Anti-KJ: A New Antibody Associated with the Syndrome of Polymyositis and Interstitial Lung Disease

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Abstract

Antibodies to aminoacyl-tRNA synthetases (anti-Jo-1, anti-PL-7, anti-PL-12) have been found in the serum of some patients with polymyositis (PM). Patients with these antibodies have an unusually high rate of interstitial lung disease (ILD) in association with their PM. Two patients (K.J. and B.T.) with severe ILD and PM were found to have antibodies to a cytoplasmic antigen, but tests to determine whether the antigen was an aminoacyl-tRNA synthetase were negative, including tests of KJ serum for inhibitory effects on the 20 synthetases. KJ immunoprecipitates did not contain tRNA, in contrast to antisynthetase sera. When IgG samples were added to a reticulocyte in vitro translation system at a concentration of 0.3 mg/ml, KJ IgG inhibited globin mRNA translation by 98%, while anti-Jo-1 IgG inhibited 62% and normal IgG had little effect. Thus, both anti-KJ and the antisynthetases are directed at antigens that are involved in translation and protein synthesis, and both are associated with the syndrome of lung disease and PM. This syndrome may be associated with antibodies to translation-related proteins in general, which may have implications for the link of PM and enteroviruses, which are mRNA viruses.

Introduction

Polymyositis (PM)[1] and dermatomyositis (DM) are inflammatory myopathies whose pathogeneses appear to involve autoimmunity (1), one manifestation of which is the frequent finding of serum autoantibodies. Routine testing with indirect immunofluorescence and Ouchterlony immunodiffusion can detect autoantibodies in the serum of almost 90% of patients with PM and DM (2). Some of these autoantibodies occur almost exclusively in patients with myositis (3). These include

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1. *Abbreviations used in this paper:* CK, creatine kinase; DM, dermatomyositis; FVC, forced vital capacity; ILD, interstitial lung disease; PM, polymyositis; SRP, signal recognition particle.

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antibodies to histidyl-tRNA synthetase (4, 5) (labeled anti-Jo-1 [6]), and antibodies to the unidentified proteins labeled PM-Scl (7) and Mi-2 (8). None of these specificities is found randomly in myositis patients, but all tend to be associated with particular clinical features, suggesting that they might help to define subgroups of patients (3, 9). Similarly, autoantibodies to ribonucleoprotein complexes are associated with characteristic clinical subgroups in systemic lupus erythematosus (SLE) (10).

PM and DM have also been divided into subgroups based on clinical criteria (11, 12), including the presence of a rash (DM), an associated malignancy, childhood onset, or an associated connective tissue disease. This clinical heterogeneity may indicate that PM and DM represent more than one disease, and it is possible that patients in one subgroup are more homogeneous. The groups defined by the myositis-associated autoantibodies, however, do not necessarily conform to the clinically defined subgroups noted above. Anti-Mi-2, for example, is exclusively seen in DM, but is seen in only 20% of that group, and may be seen in adult DM alone, DM with malignancy, or juvenile DM (8). Of the autoantibody associated clinical subgroups, that of anti-Jo-1 is the best defined. Anti-Jo-1 is the most common myositis associated antibody, being present in ∼ 20% of myositis patients in most studies (2–4, 6–7, 13–14). Most patients with anti-Jo-1 have PM, without the DM rash (2, 3, 6, 9, 13–15), although a higher proportion in DM was found in one population (16). Also, there is consistent evidence that the majority of patients with anti-Jo-1 have diffuse interstitial lung disease (ILD) along with their PM (15–20). The ILD can be severe and fatal, and dominate the patient's clinical picture. The presence of this complication has not been used to distinguish clinical subgroups of patients in most classifications. An additional genetic marker associated with this subgroup is HLA-DR3, which in one study (13) was present in 64% of anti-Jo-1 positive patients, compared to only 22% of anti-Jo-1 negative patients.

The enzyme, histidyl-tRNA synthetase, to which anti-Jo-1 is directed, is one of the aminoacyl-tRNA synthetases, a group of cytoplasmic enzymes that catalyze the attachment of a particular amino acid to its cognate tRNA (and may have other functions) (21). There is a distinct enzyme in the cytoplasm for each amino acid (as well as a second in the mitochondria). Antibodies to two of the other aminoacyl-tRNA synthetases have been reported in myositis patients, anti-threonyl-tRNA synthetase (anti-PL-7) (22, 23) and anti-alanyl-tRNA synthetase (anti-PL-12) (24). Although exceptions occurred, almost all the patients in whom these antibodies were found had myositis (9, 22, 24), and most also had ILD (9). We have recently presented evidence of antibodies to isoleucyl-tRNA synthetase in two patients and glycyl-tRNA synthetase in one (25). There were signs of myositis in all three patients, and severe ILD in one with anti-ile-tRNA synthetase. Additional

features have also been noted that were more common in patients with antibodies to synthetases than in others with myositis, including arthritis (9, 15) and Raynaud's phenomenon (9). Thus, all antisynthetases may be associated with the same clinical subgroup.

The current study began with the finding of a patient, K.J., with severe ILD and PM, and a high titer anticytoplasmic antibody on indirect immunofluorescence. A second patient, B.T., had a similar syndrome and an identical line in immunodiffusion. This antibody, however, was found not to be anti-Jo-1, anti-PL-7, or anti-PL-12. We suspected that these patients belonged to the same clinical subgroup as that of patients with antisynthetases. We felt that it was particularly important to learn more about this cytoplasmic antigen, because it could give another perspective on the features that lead to antigenicity in this syndrome. In this study we defined and partially characterized the KJ antigen, and looked for a relationship of the KJ antigen to other targets of autoantibodies in these patients.

Methods

Patients

Case 1. K.J., a 30-yr-old black woman, developed shortness of breath on exertion and new onset Raynaud's phenomenon 5 mo before hospital admission. By the time of admission, she could walk only 6–8 m before resting. She was unaware of having had any rash or muscle weakness, and did not report dry eyes or dry mouth. She had never smoked. On examination, her respiratory rate was 24/min with some distress, and there were fine bibasilar rales and an accentuated P2. Mild to moderate proximal muscle weakness was present, as well as mild pigmentary changes on the face that were not typical of DM. There were no other rashes or sclerodactyly. Creatine kinase (CK) level was 3,330 U/liter (55.51 μkat/liter) of which 3,157 U/liter (52.63 μkat/liter) was MM. Serum protein electrophoresis showed a mild polyclonal increase in gamma globulin (IgG level = 2,150 mg/dl). Tests of rheumatoid factor and antimitochondrial antibodies were negative, and C3 and C4 were normal. The erythrocyte sedimentation rate was 38 mm/h. Thyroid function tests were normal. PO_2 (aB) was 54 mmHg (7.2 kPa). The forced vital capacity (FVC) was 0.85 liter (21% of predicted) without obstruction (FEV_1/FVC = 84%). There was a diffuse interstitial pattern on chest x ray, and evidence of primary myopathy with suggestion of necrosis on electromyography. A muscle biopsy was normal. The clinical diagnosis was diffuse interstitial lung disease secondary to PM, and she was treated with prednisone at 1 mg/kg per d (90 mg/d). 3 mo later, while still taking 90 mg/d prednisone, her CK level had fallen to 320 U/liter (5.33 μkat/liter) and her strength was clinically normal. The interstitial pattern on chest x ray persisted, her pO_2 was 67 mmHg (8.9 kPa), and her forced vital capacity was 0.98 liter. 18 mo later, her CK level was normal and the lung disease had improved, although the Raynaud's phenomenon persisted. She remained on prednisone, but her dosage had been reduced to 30 mg/d.

Case 2. B.T., a 53-yr-old white housewife, was well until June, 1983 when she experienced increasing fatigue, particularly on climbing stairs, brushing her hair, and performing general household activities. She had no Raynaud's phenomenon or dry eyes, and denied smoking. Her symptoms progressed over a period of weeks until she was not able to get out of bed unaided. Her respirations were 24/min. There was generalized muscle weakness, especially of the shoulder girdle, thighs, and paraspinal muscles. Chest x ray revealed a diffuse bilateral infiltrate, especially at the bases. Laboratory studies showed a mild normocytic normochromic anemia with a hemoglobin of 11.0 g/dl and elevations of the CK to 4,000 U/liter (66.68 μkat/liter) (normal 50–250 U/liter), the AST to 150 U/liter (2.50 μkat/liter) (normal 7–40 U/liter), and ALT to 300 U/liter (5.00 μkat/liter) (normal 7–40 U/liter). There

was no myoglobin in the urine. The rheumatoid factor and antinuclear antibody were negative. Complement studies were normal. Electromyographic findings were compatible with PM and indicated insertional irritability, increased motor unit potentials, sharp spikes, and low amplitude polyphasic potentials. Histopathology of the deltoid showed muscle degeneration with necrosis and areas of regeneration. The pO_2 was 70 mmHg (9.3 kPa), the forced vital capacity was 1.5 liter (55% of predicted value), and the FEV_1/FVC ratio was 85%. A diagnosis of PM with pulmonary infiltrative disease was made. The patient was treated with 80 mg/d i.v. prednisolone. This dose was titered according to her clinical response and CK levels. Within 4 wk the CK had dropped to 500 U/liter (8.34 μkat/liter) and the patient was on 40 mg/d prednisone orally. After 12 wk of therapy, there was marked improvement in muscle strength, her CK had dropped to 300 U/liter (5.00 μkat/liter), and the prednisone dose decreased to 15 mg/d.

Evaluation for headache in 1984 revealed a right parietal meningioma and a pericallosal aneurysm. The tumor was surgically excised and the aneurysm successfully repaired. In 1985 she was rehospitalized with an exacerbation of PM. At this stage she complained of Raynaud's phenomenon precipitated by exposure to cold temperatures. Examination of the hands revealed taut skin and sclerodactyly with telangiectasiae. X ray of the chest showed diffuse pulmonary fibrosis with bibasilar honeycombing. On esophageal manometry there was a severe motility disorder of the lower two-thirds of the esophagus and the lower esophageal sphincter, with normal pharyngeal and cricopharyngeal muscles. Rheumatoid factor was still absent but the antinuclear antibody test was reported as positive with a titer of 1/160.

Sera. Serum or plasma from patient K.J. served as the standard for the KJ antibody. Other samples tested in the study came from a bank of sera collected over 15 yr from patients with myositis. Patients were considered to have myositis if they satisfied the criteria of Bohan and Peter (11, 12) for definite or probable PM or DM. Immunoglobulin from a lupus serum known to contain antibody to the ribosomal P proteins (26) (kindly provided by Dr. Keith Elkon) was also tested. Serum samples were stored at −20°C.

Aminoacylation inhibition. KJ serum was tested for antibodies to aminoacyl-tRNA synthetases by determining its ability to inhibit the aminoacylation reactions of the individual synthetases. Histidine aminoacylation inhibition was performed as described previously (14). Aminoacylation reactions for the other amino acids were set up using similar conditions. 1-4 A_{260} U of calf liver tRNA preparation (Boehringer-Mannheim, Indianapolis, IN) contained enough tRNA for most of the amino acids (although *E. coli* tRNA was used for the alanine reaction). The test amino acid was included in labeled form (^{3}H except ^{14}C-Asn and ^{35}S-Cys) and diluted with unlabeled amino acid so that the total concentration (10–20 μM) was in significant excess of the approximate tRNA accepting capacity for that amino acid. Calf liver extract, prepared using the method of Pearson (27) modified as previously described (14), was used as the enzyme source (partial purification by DEAE was used for some enzymes). The limiting concentration of synthetase preparation was determined empirically for each individual aminoacylation reaction, by preparing a dilution curve, in order that any inhibition of enzyme activity was reflected in the results. To test inhibition, 6 μl of the preparation containing the enzyme activity was preincubated with 3 μl of KJ serum at 1/10 dilution (diluted in 0.15 M NaCl, 0.01 M phosphate buffer pH 7.2 [PBS]) for 1–2 h at 4°C. The remainder of the reaction was as previously described (14), with 5-μl aliquots taken at 10 and 20 min for counting. In most cases, the 10-min readings were used for calculating inhibition. {[(Counts without serum) − (counts with test serum)]/(counts without serum)} × 100 represents the percent inhibition, and is a reflection of the amount of antibody. Greater than 40% inhibition was considered significant, based on experience with other anti-synthetases, since even low titer antibodies to other synthetases inhibit > 80% in almost every case (14, 22, 28).

Protein A-assisted immunoprecipitation. The method of Forman et al. (29) for immunoprecipitation from unlabeled cell extracts was used. 2 mg of protein A-Sepharose (Pharmacia Fine Chemicals, Piscataway,

NJ) were used per test, and washed in 0.01 M Tris buffer pH 8.0, with 0.5 M NaCl and 0.1% NP-40. 10 μl serum in 500 μl of the same buffer was incubated with the beads with gentle mixing, for at least 2 h at 4°C. The beads were then washed and used for immunoprecipitation. HeLa cell extract for immunoprecipitation was prepared according to Forman et al. (29) from cells grown in 150 cm^2 flasks, using the equivalent of 1 flask for four samples sonicated in 0.15 M NaCl in 0.05 M Tris-HCl buffer at pH 7.4. The extract was precleared with protein A-Sepharose that had not been coated with antibody, to decrease nonspecific binding. Coated protein A-Sepharose was combined with 500 μl HeLa cell extract, and incubated at least 2 h at 4°C. The phenol-extracted immunoprecipitates were analyzed on 7 M urea-10% polyacrylamide gels, which were stained with silver stain (Bio-Rad Laboratories, Richmond, CA).

In vitro translation. A nuclease-treated rabbit reticulocyte lysate system similar to that of Jackson and Hunt (30) was used (from a commercial kit from Bethesda Research Laboratories, Gaithersburg, MD). A standard translation reaction with globin messenger RNA was set up, with [^{3}H]leucine as the labeled amino acid (leucine is represented in rabbit globin [31]). IgG purified from patient serum (see below) was dialyzed against water, and concentrated. In order to measure inhibition, the level of translation in the presence of patient IgG was compared to translation in the presence of normal IgG or in the absence of added IgG. 9 μl of lysate was preincubated with 9 μl of the IgG solution in water (or water alone as control), usually at 1 mg IgG/ml. Since it is possible that the IgG could have contamination with ribonucleases, 1 U/μl of placental ribonuclease inhibitor was also added, which was reported to be effective for this purpose (32). The antibody was preincubated with all reaction components except the mRNA for 30 min at 4°C. The mRNA was then added to start the reaction (completing a 30-μl reaction volume), which was otherwise carried out as in kit directions, including addition of pancreatic ribonuclease at 60 min. Two aliquots of 5 μl each were taken at 75 min and counted as for aminoacylation except that an additional bleaching step was added (formic acid and peroxide [33]).

For some experiments, instead of adding IgG directly to the reaction mixture, protein A-Sepharose beads coated with antibody were preincubated with the lysate. 5–7 mg of protein A-Sepharose were washed and preincubated with 100 μl of serum in 500 μl of 0.02 M Tris buffer pH 7.2 with 0.15 M NaCl (or, where indicated, 500 μl of IgG at 1 mg/ml). The Sepharose was washed again three times in buffer and two times in water, and 30–40 μl lysate and an equal volume of sterile water were added to the pelleted Sepharose. The tube was stirred frequently over 30 min incubation at 4°C. The Sepharose was then centrifuged out and the lysate was used for translation, and compared to lysate that had been treated with Sepharose coated with other sera or no serum. For further confirmation that an antigen/antibody reaction was responsible for the observed effects, the 5–7 mg of protein A-Sepharose beads were coated with 100 μl of serum and incubated with HeLa extract (or HeLa extract buffer as control) prepared as in the immunoprecipitation procedure described above, and the washing steps repeated, before incubation with the lysate. Anti-KJ-coated Sepharose, with and without incubation with HeLa extract, was compared to Sepharose coated with normal IgG or anti-Jo-1 controls.

Partial purification of the KJ antigen. The KJ antigen was purified from bovine liver, obtained fresh, transported on ice to the laboratory, and either used immediately or frozen and stored at −40°C until used. Extract was prepared at 4°C by homogenization by blender (at medium speed) in PBS with 0.01 M sodium azide and 0.001 M PMSF (2 vol PBS/weight of tissue), followed by centrifugation in a Sorvall SS-34 rotor at 20,000 rpm × 1 h. The major step in purification was affinity chromatography, which was performed using a method similar to that previously described (8, 14). The IgG fraction was purified from 50 ml of KJ serum using DEAE (see below). KJ IgG was coupled to Affi-gel (Bio-Rad Laboratories) hydroxysuccinamide-agarose in 0.1 M bicarbonate buffer at pH 8.3, with > 90% coupling. The immunoadsorbent was washed extensively, including with the intended eluting agent (3 M MgCl$_2$). Later experiments were performed using a second column

prepared in a similar manner (from 75 ml of serum taken at a time when the titer was slightly lower). After liver extract was applied to the column in excess of adsorbing capacity, the column was extensively washed with 0.5 M NaCl in 0.05 M Tris buffer at pH 7.2 with 0.01 M Na azide and 0.0001 M PMSF (TBS), and eluted with 3 M MgCl$_2$. Fractions containing protein (as determined by optical density at 280 nm) were pooled and concentrated (by ultrafiltration) and applied to a Superose 12 gel filtration column using Fast Protein liquid chromatography (FPLC; Pharmacia Fine Chemicals) with TBS as running buffer. Antigenic activity was monitored by ELISA.

ELISA. To detect the presence of KJ antigen in purified preparations and column fractions, a standard indirect ELISA was used, similar in design to that which was described previously (8, 14). 10–15 μl of pooled active fractions from FPLC were added to the microtiter well, followed by PBS to bring the volume to 100 μl. After overnight incubation, plates were washed in PBS with 0.05% Tween-20 and blocked with 2% BSA in PBS. The ELISA activity of KJ serum against each fraction was compared to that of normal, BT, and anti-Ro/SSA sera for the same fraction (sera were diluted 1/300 in blocking solution). After incubation and washing, goat anti-human IgG (gamma chain specific) (or F(ab')$_2$ fragments of this antibody) conjugated to alkaline phosphatase (Sigma Chemical Co., St. Louis, MO) was applied, diluted in the washing solution. After incubation and washing, *para*-nitrophenyl phosphate substrate was applied. ELISA for anti-Ro/SSA was performed as previously described (34).

Polyacrylamide gel electrophoresis and Western blotting. SDS-PAGE was performed according to Laemmli (35), modified as previously described (8, 14). For protein staining, the silver stain was used. Most samples were dialyzed against water and concentrated, before addition of 1/3 vol of fourfold concentrated sample buffer, (containing beta-mercaptoethanol except as noted). Western blotting was performed according to Towbin (36), with some modification similar to that described previously (14). Proteins were transferred to nitrocellulose in 0.025 M Tris-0.192 M glycine buffer at pH 8.3 with 20% methanol. The nitrocellulose was then blocked with 5% BSA in 0.01 M Tris, 0.15 M NaCl, pH 7.4. Test sera were diluted 1/100 in blocking buffer. The nitrocellulose was washed with 0.01 M Tris-0.15 M saline buffer pH 7.4 with and without 0.05% Triton X-100. Conjugate (same as for ELISA) was then applied, diluted 1/500 in blocking buffer, followed by BCIP/NBT alkaline phosphatase substrate (Kierkegaard-Perry).

Isolation of ribosomes. Ribosomes derived from microsomes were isolated from rat liver by the method of Moldave and Sadnik (37). In this method, microsomes were obtained by homogenization in 0.35 M sucrose (in 35 mM KHCO$_3$, 20 mM K$_2$HPO$_4$, 25 mM KCl, 4 mM MgCl$_2$ buffer at pH 7.6), and centrifugation for 2 h at 100,000 g. The sediment was resuspended in 0.5 M NH$_4$Cl (in 50 mM Tris HCl buffer at pH 7.6 with 10 mM MgCl$_2$, and 6 mM mercaptoethanol), treated with deoxycholate (final concentration 1.5%), and centrifuged through a discontinuous sucrose gradient (0.5 and 1.0 M sucrose). The postmicrosomal supernatant was further centrifuged for 16 h at 80,000 g to remove remaining subunits. The resulting supernatant was referred to as the postribosomal supernatant. The ribosomes were resuspended in 0.35 M sucrose (in 50 mM Tris buffer at pH 7.6 with 50 mM NH$_4$Cl, 4 mM MgCl$_2$, and 1 mM DTT).

Fluorescence inhibition. Since Western blots and immunodiffusion were not sensitive enough to detect the KJ antigen in subcellular fractions (ribosomes, postribosomal supernatant, whole extract, etc.), the technique of fluorescence inhibition was used. In this experiment, KJ serum was absorbed with the test extract before using the KJ serum in indirect immunofluorescence. Indirect immunofluorescence titer would be decreased if antigen were present in the test extract, and able to bind the anti-KJ antibodies. This method was also used to confirm that the cytoplasmic antigen had been recovered from the affinity column (by adding affinity purified antigen to KJ serum before using the serum in indirect immunofluorescence). Since KJ serum reacted in immunodiffusion with the affinity purified antigen, this evidence that the affinity purified antigen preparation contained the cytoplasmic

antigen provided further evidence that the antibody detected by immunodiffusion was the same as the antibody giving a cytoplasmic pattern of indirect immunofluorescence.

KJ serum was diluted 1/100 in PBS. One part diluted KJ serum was mixed with nine parts test extract (or PBS for control), and the samples were tested by indirect immunofluorescence using HEp-2 cell substrate. Any decrease in fluorescence activity represented the presence of KJ antigen in the extract. Active extracts were then serially diluted and retested to estimate the KJ antigen content. The KJ serum sample was used at 1/1,000 final dilution because it was not maximally active, but was still strong. The indirect immunofluorescence procedure, using commercial HEp-2 slides (Breit Laboratories, West Sacramento, CA), was otherwise performed as usual (2).

Purification of IgG. In some experiments, human Cohn fraction II (Sigma) was used for normal human IgG, while in others, as indicated, IgG was purified from test serum as from normal serum. Standard anti-Jo-1 IgG was purified from one of the three sera out of 24 with the highest titer of anti-Jo-1 as determined in a recent study (14) using both ELISA and histidine aminoacylation inhibitory activity. IgG was purified from test sera by one of two methods. First, serum was dialyzed against 0.2 M Tris-HCl buffer at pH 7.4, and applied to a similarly equilibrated DEAE column (DE-52; Whatman Inc., Clifton, NJ) of three- to fivefold volume, with collection of the effluent as IgG. This method was also used for preparation of IgG for the affinity column. In the second method, serum was applied to protein A-Sepharose (Pharmacia) (approximately fivefold gel/serum ratio) that had been equilibrated with TBS. The column was washed with TBS, and IgG was then eluted with glycine-HCl buffer at pH 3.0, and dialyzed against water for translation studies. For absorption of the IgG preparation with protein A-Sepharose, protein A purified IgG was mixed 1:1 with TBS

and separated into fractions 1 and 2. A portion of fraction 1 was applied to a fresh protein A-Sepharose column. The flow-through fraction from this column and fraction 2 were dialyzed against water and concentrated (the flow-through of fraction 1 was concentrated twice as far as fraction two).

Other methods. Protein concentrations were determined by the Bio-Rad protein assay, based on the method of Bradford (38), except for IgG concentrations, which were determined by optical density at 280 nm. Ouchterlony immunodiffusion and indirect immunofluorescence were performed as previously described (2).

Results

Serological screening studies. Indirect immunofluorescence with KJ serum was negative using a mouse kidney substrate, but strongly positive for cytoplasmic fluorescence using HEp-2 cells (Fig. 1). The anticytoplasmic antibody titer was 1/29,160 before therapy, and still showed a titer of 1/3,240 one year later. No antibodies to single stranded or double stranded DNA were present. Ouchterlony immunodiffusion of KJ serum against bovine spleen extract showed a precipitin line that was nonidentical with standard sera for antibodies to Ro/SSA, La/SSB, U₁RNP, Sm, Jo-1, Mi-2, Ku, and PM-Scl, as well as antiribosomal P. This line was compared to unidentified lines that have been found previously in routine testing of other myositis sera, and only one additional patient, BT, gave an identical precipitin line. Indirect immunofluorescence on HEp-2 cells with BT serum showed an exclusively cytoplasmic

Figure 1. Indirect immunofluorescence of KJ serum on acetone fixed HEp-2 cells. Results with 1/40 dilution are shown, but this sample could be titered to 1/3240.

pattern to a dilution of 1/3,240, with no staining of the nucleus.

Testing for anti-synthetase antibodies. Because of the presence of myositis in association with interstitial lung disease in both patients, it was suspected that the anticytoplasmic antibody was directed at an aminoacyl-tRNA synthetase. Both KJ and BT sera were negative for anti-Jo-1 by the sensitive ELISA method with affinity purified Jo-1 as antigen (14). KJ and BT sera were also tested for antibody to histidyl-tRNA, threonyl-tRNA, and alanyl-tRNA synthetase by aminoacylation inhibition, and the difference from controls was not considered significant for either serum (all reported patient sera with such antibodies inhibit the respective enzyme). There was slightly more inhibition of threonyl-tRNA synthetase by BT serum (by ∼ 15%), but still well below the 25% significance level (all previous sera found to contain antibodies to this enzyme have inhibited > 80% (28). Thus, if anti-KJ was an antisynthetase, it was not one that had been previously described.

Aminoacylation inhibition data for KJ serum for the 20 amino acids is shown in Table I. The level of significant inhibition, based on screening a large number of sera for anti-Jo-1 and anti-PL-7 by this method, was considered to be 40%. The largest amount of inhibition by KJ serum for any of the reactions tested was 16.8% (for proline). Thus, there was no evidence of significant inhibition of the activity of any of the synthetases, indicating that there was no evidence for antibody. Since later evidence demonstrated that anti-KJ inhibited the function of the KJ antigen, it was concluded that the cytoplasmic KJ antigen was not one of the aminoacyl-tRNA synthetases.

Immunoprecipitation. As seen in Fig. 2, specific immunoprecipitation of tRNA by KJ serum could not be demonstrated. Previous studies have consistently found that autoantibodies to any of the five antigenic aminoacyl-tRNA synthetases immunoprecipitate their respective cognate tRNAs (4, 22–25, 32). This is demonstrated in Fig. 2, in which anti-Jo-1 and anti-PL-7 sera were found to precipitate very strong sharp bands containing different tRNAs. Lack of precipitation of tRNA supports the conclusion that KJ antigen is not an aminoacyl-tRNA synthetase. It also may be concluded that KJ antigen does not form stable complexes with tRNA under the conditions of the immunoprecipitation, as do certain other antigens to which myositis sera have been found (32). As seen in Fig. 2, BT precipitates a faint single band of very small RNA which has not been identified (some background tRNA is seen, but the smallest, furthest migrating band is significant and reproducible). This could be due to a coexistent antibody to a tRNA-related antigen, but is unlikely to be the anti-KJ in BT serum, because KJ serum did not show this tRNA despite its much higher titer of anti-KJ (see ELISA data below).

KJ serum immunoprecipitated bands of RNA corresponding to those of the Ro/SSA RNAs, as evident by comparison to the standard anti-Ro/SSA serum in Fig. 2. The finding that KJ serum had anti-Ro/SSA in addition to anti-KJ antibodies was confirmed by ELISA. KJ serum had 5.5×10^5 activity units on anti-Ro/SSA ELISA, significantly higher than the average normal of 8.0×10^3 U. No anti-Ro/SSA precipitin line was present by immunodiffusion, but it is common for sera with this level of anti-Ro/SSA ELISA activity to fail to form an anti-Ro/SSA precipitate (34). BT serum did not precipitate the Ro RNAs, and BT serum was negative for anti-Ro/SSA by ELISA. The precipitation of strong Ro RNA bands by KJ

Table I. Aminoacylation Inhibition by KJ Serum

Amino acid	CPM-NS	CPM-KJ	%INH
Ala	2,180	1,857	14.8
Arg	223,627	201,196	10.0
Asn	7,190	7,194	−0.1
Asp	5,531	5,955	−7.7
Cys	2,009	2,113	−5.2
Gln	5,531	5,131	7.2
Glu	9,132	9,577	−4.9
Gly	2,144	2,320	−8.2
His	4,770	4,366	8.5
Iso	4,188	4,431	−5.8
Leu	2,496	2,517	−0.9
Lys	19,655	20,489	−4.2
Met	7,619	7,028	7.8
Phe	6,298	6,132	2.6
Pro	36,439	30,308	16.8
Ser	15,527	15,797	−1.7
Thr	2,923	3,370	−15.3
Try	14,766	14,133	4.3
Tyr	14,436	13,953	3.3
Val	37,174	38,029	−2.3

The effect of KJ serum on the activity of the 20 aminoacyl-tRNA synthetases is shown. CPM, counts per minute in the TCA precipitate, which represents the amount of aminoacyl-tRNA formed and reflects the activity of the respective enzyme under the conditions of the assay. CPM-NS, CPM when no serum is added; CPM-KJ, CPM in the presence of 1/100 parts KJ serum (3 μl 1/10 diluted serum added to the reaction mixture). %INH, % inhibition, calculated as: [(cpm-NS − cpm-KJ)/cpm-NS] × 100.
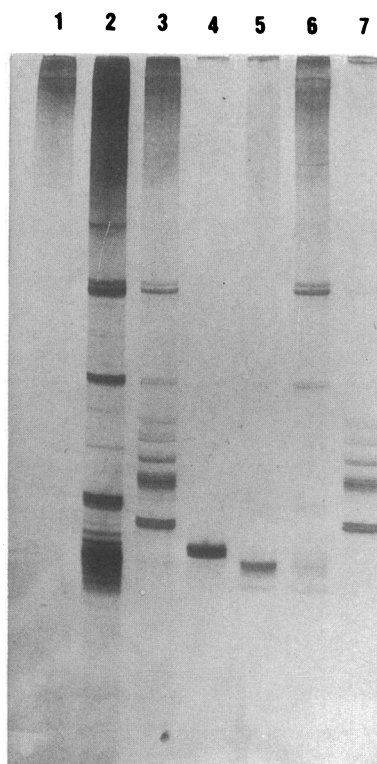

Figure 2. 7 M urea, 10% polyacrylamide gel electrophoresis of protein A-immunoprecipitates, stained with silver stain. Lane 2 contained total nucleic acids. Other lanes represent phenol-extracted immunoprecipitates obtained using (*a*) normal serum; (*b*) KJ serum; (*c*) serum from a PM patient (CD) with anti-Jo-1 by ID and ELISA (this serum inhibits histidyl-tRNA synthetase); (*d*) serum from a PM patient (VP) with anti-PL-7 by ELISA (this serum inhibits threonyl-tRNA synthetase); (*e*) BT serum; (*f*) serum from a patient with SLE with anti-Ro/SSA.

serum, with weak anti-Ro/SSA activity, serves as an internal positive control, indicating sensitive detection of precipitated RNA, and supporting the negative result for KJ with respect to tRNA.

Fig. 2 shows that both KJ and BT sera immunoprecipitated bands of ~ 5.0 and 5.8 S that are likely to be the small ribosomal RNAs. There is only a modest amount of precipitation of these RNAs with KJ and BT sera despite the abundance of these RNAs in the extract (as shown in the total nucleic acid lane), and in some runs these RNAs are not evident at all. These findings suggest that precipitation of the ribosomal RNAs by KJ and BT may have represented a partial or intermittent association of the antigenic protein with the ribosome.

In vitro translation. The translation of globin mRNA, expressed as the amount of protein synthesized in 1 h, was unaffected by the addition of 9 μg of normal human IgG in water in the experiment shown in Table II, and was taken as baseline. In other experiments, some nonspecific inhibition (up to 20–30%) may be seen, despite the presence of ribonuclease inhibitor. However, IgG containing antibody to histidyl-tRNA synthetase (anti-Jo-1) did inhibit translation to a considerable degree (62%). Increasing the amount of anti-Jo-1 IgG added by threefold increased the inhibition (to 84% over normal IgG). Addition of antibody to threonyl-tRNA synthetase (anti-PL-7), from the same sample shown in Fig. 2, did not inhibit translation to a significant extent. This was surprising since this anti-PL-7 serum (and all others tested to date [22, 28]) efficiently inhibited threonyl-tRNA synthetase activity, and threonine is present in rabbit globin (31). IgG isolated from a serum with antibody to Mi-2, a DM associated nuclear antigen (8), also did not inhibit translation in the experiment shown. On the other hand, there was dramatic inhibition of translation by IgG from patient K.J., with a 98% decrease in amount of product formed (99% with 27 μg). The stronger inhibition found with KJ antibody compared to Jo-1 antibody was consistent and reproducible. BT IgG also inhibited strongly; 96.4% with addition of 18 μg IgG, 92.5% with 9 μg. (These determinations were made in a separate experiment and were not included in the table.) Despite the lower titer of anti-KJ in BT serum, inhibition still exceeded that of anti-Jo-1.

Further studies were done to confirm that the inhibition was due to the anti-KJ antibody in the DEAE-purified IgG

preparations of KJ and BT sera. First, the IgG from the two serum samples was purified using protein-A Sepharose affinity chromatography. KJ IgG continued to inhibit strongly (98.3% at 9 μg) as did BT IgG (67.3%) compared to normal IgG. Thus, inhibition of in vitro translation by KJ and BT IgG was independent of the method of purification of the IgG. This inhibitory activity of the KJ IgG preparation could also be absorbed out of the preparation by protein A-Sepharose. While normal IgG inhibited 0.8% and KJ IgG inhibited 94.7% initially, after absorption with protein A-Sepharose (as described in Methods), the remainder of the preparation formerly containing normal IgG inhibited 0% while that which formerly contained the KJ IgG inhibited 2.8%. Thus, protein A-Sepharose removed the translation-inhibiting factor from the KJ IgG preparation.

A third line of evidence that the KJ antibody itself was responsible for the inhibition was obtained by absorption experiments (Table III). The reticulocyte lysate used for translation was pretreated with protein A-Sepharose coated with antibody. Lysate absorbed with Sepharose coated with normal antibody was usually 20–40% less effective than untreated lysate at translation of mRNA, probably reflecting nonspecific inhibition. The level of translation after incubating the lysate with Sepharose coated with normal antibody was used as base-

Table III. Inhibition of In Vitro Translation by Absorption

Sample	INCUB	CPM	% INHIB	Change
No IgG	—	44,356	—	—
Normal 1	B	45,720	0	—
Normal 2	B	66,757	0	—
Normal 3	B	50,401	0	—
Anti-Jo-1	B	8,695	80.4	—
KJ (1/4)	B	14,174	68.0	—
BT	B	2,925	93.4	—
Normal 1	H	44,285	0.2	+0.2
Normal 2	H	32,718	26.2	+26.2
Normal 3	H	29,315	33.9	+33.9
Anti-Jo-1	H	16,808	62.1	−18.3
KJ (1/4)	H	43,578	1.8	−66.2
BT	H	20,401	54.0	−39.4

IgG purified from test serum was used to coat protein A-Sepharose. After washing, the Sepharose was incubated for 2 h either with HeLa extract (*H*) or with HeLa extract buffer (*B*). The Sepharose was then incubated with reticulocyte lysate. The Sepharose was then sedimented, and the lysate removed for use in in vitro translation as described in Methods. Sample, IgG source used for incubation of Sepharose beads. Samples were IgG purified from three sera from normal subjects, IgG containing anti-Jo-1, and KJ (coated at 1/4 of the concentration of the other IgG samples tested, 250 μg vs. 1 mg) and BT IgG. INCUB, material used for first incubation of antibody coated Sepharose. CPM, average of duplicate translation runs using lysate absorbed with Sepharose coated with the sample indicated (counts per minute in a 5-μl aliquot). % Inhib, percent drop in CPM when the lysate was absorbed with the Sepharose coated with the sample as compared to coating with no IgG. Change, Difference in % inhibition that resulted from incubation with HeLa extract as compared to incubation with buffer. (−) indicates that there was less inhibition when the beads were incubated with HeLa extract, and (+) indicates that there was more inhibition after incubation with HeLa extract.

Table II. In Vitro Translation after Direct Addition of Purified IgG to Reticulocyte Lysate before Addition of mRNA

Sample	9 μg: CPM	% INHIB	27 μg: CPM	% INHIB
Normal IgG	138,951	—	108,109	—
Jo-1 IgG	52,246	62	16,908	84
PL-7 IgG	134,316	0	121,154	0
Mi-2 IgG	161,990	0	117,379	0
KJ IgG	2,376	98	1,161	99

Each sample was tested after addition of either 9 μg or 27 μg of IgG. Jo-1, PL-7 and Mi-2 samples represent IgG purified from sera containing those antibodies. CPM, counts per minute in a 5-μl aliquot of the reaction mixture after 1 h of reaction. % INHIB, % inhibition of the CPM present after incubation with the respective amount of normal IgG. CPM when no IgG was added was 137,085. BT serum, which was tested in a different run and was therefore not included in the table, inhibited 92% at 9 μg IgG added.

line. As shown in Table III, both KJ and BT strongly inhibit by this method. Sepharose coated with anti-Jo-1 also inhibited translation as expected, with anti-KJ having a greater effect per milligram added in most experiments. For example, in one typical experiment (not shown), anti-Jo-1 coated beads inhibited 20%, while anti-KJ coated beads inhibited 81%. In these experiments, the KJ IgG sample does not come in contact with the mRNA, yet still inhibits translation, indicating that the effect was on the lysate itself.

If the factor in KJ serum responsible for inactivating the lysate was in fact the antibody, it should be blocked by antigen. This was confirmed by incubating the antibody-coated protein A-Sepharose with HeLa extract before using it to absorb the lysate. As shown in Table III, when KJ-coated Sepharose was incubated with HeLa extract, the antibody was blocked and the absorption did not inactivate the reticulocyte lysate (1.8% inhibition), but Sepharose beads incubated with buffer alone were still effective (68% inhibition). Sepharose coated with BT IgG (in fourfold higher amount) was highly active at inhibiting the lysate (93.4%), but this effect was partially blocked after incubation with HeLa extract (54% inhibition). A similar effect of incubation with HeLa extract was seen using an anti-Jo-1 IgG, but no effect or an opposite effect of this step was seen with Sepharose coated with normal IgG (Table III).

Testing isolated ribosomes for KJ. The findings that anti-KJ reacted with a cytoplasmic antigen, inhibited translation, did not inhibit the synthetases, and partially immunoprecipitated the small ribosomal RNAs suggested that KJ antigen could be a ribosomal protein. To investigate this, isolated ribosomes were tested for the presence of the KJ antigen by a fluorescence inhibition test (Table IV). The ribosomes (or control preparations) were incubated with KJ serum (or other test serum), and the serum was tested by indirect immunofluorescence to determine whether the amount of anti-cytoplasmic activity was decreased. Incubation with isolated ribosomes prepared in 0.5 M NH4Cl had no effect on KJ serum immunofluorescence activity, but incubation with the same amount of isolated ribosomes markedly inhibited antiribosomal P immunofluorescence. On the other hand, incubation with a postribosomal supernatant very efficiently inhibited the fluorescence of KJ serum, but showed no inhibition of the immunofluorescence of the anti-P sample, indicating that the ribosomes had been removed. Thus, the anticytoplasmic antibody in KJ serum does not seem to be directed at an integral

ribosomal protein. Considering the immunoprecipitation results, KJ antigen may have some association with the ribosome at low ionic strength, accounting for the immunoprecipitation results (alternatively, there may be a second antibody). None of the buffers or media had any inhibitory activity of their own.

Purification of KJ. After affinity chromatography, the concentrated, dialyzed affinity eluate had KJ antigen activity, as demonstrated by ELISA using KJ or BT serum. In addition, this material could completely inhibit the KJ cytoplasmic immunofluorescent pattern, and produced a precipitin line with KJ serum. The affinity eluate still contained a large number of proteins (see below) despite extensive washing of the affinity column before elution, and it was therefore further purified and analyzed with FPLC, using gel filtration on Superose 12. Fig. 3 shows the elution profile (OD at 280 nm, reflecting total protein), on which is superimposed the ELISA antigenic activity of each fraction for each of four sera; KJ, BT, a normal serum, and à serum with anti-Ro/SSA. Only the late eluting peaks had activity against KJ that was not seen with normal serum. Antigenic activity against KJ serum formed a single peak, but antigenic activity against BT serum eluted in two smaller peaks within the KJ peak, corresponding to the split peak of OD_{280}. Despite the presence of anti-Ro/SSA in KJ serum, there was no evidence of Ro/SSA antigenic activity in any of the gel filtration fractions, nor in the whole affinity eluate. Low level background activity against all sera tested corresponded to the other OD_{280} peaks, indicating that no specific antigen activity was present in them. Pooled active fractions from gel filtration were used for analysis on PAGE.

Analysis by PAGE. Fig. 4 A reveals that a number of polypeptides were present in the affinity eluate (lane 2). The strongest band present in the whole eluate on PAGE (seen in Fig. 4 A and in all preparations examined) is ~ 80–85 kD. The FPLC fractions containing this material were not immunologi-
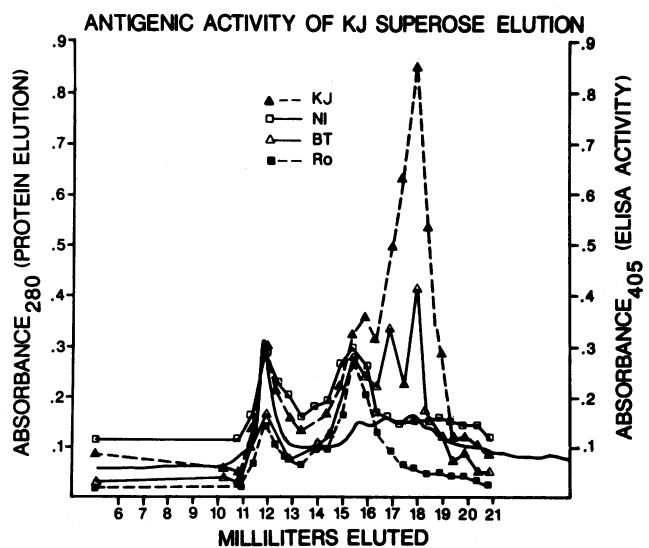
Table IV. KJ Antigen Activity of Ribosomes

Sample	Protein	KJ activity	Specific activity for KJ	Ribosomal-P activity	Specific activity for ribo-P
Postribosomal supernatant	15	12	0.80	0	0
Ribosomes	11.5	<1	0.087	>8	>0.70

KJ and P-protein antigen activity was determined by their inhibition of cytoplasmic fluorescence of the respective sera. 45 µl of fraction (or its dilution) was added to 5 µl of 1/100 dilution of KJ serum or 1/50 dilution of anti-P, and preincubated before performing fluorescence. The reciprocal of the highest dilution of fraction still showing some inhibition = inhibitory activity per ml. This was divided by the protein concentration (mg/ml) to give the specific antigen activity/mg.

Figure 3. Gel filtration chromatography of KJ affinity column eluate on Superose 12. Protein is detected by absorbance at 280 nm. ELISA activity of each fraction (using 10 µl of each fraction + 90 µl PBS to coat each well) was determined for each of four sera (KJ, normal, BT, and a standard serum for anti-Ro/SSA). Sera were used at 1/300 dilution.
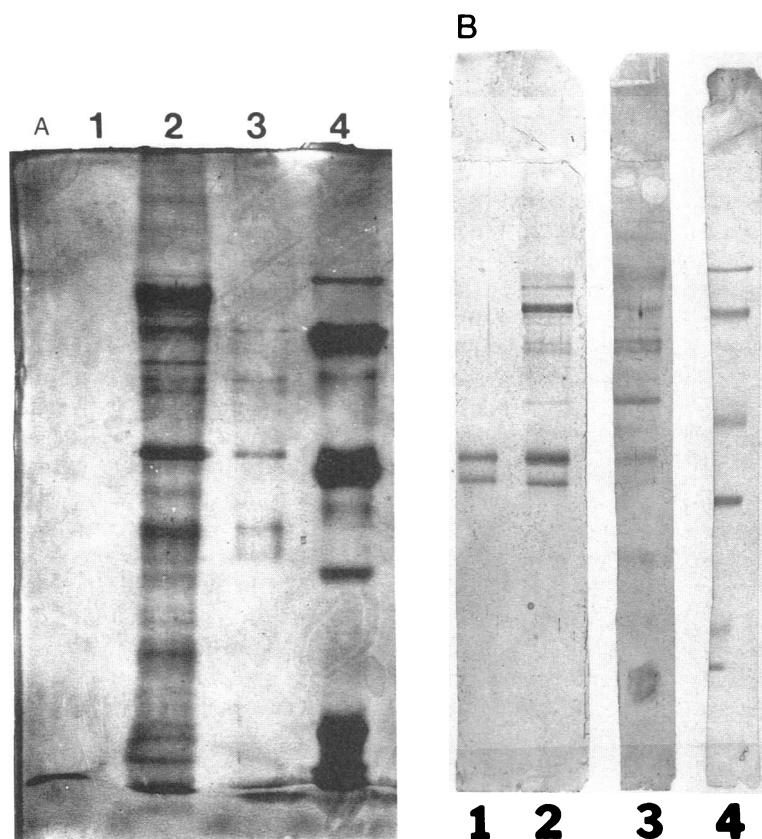
Figure 4. (A) 12% SDS-PAGE of affinity purified KJ stained with silver stain. Lane 2 contains the whole KJ affinity eluate. Lane 3 contains the fractions from Superose 12 chromatography that were active against KJ serum in ELISA (17-18.5 ml), pooled and concentrated. Lane 4 contains molecular weight markers (94, 67, 43, 30, 20.1, and 14.4 kD). (B) Western blot of polyacrylamide gel run with gel in A. Lanes 1 and 2 were developed with KJ serum, 1/100. Lane 3 was developed with normal serum, 1/100. Lane 4 contained molecular weight markers and was developed with amido black. Whole KJ affinity eluate was run in lanes 2 and 3 (as in lane 2 in A), while pooled active fractions (as in lane 3 in A) were run in lane 1. Bands at 32, 36, and 68–70 kD were stained more heavily by KJ than normal serum, but only the fractions with the 32- and 36-kD bands were active in ELISA.

cally active in ELISA, and the pooled active fractions did not contain this band (lane *3*). In addition to this band, the whole eluate contained prominent bands at ~ 24 kD, 30–35 kD, 45 kD, a doublet at 55–60 kD, and a doublet at 68–70 kD, as well as other weaker bands. A substantial further purification was evident in comparing the whole eluate to the pooled active fractions from Superose 12 chromatography (lane *3*). The latter contained two discrete bands, one ranging between 30 and 33 kD, and the other between 33 and 36 kD, in addition to fainter bands at 45 kD, 70 kD, and a doublet at 55–60 kD. After transfer to nitrocellulose (Fig. 4 *B*), there was prominent staining with KJ serum of the bands at molecular weights of 30 kD and 34 kD, in both the whole affinity eluate and the active Superose fractions. Although faint background staining is seen with normal serum in the experiment shown, the 30- and 34-kD bands stain with KJ serum strongly and consistently in all runs, and usually show little background. Also evident in Fig. 4 *B* is staining of the 70-kD band. There is no corresponding FPLC fraction with KJ or Ro/SSA ELISA activity eluting from the Superose column in this molecular weight range. This band is not consistently demonstrable in blots of whole KJ affinity eluates, possibly reflecting proteolytic breakdown. The Western blot confirms that the 80–85-kD band does not contain antigenic activity. The 24-kD, 45-kD, and 55–60-kD bands also appear to lack antigenic activity when compared to normal.

Discussion

We report two patients with ILD and PM, who have an autoantibody directed at a cytoplasmic antigen that is involved in the process of translation of mRNA, the central process in protein synthesis. This same clinical syndrome of lung disease and PM also affects many patients with antibodies to amino-acyl-tRNA synthetases. Since the synthetases also play a necessary role in translation, there is a functional relationship between the antigens that have been associated with this syndrome: His-, Thr-, and Ala- tRNA synthetases, and KJ.

Substantial evidence was presented that KJ antigen is not a synthetase. Lack of inhibition of synthetase activity is strong evidence in favor of this conclusion. We have found inhibition of histidyl-tRNA synthetase activity by all sera with anti-Jo-1 (detectable by ID) that we have tested, now including sera from over 30 patients, and by all sera with threonyl-tRNA synthetase. In general, this method could fail to detect an antibody that combined with the antigenic enzyme without inactivating it, or an antibody reacting exclusively with one form of the enzyme (for example, the mitochondrial form), allowing the reaction to be catalyzed by others. However, the results of the translation inhibition studies indicate that anti-KJ can completely abolish the function of KJ antigen. Studies with anti-Jo-1 and anti-PL-7 indicate that translation inhibition is a less sensitive method of detecting functional inhibition of synthetases than is aminoacylation inhibition, possibly because the concentration of synthetase can be specifically controlled in the latter. There is also supportive data provided by immunoprecipitation. All recognized autoantibodies to synthetases have precipitated the cognate tRNA for the respective amino acid, while KJ did not precipitate tRNA. Failure to precipitate tRNA does not in itself rule out the presence of an antisynthetase, but combined with the enzyme inhibition data, there is a strong case against the KJ antigen being a synthetase.

The evidence presented also indicates that the KJ antigen plays an important role in protein synthesis, since in vitro translation of globin mRNA is prevented by binding of antibody to KJ antigen, or removal of KJ antigen from the reticulocyte lysate. It is unlikely that complicating factors such as

ribonucleases in the IgG preparation accounted for this effect. Evidence for this included: (*a*) the effect was independent of the method of IgG purification; (*b*) it could be produced either by absorption of the lysate or by direct addition of antibody; (*c*) the effect of antibody could be blocked by extract containing the antigen; and (*d*) IgG from both sera containing the antibody had this effect, but not with IgG from myositis sera containing nontranslation related antibodies or from normal sera. The level of inhibition of translation that was observed with anti-KJ (from either KJ or BT) was stronger than was generally seen with antisynthetases. Inhibition with high titer anti-Jo-1 was less complete, even when threefold more antibody was added, and no inhibition at all was seen with anti-PL-7 sera. This may be related to the relative amounts of the antigens and antibodies, or it might reflect an aspect of the respective functions of the antigens, implying that the role of the KJ antigen in translation is more direct. Mathews et al. (32) also found that anti-PL-7 serum did not inhibit translation, while Elkon et al. (39) found that antibody to the ribosomal P proteins, which are more directly involved in formation of the polypeptide chain, strongly inhibited translation.

Although anti-KJ antibody can clearly disturb protein synthesis in vitro, it is unlikely that this occurs in vivo, or that inhibition of protein synthesis is involved in disease pathogenesis, since the antibody would not ordinarily enter the living cell. Analysis of the effects of KJ antibody on in vitro protein synthesis was useful, however, to study the function of the KJ antigen, and should prove to be useful for the study of other anticytoplasmic antibodies. Others have reported inhibition of in vitro translation by autoantibodies (32, 39, 40). Reedy et al. (40) reported preliminary results showing some inhibition of translation by anti-Jo-1 sera. Our study confirms that antibody to Jo-1 interferes with translation of globin mRNA, presumably by preventing the formation of charged tRNA^{his}. Reedy et al. also found evidence of partial inhibition of translation by a number of diluted myositis sera without anti-Jo-1, and enhancement of translation by others, but they did not study isolated IgG, they did not analyze the individual specificities of the inhibiting antibodies (other than anti-Jo-1), and the effects noted were small compared to those shown here for anti-KJ. Mathews et al. (32) studied the effects of IgG from myositis sera on in vitro translation, and found that anti-Jo-1 IgG inhibited translation. IgG with anti-Fer, another antibody that was found in myositis and that immunoprecipitates tRNA, did not inhibit translation.

Our data clearly show that KJ antigen is not ribosomal P antigen. It is possible that KJ is a different protein with affinity for the ribosome, a possibility supported by the immunoprecipitation results. Most proteins involved in translation, including some synthetases (21), associate at one point or another with the ribosome in the course of performing their function. However, the evidence presented makes it unlikely that KJ antigen is an integral part of the ribosome. It is still present in extracts after removal of ribosomes by centrifugation, and it is not a part of ribosomes isolated at 0.5 M salt according to immunofluorescence inhibition data. The KJ precipitin line in Ouchterlony immunodiffusion was nonidentical with that of anti-P, using an extract prepared in 0.15 M salt, suggesting that the two antigens exist on different particles.

Since it does not appear to be a synthetase or an integral ribosomal protein, but does inhibit translation, KJ antigen is most likely to be a translation factor. The inhibition results that were observed could have been produced by inhibition of crucial initiation or elongation factors. Thus far, we have been unable to identify KJ antigen as a described translation factor. Further analysis of its structure may be helpful in this regard. The FPLC, ELISA, and Western blot data indicate that the most strongly antigenic polypeptides are of ~ 30–32 kD and 34–36 kD, but these may be breakdown products, possibly of the 70-kD antigenic band seen in some whole affinity eluates. These antigenic polypeptides may be present in vivo in a complex with the 80–85-kD band, and possibly others.

The reason for the split peak of ELISA activity seen on analysis of gel filtration fractions with BT serum is unclear. Both parts of the split peak of BT fall within the KJ peak, and it is possible that the apparent difference in reactivity is an effect of the relative titers. Since BT and KJ sera have an identical precipitin line in Ouchterlony, they must react with the same complex, although it is conceivable that they are reacting with different portions of it. It is possible, because of the fine resolution of the FPLC, that the two peaks represent the 30- and 34-kD bands. There may be additional reactivity not evident in Western blot because of denaturation of the antigen. The other band seen in active fractions, of 45 kD, was present in other, inactive fractions, and was not stained on blot, but the possibility that it is involved must be kept in mind.

The question of whether the KJ antigen is related to tRNA is an important one, as discussed below. Our data indicate that KJ serum does not immunoprecipitate tRNA, so that the antigen is not itself tRNA, nor does it form stable complexes with tRNA under the conditions of the immunoprecipitation. Other autoantibodies to nucleoprotein complexes tend to immunoprecipitate the nucleic acid present. Establishing the molecular identity of the antigen will help to settle this question. It is unlikely, however, that the lack of precipitation of tRNA by KJ serum was due to low sensitivity of the method. This method of immunoprecipitation, involving silver stain of immunoprecipitates from extracts that had not been radioactively labeled, was shown by Forman et al. (29) to be almost as sensitive on a qualitative basis as ^{32}P immunoprecipitation (it can identify almost all positive sera), and KJ serum had a high titer of anti-KJ. Anti-Jo-1 and anti-PL-7 were easily detected in other sera, as was the weak anti-Ro/SSA in KJ serum itself. It is also unlikely that the lack of precipitation of tRNA was due to a lack of KJ antigen in HeLa extract. KJ antigen was present in HEp-2 cells (another human line) by fluorescence, and proteins necessary for translation should be required by all active cells. While KJ serum did not precipitate tRNA, BT serum did precipitate a faint band of tRNA. This was most likely due to a coexistent antibody, since the ELISA data and the immunofluorescence data indicate that BT contains a lower titer of anti-KJ than does KJ serum, and one would have expected to see it with KJ serum also if the anti-KJ were responsible.

Anti-KJ was found in only two patients, and it is difficult to draw conclusions about clinical associations with such a small number. However, of the entire group of antisynthetase negative myositis patients, patients with the myositis/lung disease syndrome (PM/ILD) in association with Raynaud's phenomenon are not common, particularly in the absence of scleroderma. An association has also been reported of lung disease with anti-PM-Scl (7, 9, 41) and anti-nRNP (9, 42) in the setting of overlap syndromes, but these antibodies also were not present here. Although B.T. did develop manifesta-

tions of scleroderma, her disease began as PM with ILD, and remained so for 2 yr. (We do not know when anti-KJ developed in B.T.) Thus, the association of anti-KJ with two patients with this syndrome is striking. The presence of anti-Ro/SSA in KJ serum is interesting, and we have observed it also in association with anti-synthetases.

Patients with the PM/ILD syndrome may represent a separate subgroup within adult PM (9), that is a more uniform group than adult PM patients as a whole. They share an association with antibodies to cytoplasmic antigens that have the functional relationship described here, in that they are all involved in translation. Antibodies to individual synthetases do not cross-react with other synthetases (or with KJ), indicating that it is not a shared epitope that is targeted, and it may be an aspect of their related function that causes these otherwise distinct proteins to become antigenic to patients with the same syndrome. It has been suggested that this aspect is a relationship to tRNA (22). The Mas and Fer specificities, both described in myositis, are both tRNA related antigens but they do not appear to be synthetases. KJ, however, does not appear to be a synthetase, nor is there evidence of association with tRNA (although as discussed above, our negative evidence cannot rule this out). This may indicate that the function in translation is related to the development of immunogenicity. It is interesting in this regard that the signal recognition particle (SRP) has been repeatedly shown to be an antigen in polymyositis patients (43–46). The SRP targets secretory proteins into the endoplasmic reticulum, recognizing them as they are being translated, and can have effects on their translation. Anti-SRP is associated with PM, but not with a high frequency of ILD (46). Understanding the reason for the focus of these antibodies on cytoplasmic antigens and translation may provide insights into the etiologic and pathogenetic mechanisms of PM.

It has been suggested previously (4) that the reason antibodies to synthetases develop in myositis patients is that the synthetases can specifically interact with the RNA of certain viruses. There is some experimental support for such interactions. It has been reported that the RNA of two picornaviruses (Mengo and encephalomyocarditis) can accept a specific amino acid (histidine and serine, respectively) as if there were a tRNA-like structure as part of their genome (47, 48). An autoimmune response against the synthetase may result if a stable complex of synthetase and virus (such as that formed between the synthetase and its natural tRNA substrate) is presented to the immune system as a foreign antigen (4); if the complementarity of virus and synthetase leads to cross-reaction of antiidiotypic antibodies to the antiviral antibodies with the synthetase (49); or possibly by other mechanisms. There is a considerable amount of independent evidence (50–54) for a viral etiology for at least some forms of PM (particularly for Coxsackie, also a picornavirus), and thus this hypothesis has been especially attractive. Picornaviruses are small RNA viruses whose RNA serves directly as a positive strand of messenger RNA. An early step in picornavirus infection of a cell is the shutdown of translation of host mRNA (55). Shortly thereafter, the host cell protein synthesizing machinery begins to efficiently translate viral mRNA, but it is still unable to translate host mRNA. There may be differences in the mechanism by which different picornaviruses exert these effects. Manipulation of this intricate mechanism in this precise manner by the virus would likely involve specific interactions of viral RNA and protein with host RNA and protein, but would not seem to require interaction with nuclear proteins or DNA. Thus, if viral/host interactions were the basis for selection of particular proteins to be antigens, and if picornaviruses (or viruses with a similar mechanism) were responsible for the PM/ILD syndrome, it would not be surprising to find antibodies to tRNA or translation-related proteins in this syndrome.

In summary, two patients with polymyositis, interstitial lung disease, and Raynaud's phenomenon, were found to have an autoantibody, labeled anti-KJ, that can strongly inhibit translation of mRNA. KJ antigen did not appear to be either an integral ribosomal protein, nor a synthetase, and it may be a translation factor. The functional relationship of KJ antigen and the synthetase antigens, and similar clinical presentation of the patients, may relate to similar viral triggers for the generation of the antibodies and the clinical syndrome.

Acknowledgments

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