is increasing interest and support for an alternative explanation for the generation of AuAbs and the selection of particular antigens. It has been found that many autoantigens (AuAgs) redistribute to and cluster on the surface of cells undergoing apoptosis [68]. In addition, many autoantigens are cleaved by apoptotic proteases during apoptosis, potentially producing unique fragments of the antigens with potential new epitopes [69–71]. However, as pointed out by Rosen et al. [71] and by Casciola-Rosen et al. [65••], caspase cleavage may not be the mechanism for generation of these unique fragments and epitopes. Not all antigens are cleaved by caspases, and caspase cleavage products would be expected to have been seen and tolerated by the immune system. In contrast, Casciola-Rosen et al. [65••] found that granzyme B might be more important in this regard. Granzyme B, which is involved in induction of apoptosis during lymphocyte cytotoxicity, was found to cleave most AuAgs, and to produce unique fragments during cleavage. For non-AuAbs, granzyme B either did not cleave at all, or did not produce unique fragments. Even those antigens that can be cleaved by caspases were cleaved by granzyme B to generate different fragments. For example, Utz et al. [72] had previously found that the 72 kd protein of SRP was cleaved by caspases during apoptosis. Although the 54 kd protein was previously felt to be the major antigenic component of SRP, the 72 is also antigenic for some patients. Casciola-Rosen et al. [65••] found SRP72 to be cleaved by granzyme B to produce different products. Other myositis-related AuAgs cleaved by caspases but also granzyme B were Mi-2, and the new hPMS1 and hPMS2, as well as the MAA U1-70_kd protein. In addition, the MSAs Jo-1, OJ, and PL-12, and the MAAs Ku70 and PM-Scl, were cleaved by granzyme B but not by caspases. Five of the eight tested AuAgs that were not cleaved by granzyme B were MSAs or MAAs: PL-7, EJ, R060, Ro52, and Ku80. In all, 29 AuAgs were tested, and 21 were cleaved by granzyme B, with unique fragments generated, whereas 19 non-AuAgs were tested, and none were cleaved with unique fragments ($P<0.0001$).

Although these observations are of great interest, it remains to be explained how the particular disease manifestations are associated with particular AuAbs. This would be clear if the antibodies were themselves responsible for tissue injury, but this is not yet established. There is more evidence for AuAbs being involved in tissue injury in DM than in PM, but even in DM, it is not yet known whether the AuAbs to cellular antigens that have been associated with the disease are the same ones involved in tissue injury.
References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

• Of special interest
•• Of outstanding interest


8 A new antisynthetase was described for the first time since 1990. It was more closely associated with interstitial lung disease than with myositis.


The pathology associated with anti-Jo-1 antibodies looks superficially similar to that seen with antibody-negative myositis patients, but this careful analysis revealed important differences. As with dermatomyositis, perifascicular atrophy and perimysial inflammation are commonly seen, but no decrease in muscle capillaries was seen. All anti-Jo-1 patients also showed fragmentation of perimysial connective tissue.


Update on myositis-specific and myositis-associated autoantibodies

Targoff 481


This study suggests that the PM-Scl complex, previously described as containing 11 proteins, is probably the homologue of the yeast exosome, with homology demonstrated for at least three components.


This exciting study describes a characteristic of most autoantigens, the ability to be cleaved by granzyme B into unique fragments, which distinguishes this group of proteins from other proteins and provides a hypothesis for generation of autoantibodies in connective tissue diseases. Included in their study were several MSAs and MAAs. Nine of the 14 MSAs or MAAs tested showed granzyme B cleavage.


