ANTI–THREONYL-tRNA SYNTHETASE, A SECOND MYOSITIS-RELATED AUTOANTIBODY

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In the autoimmune disease systemic lupus erythematosus (SLE),[1] antibodies reactive with nuclear and cell surface components are a general finding (1, 2). By contrast, in myositis, an inflammatory disease of muscle, autoantibodies are often directed at cytoplasmic components (3). The most common target, Jo-1, was recently identified as the enzyme, histidyl-tRNA synthetase (4). Antibodies to Jo-1 are found in 25–30% of myositis patients (5–7), particularly in patients with myositis and interstitial lung disease, where the frequency is ~70% (6, 7). In this paper we identify a second, less common autoantibody found chiefly, though not exclusively, in myositis. We show that it is directed at another charging enzyme, threonyl-tRNA synthetase, and that the antigenic site recognized by the human autoantibody differs from that recognized by an experimental antibody raised in rabbits. We discuss the association between myositis and autoantibodies to tRNA-related antigens.

Patients and Methods

Patients. Five examples of the PL-7 precipitin system were studied: Three were obtained in a survey of 84 patients with myositis, the fourth from a study of 10 myositis sera giving cytoplasmic immunofluorescence on the HEp-2 cell line, and the fifth by screening sera from >1,000 patients with other forms of systemic autoimmune disease (8), using counterimmunoelectrophoresis (CIE) (9).

Immunofluorescence. Indirect immunofluorescence was performed with HEp-2 cells as substrate using fluorescein-labeled anti-human immunoglobulin provided by the supplier (Immunoconcepts, Inc., Sacramento, CA). Serum was diluted 1:40 in phosphate-buffered saline (PBS). Slides were read on a Nikon Optiphot ultraviolet microscope equipped with filters for fluorescein.

Preparation of IgG. All experiments used IgG isolated from serum by chromatography on DEAE-Sephadex A-50 columns run in 0.01 M Na phosphate at pH 7.2. Fractions with absorbance >0.8 at 280 nm were pooled, restored to 150 mM NaCl by the addition of salt, and held at −20°C. As used, IgG stocks were generally 0.7–1 mg/ml.

Cell Labeling. For [^{35}S]methionine labeling, subconfluent HeLa cell monolayers were incubated for 5 h with [^{35}S]methionine (New England Nuclear, Boston, MA) at 0.5 mCi/

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[1] *Abbreviations used in this paper:* BSA, bovine serum albumin; CIE, counterimmunoelectrophoresis; DTT, dithiothreitol; PBS, phosphate-buffered saline; SDS, sodium dodecyl sulfate; SLE, systemic lupus erythematosus; SS, systemic sclerosis.

ml in methionine-free medium (3 ml per 10-cm plate). The cells were washed once with ice-cold PBS, harvested, and lysed in 2 vol (~0.1 ml/plate) of buffer A (10 mM Tris-HCl, pH 7.5, 0.14 M NaCl, 1.5 mM $MgCl_2$, 0.5% Nonidet P-40). The extract was frozen and thawed three times and stored at −70°C.

For ^{32}P labeling, HeLa cells in suspension culture were washed twice in phosphate-free medium, resuspended at 5×10^5 cells/ml in phosphate-free medium containing 1% serum, and incubated for 17 h with [^{32}P]phosphate (New England Nuclear) at 30 mCi/100 ml culture. Cells were then harvested, washed, resuspended in buffer A (2 ml/100 ml culture), frozen and thawed three times, and stored at −20°C.

Immunoprecipitation. Unless otherwise specified, all procedures were carried out at 0–4°C in Eppendorf Microfuge tubes as previously described (10), using the Brinkman Microfuge (Brinkman Instruments, Inc., Westbury, NY). Immune complexes were recovered by adsorption to protein A in the form of a 10% suspension of *Staphylococcus aureus* (IgGSORB; Tufts Medical Center, Boston, MA) in buffer C (50 mM Tris-HCl, pH 7.4, 150 mM NaCl, 5 mM EDTA, 0.5% Nonidet P-40, 0.5% sodium deoxycholate, 0.1% sodium dodecyl sulfate [SDS]) containing bovine serum albumin (BSA), 2 mg/ml. For larger preparative immunoprecipitations, protein A-Sepharose CL-4B (Pharmacia Fine Chemicals, Piscataway, NJ) was easier to handle.

The cell extract was centrifuged for 5 min, precleared with 1/10 vol of IgGSORB in buffer C with BSA for 5 min, and diluted 1:1 in buffer C with BSA. Aliquots of 25 μl were incubated with 10 μl of IgG for 20 min, then with 50 μl IgGSORB for 10 min, with regular mixing in both cases. After centrifugation for 20 s, the pellet was washed six times in 0.8 ml buffer C. Proteins were detached from the pellet by boiling in 2× gel sample buffer and resolved by electrophoresis on 15% SDS-polyacrylamide gels (11) and fluorography (12). For analysis of RNA the pellet was boiled in 400 μl of a buffer containing 10 mM Tris-HCl, pH 7.4, 250 mM NaCl, 0.5% SDS, and 25 μg/ml calf liver tRNA, followed by phenol and chloroform extractions and precipitation with ethanol. RNAs were resolved using 7 M urea/10% polyacrylamide gels and were visualized by autoradiography, in some cases with the assistance of intensifier screens.

Analysis of RNA and Protein. Immunoprecipitated RNA was eluted from the gel by crushing and soaking in 10 mM Tris-HCl, pH 7.4, 1 mM EDTA, 0.5% SDS, extracted with phenol and chloroform, and recovered by ethanol precipitation. Fingerprints were prepared by digestion with ribonuclease T_1, followed by electrophoresis on cellulose acetate paper in the first dimension, and homochromatography on DEAE-cellulose thin layers using homomix C in the second dimension (13). Minor nucleotides were separated by two-dimensional chromatography (14) after digestion with a mixture of ribonucleases A, T_1, and T_2 (13). Radioactivity was detected by autoradiography.

For partial proteolysis, immunoprecipitated protein was eluted from the dried gel after fluorography by boiling in a solution containing 0.5% SDS, 10 mM 2-mercaptoethanol, 10 mM Tris-HCl, pH 7.4. Aliquots of the proteins to be compared, containing approximately equal radioactivity, were incubated with varying quantities of protease from *S. aureus* strain V8 (15). After digestion for 15 min at 37°C, the products were resolved by electrophoresis in SDS–20% polyacrylamide gels (11).

Antigen Depletion. Protein A-Sepharose, equilibrated and suspended at 15% vol/vol in buffer D (10 mM Tris-HCl, pH 7.4, 140 mM NaCl, 1.5 mM $MgCl_2$, 0.5 mM dithiothreitol [DTT]), was incubated with 0.75 ml IgG for 20 min. After six washes in buffer D the protein A-Sepharose was incubated with 100 μl of HeLa cell extract for 30 min. Both incubations were at 4°C with constant mixing. The final supernatant was held at −70°C. In aminoacylation assays the volume of extract used was adjusted according to its absorbance at 280 nm to allow for dilution (1.6–2-fold) during the depletion procedure.

Aminoacylation of tRNA. An extract containing aminoacyl-tRNA synthetases was prepared using a modification of the method described previously (16). HeLa cells grown in suspension culture were harvested, washed twice in cold PBS, suspended in 2.5 vol of buffer E (10 mM Tris-HCl, pH 7.4, 1.5 mM $MgCl_2$, 15 mM KCl, 0.5 mM DTT), and allowed to swell for 10 min on ice. After disruption using a Dounce homogenizer, NaCl was added to 0.14 M, and the lysate was centrifuged at 10,000 *g* for 10 min and then at

100,000 *g* for 2 h. The resultant supernatant, avoiding the fatty layer, was dialyzed for 16 h against two changes of buffer E.

Aminoacylation reactions contained the following, in a volume of 25 μl: 2.5–5 μl of the HeLa cell extract; 90 μg calf liver tRNA (Boehringer Mannheim Biochemicals, Indianapolis, IN); 3 μCi radioactive amino acid plus 16 μM of the same amino acid in unlabeled form; 5 mM adenosine triphosphate, 0.5 mM guanosine triphosphate, and 0.5 mM cytidine triphosphate; 10 mM Tris-HCl, pH 7.4; 10 mM $MgCl_2$, 0.1 mM EDTA, and 0.5 mM DTT; and varying amounts of IgG. The final concentration of monovalent cation (KCl and NaCl) was adjusted to 60 mM. Incubation was at 37°C, and 5-μl samples were removed at various times, usually 0, 3, and 10 min, for determination of the amount of amino acid bound to tRNA by trichloroacetic acid precipitation and liquid scintillation counting (4).

Results

Antibody Frequency and Clinical Association. A precipitating antibody known as PL-7 was identified in three sera from a series of 84 patients with myositis studied at Hammersmith Hospital, and a fourth example was found among 10 American myositis sera selected because they gave cytoplasmic immunofluorescence. A fifth isolate came from a patient with a connective tissue disease without apparent myositis (Table I). All five isolates gave similar patterns of cytoplasmic immunofluorescence on HEp-2 cells (Fig. 1). By CIE with rabbit thymus extract, all five sera gave an identical precipitin line distinct from the lines given by other autoantibody systems, and no further examples were detected in a survey of >1,000 patients with various forms of systemic autoimmune disease (8).

Immunoprecipitation of the Antigen. HeLa cell extracts labeled with [^{35}S]methionine or with [^{32}P]phosphate were used in protein A–mediated immunoprecipitation reactions. All five PL-7 isolates recognized a protein of 80,000 daltons (Fig. 2*A*, lanes *2–4*). This protein was not recognized by IgG from healthy controls (lane *5*) or patients with other autoantibodies (lane *6*). The 80,000 dalton protein was also phosphate labeled (8). Three to four bands of RNA in the transfer RNA size range were coprecipitated in all cases (Fig. 3*A*, lanes *2–4*). In some experiments, up to four additional weaker bands of slightly greater mobility were also seen (Fig. 3*B*). The PL-7 RNAs represent a subset of the tRNA-sized molecules present in the HeLa cell extract (Fig. 3*A*, lane *6*) and were electrophoretically distinct from $tRNA^{His}$ precipitated by anti-Jo-1 antibodies (Fig. 3*A*, lane *5*). In further experiments, the PL-7 RNAs were immunoprecipitated from cytoplasmic but not nuclear extracts (data not shown).

Nature of the Antigen. These findings are consistent with the antigen being a

TABLE I

Clinical Features in Five Patients with PL-7 Antibody

1. Female, age 49, developed an acute hepatitis followed by a 6 mo episode of myositis and persistent arthritis, tendinitis, and Raynaud's phenomenon.
2. Female, in her 40s, with dermatomyositis.
3. Male, age 57, developed polymyositis on top of longstanding rheumatoid arthritis.
4. Female, age 55, with 5 yr history of dermatomyositis and interstitial lung disease.
5. Female, age 39, developed arthritis and, subsequently, Raynaud's phenomenon, reflux esophagitis with stricture, pleurisy, pericarditis, maculopapular rash, and hyperpigmentation. DNA binding was normal; muscle weakness was not a feature.

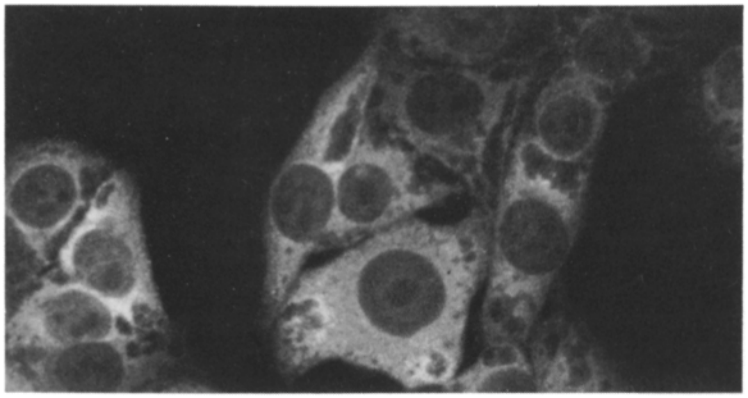

FIGURE 1. Localization of PL-7 antigen. Indirect immunofluorescence on a substrate of HEp-2 cells using PL-7 antibody. × 200.

ribonucleoprotein complex. To discover whether the antigenic component is RNA or protein in nature we carried out two types of experiments. First, ^{35}S-labeled extracts were digested with ribonuclease before immunoprecipitation. As shown in Fig. 2*B*, the 80,000 dalton protein remained precipitable after this treatment (lane *3*). Second, ^{32}P-labeled RNA isolated by phenol extraction was not precipitable by PL-7 antibody (Fig. 3*B*, lane *2*) unless an extract of unlabeled HeLa cells was added (Fig. 3*B*, lane *3*). These experiments suggest that the PL-7 antigen is a protein capable of associating with one or more RNA molecules.

Characterization of the RNA. The immunoprecipitated RNA was eluted from the gel and subjected to digestion with ribonuclease T_1 for "fingerprinting", or with a combination of ribonucleases A, T_1, and T_2 for analysis of nucleotide composition. The fingerprint, shown in Fig. 4*A*, differed from that given by Jo-1 RNA (4) and appeared to contain two sets of spots. The major set was roughly of the complexity expected for an RNA of 70–80 nucleotides length, the size of tRNA. At least one oligonucleotide streaked in the second dimension, suggesting that it contained an unstable nucleotide. The presence of modified nucleotides characteristic of tRNA is shown in Fig. 4*B*, where minor spots can be seen in addition to the major spots for Ap, Up, Cp, and Gp. Several of the same minor nucleotides were also observed in the digest of Jo-1 tRNA (Fig. 4*C*), though in different proportions. As expected, minor nucleotides were undetectable in digests of ribosomal 5S RNA (Fig. 4*D*). From these data we conclude that the predominant RNA in the PL-7 immunoprecipitate is a single species of tRNA probably containing variable numbers of minor bases that give rise to several bands upon gel electrophoresis. The results presented below indicate that this RNA is $tRNA^{Thr}$. It is not clear whether the minor spots in the fingerprint pattern come from a second, isoaccepting $tRNA^{Thr}$ species or from an unrelated RNA.

tRNA Charging. We previously (4) identified the Jo-1 antigen as histidyl-tRNA synthetase by demonstrating that the antibody inhibits the charging of tRNA with histidine. Following this analogy, we investigated the specificity of PL-7 antibody in the same way, testing each amino acid in turn since, in this case, we did not know the identity of the tRNA. Initial experiments revealed no

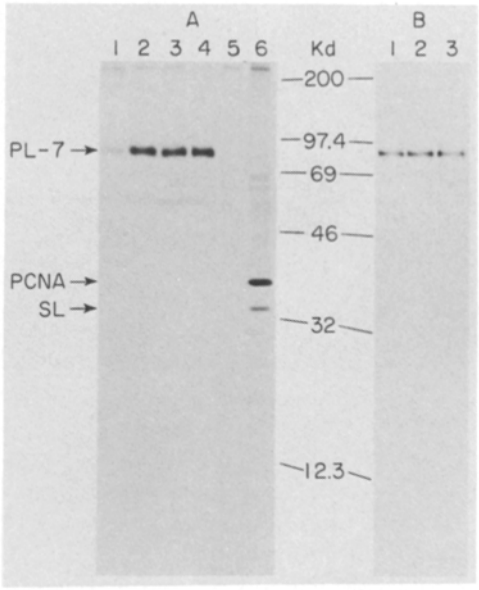

FIGURE 2. Immunoprecipitation of PL-7 protein. (*A*) Proteins were precipitated from ^{35}S-labeled HeLa cell extracts with the following antibodies: (*1*) rabbit anti-threonyl-tRNA synthetase; (*2–4*) three isolates of PL-7; (*5*) normal human IgG; (*6*) antibodies to PCNA and SL antigens (8, 17). (*B*) Proteins were immunoprecipitated by PL-7 antibody from HeLa cell extracts that were (*1*) preincubated for 5 min at 37°C with 0.5 mg/ml ribonuclease A; (*2*) preincubated without ribonuclease; (*3*) not preincubated.

consistent effect with any amino acid, but, when the sensitivity of the assay was raised by decreasing the amount of charging extract and increasing the amount of antibody in the reaction, a profound inhibition (~95%) of threonine charging became apparent (Fig. 5). Control antibodies such as anti-Jo-1 (Fig. 5), anti-La, or normal IgG (not shown) did not block aminoacylation with threonine, although, as expected, anti-Jo-1 did inhibit histidinylation of tRNA. At the enhanced level of sensitivity used in these experiments, fluctuations of up to ±50% were seen in the charging of several other amino acids (Fig. 5), but none of them was inhibited as markedly as threonine. We assume these smaller inhibitions are due to nonspecific effects or possibly to the presence of lesser amounts of other antibodies.

As shown in Fig. 6*A* the addition of increasing amounts of PL-7 IgG led to a progressive decrease in the rate of tRNA aminoacylation with threonine. All five isolates of PL-7 IgG displayed the inhibitory effect on threonine charging. To exclude the possibility that the block was due to the addition of a nonspecific inhibitor, we carried out the depletion experiments shown in Fig. 6, *B* and *C*. When the charging extract was treated with immobilized PL-7 antibody to remove the corresponding antigen, subsequent aminoacylation with threonine was greatly reduced (Fig. 6*B*), but there was no appreciable effect on charging with histidine (Fig. 6*C*). The converse was seen when the charging extract was depleted of Jo-1 antigen. In this type of experiment the fluctuations seen with other amino acids were much less evident. Further experiments (not shown)

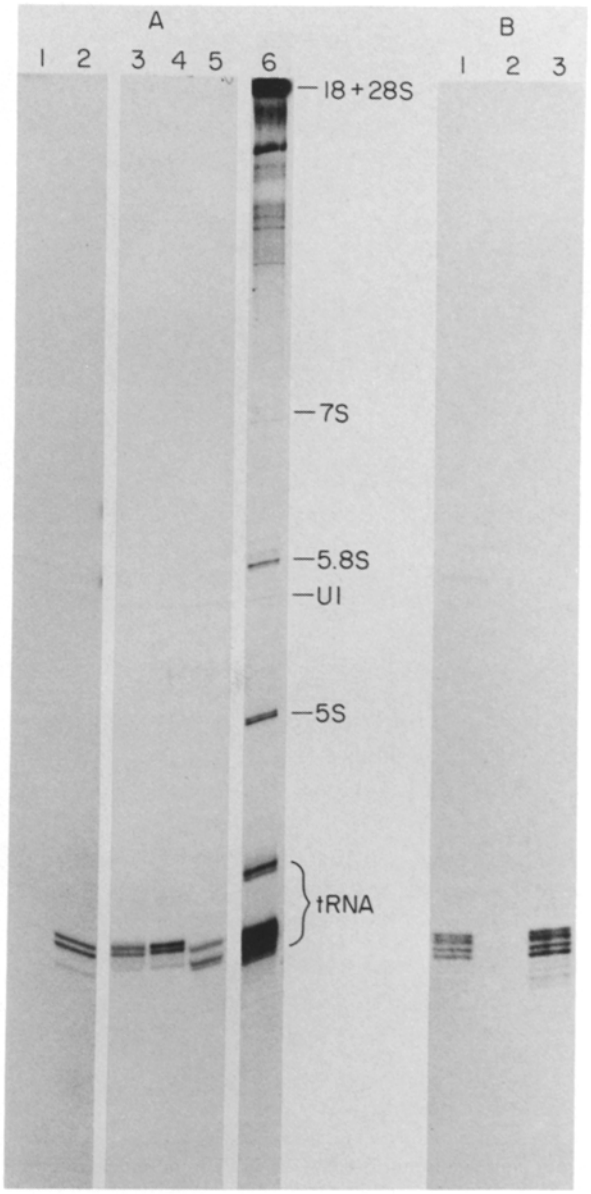

FIGURE 3. Immunoprecipitation of PL-7 RNA. (*A*) Immunoprecipitations from ^{32}P-labeled HeLa cell extracts were carried out with (*1*) normal IgG; (*2–4*) three PL-7 isolates; (*5*) anti-Jo-1 IgG. Lane *6* contains total cellular RNA as marker. (*B*) Immunoprecipitations were carried out with PL-7 IgG from (*1*) untreated ^{32}P-labeled HeLa cell extract; (*2*) ^{32}P-RNA extracted with phenol and chloroform; (*3*) ^{32}P-RNA plus an equivalent amount of unlabeled HeLa cell extract.

ruled out the possibility that PL-7 antibody discharges preformed threonyl-tRNA, so we concluded that most probably the PL-7 antigen is threonyl-tRNA synthetase.

Antibody to Threonyl-tRNA Synthetase. To confirm this identification we carried

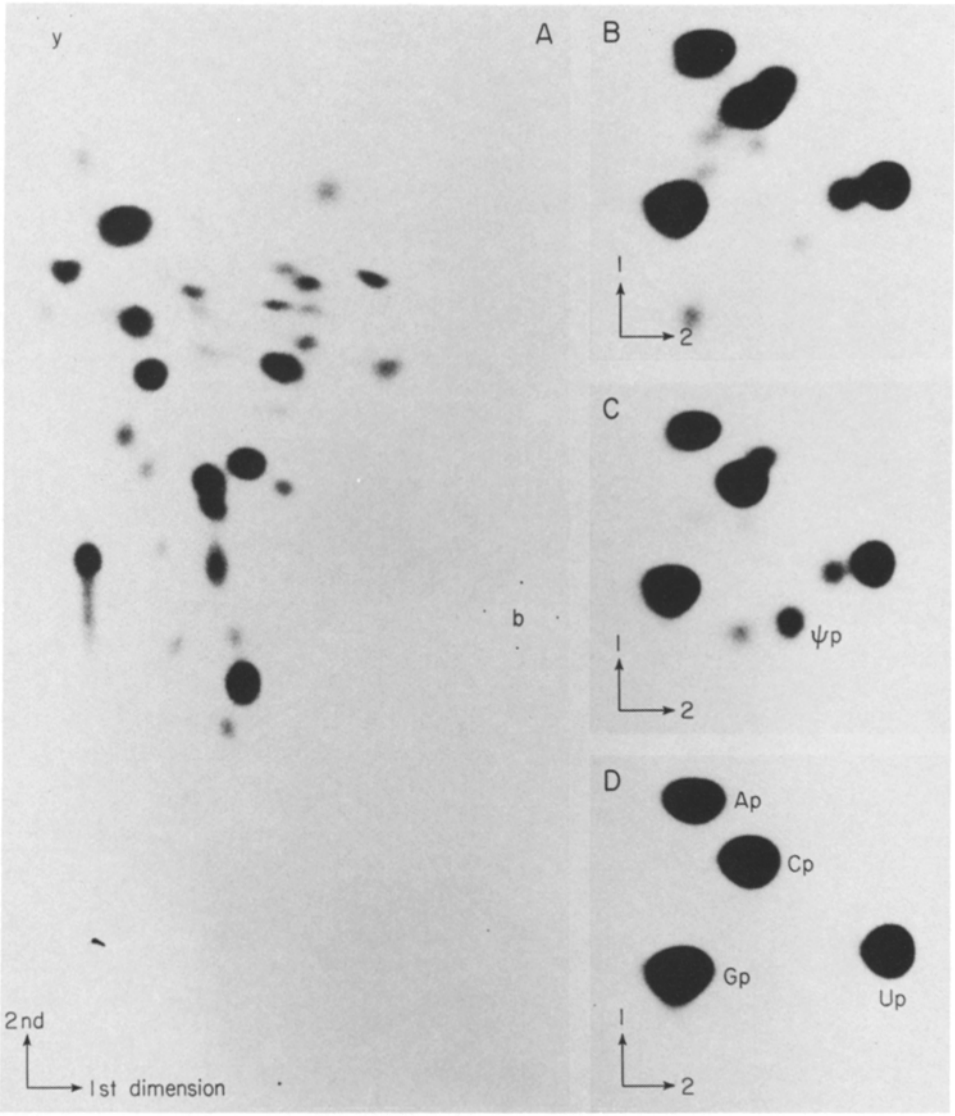

FIGURE 4. Analysis of immunoprecipitated RNA. (*A*) Fingerprint of PL-7 RNA digested with ribonuclease T_1. (*B–D*) Nucleotide composition of PL-7 RNA, Jo-1 RNA, and ribosomal 5S RNA, respectively.

out immunoprecipitations with an antibody (18) raised in rabbits against purified rat threonyl-tRNA synthetase (19), generously provided by Dr. S. Arfin. As expected, extracts depleted by pretreatment with this antibody showed greatly reduced aminoacylation with threonine, but no diminution in the charging of other amino acids (not shown). The rabbit antibody immunoprecipitated a ^{35}S-labeled protein with the same electrophoretic mobility as PL-7 (Fig. 2*A*, lane *1*), and extracts precleared with the human autoantibody contained no protein reactive with either the human or rabbit antibody on subsequent immunoprecip-

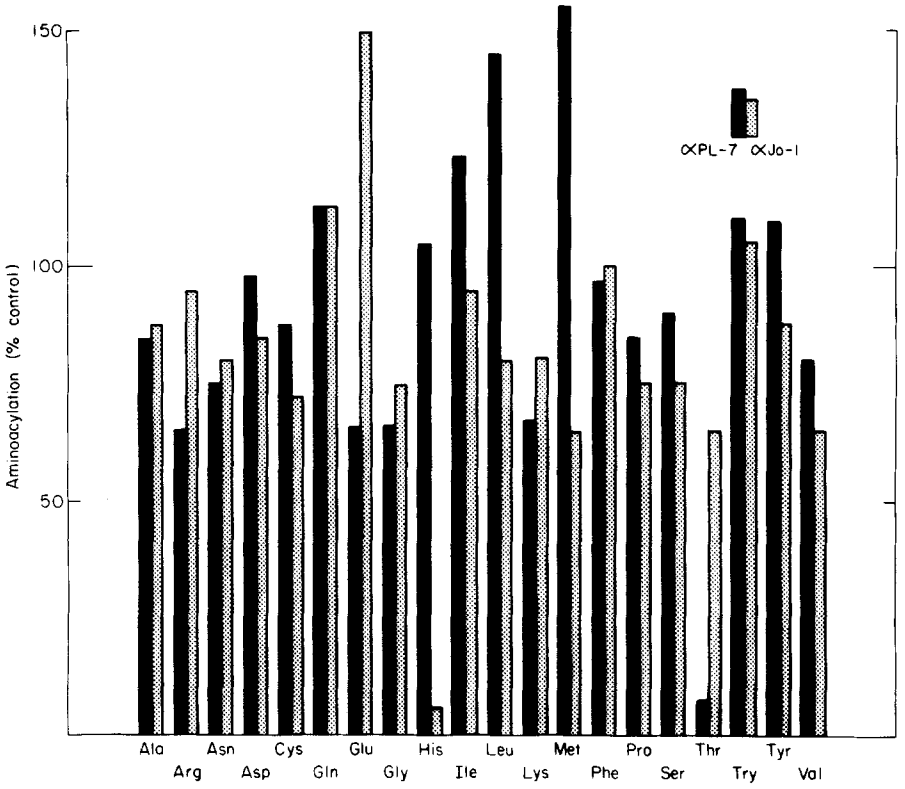

FIGURE 5. Screening for an inhibitory effect of PL-7 antibody on aminoacylation of tRNA. Four charging reactions were set up with each labeled amino acid: two control reactions contained no IgG or 10 μl of normal IgG, and two experimental reactions contained 10 μl of anti-Jo-1 or PL-7 IgG. The extent of aminoacylation in the presence of each autoantibody is expressed as a percentage of the average of the two control reactions.

itation (Fig. 7*A*, lanes *3* and *4*). Preclearing with the rabbit antibody reduced the precipitation of the 80,000 dalton protein by either antibody (Fig. 7*A*, lanes *5* and *6*), but did not eliminate it, presumably because of the lower activity of the experimental antibody (see Fig. 2*A*). These precleared extracts contained undiminished amounts of other cellular antigens such as PCNA (12, 13) (not shown). In a more direct test of identity, the peptide composition of the immunoprecipitated proteins were compared. Partial proteolysis using *Staphylococcus aureus* V8 protease (15) gave similar polypeptide patterns with material immunoprecipitated by either rabbit antibody (Fig. 7*B*, lanes *5–8*) or human antibodies (lanes *1–4* and *9–12*). These results confirm that the PL-7 antigen is threonyl-tRNA synthetase.

Different Antigenic Sites. Surprisingly, in contrast to PL-7 autoantibody, the rabbit antibody failed to immunoprecipitate tRNA from a ^{32}P-labeled extract although the amount of protein precipitated by the two antibodies was comparable (Fig. 8*A*). The experiments shown in Fig. 8*B* were carried out to exclude the possibility that the rabbit IgG preparation contained ribonuclease or some

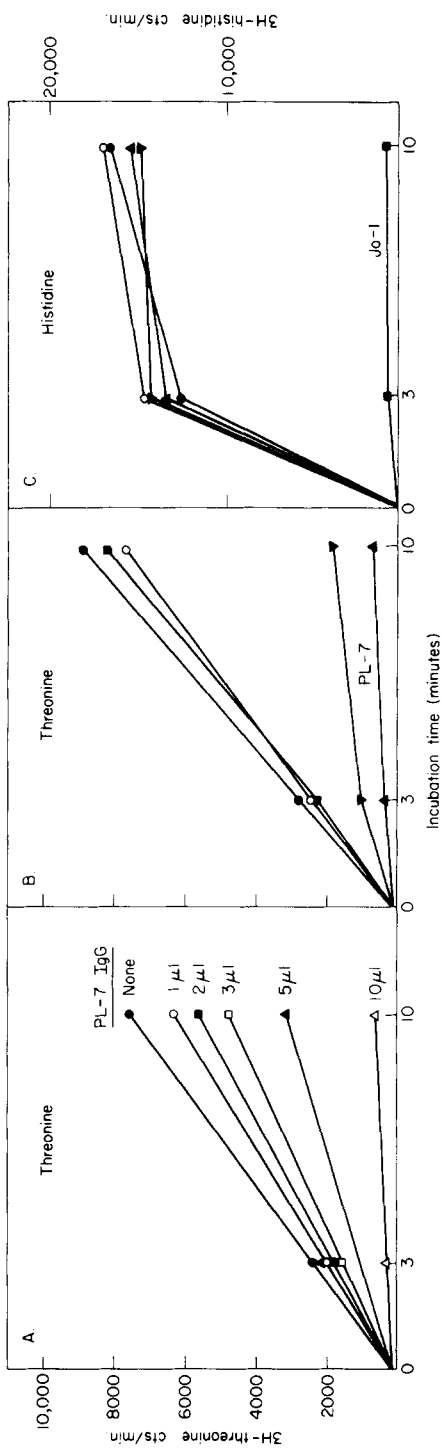

FIG. 6. Kinetics and specificity of inhibition of threonine charging by PL-7 antibody. (*A*) Increasing amounts of PL-7 IgG were added to aminoacylation reactions containing [^{3}H]threonine. (*B* and *C*) Antigen-depleted extracts were assayed for aminoacylation activity with [^{3}H]threonine (*B*) and [^{3}H]histidine (*C*): PL-7 depleted, using two IgG isolates (▲, ▼); Jo-1 depleted (■); mock-depleted (no IgG, ●); and untreated (○).

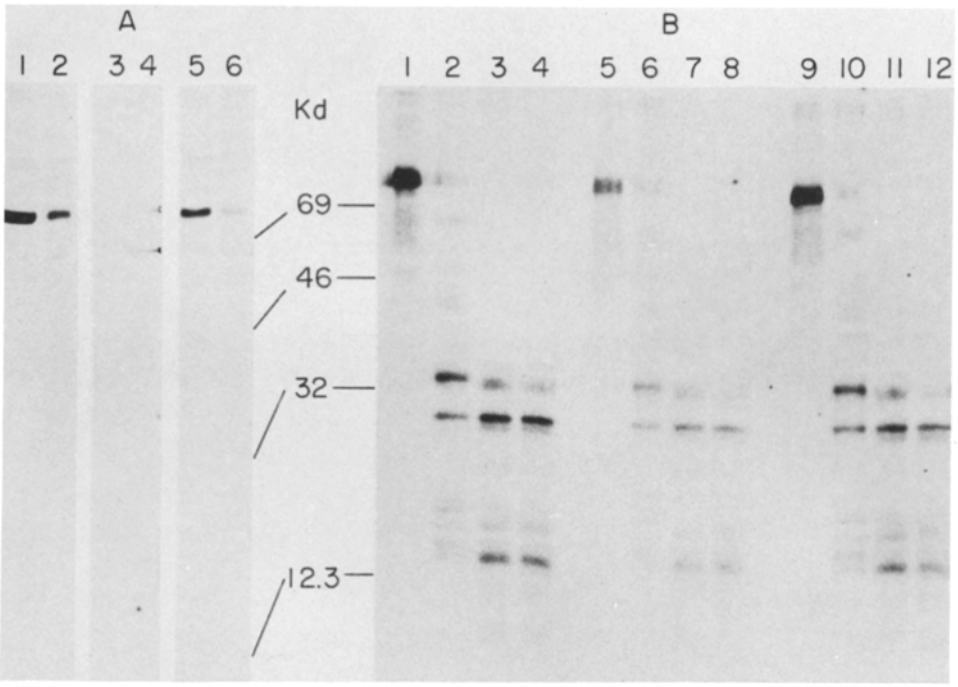

FIGURE 7. Identity of PL-7 antigen with threonyl-tRNA synthetase. (*A*) Immunoprecipitations were carried out with PL-7 antibody (lanes *1*, *3*, and *5*) and anti–threonyl-tRNA synthetase (lanes *2*, *4*, and *6*) from precleared [^{35}S]methionine-labeled HeLa cell extracts. The extracts were precleared with control IgG (lanes *1* and *2*), PL-7 IgG (lanes *3* and *4*), or rabbit anti–threonyl-tRNA synthetase (lanes *5* and *6*). (*B*) Peptides were released by partial proteolysis from the 80,000 dalton protein immunoprecipitated by rabbit anti–threonyl-tRNA synthetase (lanes *5–8*) and two isolates of PL-7 antibody (lanes *1–4* and *9–12*). Each set shows, from left to right, digests with 0, 1, 10, and 100 ng of V8 protease.

other interfering agent. The addition of human placental ribonuclease inhibitor to the rabbit IgG preparation did not lead to the appearance of tRNA in the immunoprecipitate (Fig. 8*B*, lane *2*), nor did the rabbit antibody prevent immunoprecipitation of tRNA by PL-7 antibody (lane *3*). The slight reduction in $tRNA^{Thr}$ precipitated in the presence of the rabbit antibody (compare Fig. 8*B*, lanes *3* and *5*) was also seen with $tRNA^{His}$ precipitated by anti-Jo-1 antibody (lanes *4* and *6*) and is therefore presumably due to overloading of the protein A adsorbent. Since the human autoantibody precipitates tRNA with the enzyme whereas the rabbit antibody does not, we conclude that the two antibodies bind to different sites on the antigen.

Discussion

We have shown that the PL-7 antibody is directed at threonyl-tRNA synthetase. It precipitates a set of tRNAs bound to an 80,000 dalton protein and it specifically blocks the charging of tRNA with threonine. The protein moiety of the ribonucleoprotein complex is the antigenic component, and its molecular weight is in close agreement with measurements on the pure enzyme by Dignam et al.

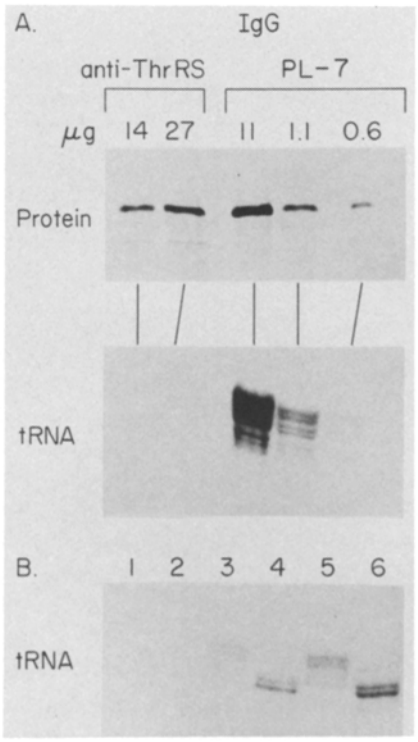

FIGURE 8. Contrasting reactivities of rabbit and human antibodies to threonyl-tRNA synthetase. (*A*) Immunoprecipitations using ^{35}S- and ^{32}P-labeled extracts were carried out with the amounts of rabbit anti–threonyl-tRNA synthetase and PL-7 IgG specified. (*B*) Immunoprecipitations from ^{32}P-labeled extracts were carried out with 10 μg rabbit anti–threonyl-tRNA synthetase (lanes *1–4*) in the presence of the following supplements: (*1*) none; (*2*) ribonuclease inhibitor, 60 U (Rnasin; Promega-Biotec, Madison, WI); (*3*) 1 μg PL-7 IgG; (*4*) 1 μg anti-Jo-1 IgG. (*5* and *6*) 1 μg PL-7 IgG (*5*) and 1 μg anti-Jo-1 IgG (*6*) in the absence of the rabbit antibody.

(19). These workers obtained an estimate of 80,000 daltons with the gel system used by us and 85,000 daltons with another gel system; the native enzyme behaved as a dimer of apparently identical subunits with an aggregate molecular mass of 154,000 daltons. Antibodies raised in rabbits against the purified enzyme recognize a protein that is identical with the PL-7 antigen in size and peptide composition, and by the immunological criterion of the preabsorption test.

Although a rather uncommon specificity, four of the five PL-7 isolates were from cases of myositis. Likewise, the more frequent anti-Jo-1 antibody, directed at histidyl-tRNA synthetase, is closely associated with myositis, where it marks a subset of patients with fibrosing alveolitis and other overlap features (6, 7). From our own work (20), at least three additional RNAs of tRNA size are immunoprecipitated by autoantibodies. Two of these, Mas and Fer, were found only in myositis (Table II). A third antibody system, initially named tRNA-1 (21) and also represented by serum Ha (22), appears to involve alanyl-tRNA synthetase.[2]

[2] Bernstein, R. M., C. C. Bunn, and M. B. Mathews. Coexistence of antibodies to alanyl-tRNA synthetase and tRNA in autoimmune sera. Manuscript in preparation.

This antibody has been found in one case of systemic sclerosis (SS) with myositis, two cases of SLE (one with myositis), one case of juvenile rheumatoid arthritis, and in a healthy control; thus, only two of the patients had myositis (22).[2] Two other tRNAs immunoprecipitated by myositis sera have been reported (21, 22) and are listed below the line in Table II, since they may overlap with those discussed above. Thus, of the five or more independent antibody systems involving RNA of tRNA size, all have been found in myositis, and all but one have been found chiefly or only in this condition.

Considering the large number of sera from other connective tissue diseases that have been studied by immunoprecipitation (8, 21, 23), a link between myositis and tRNA-related antigens is now clearly established. However, the correlation between myositis and tRNA is far from absolute. First, the tRNA-1 antibody was found equally often in other diseases. Second, the Mas RNA may not be a tRNA although it is similar in size. Third, while most myositis sera contain autoantibodies of some sort (3, 24), no more than a third have been shown to recognize tRNA-related antigens. In particular, the Pm-Scl, Mi-1, and RNP antibodies do not recognize tRNA. The Pm-Scl antigen appears to be part of a complex containing three polypeptides of molecular masses 26,000, 31,000, and 36,000 daltons (8), the Mi-1 antigen has not been detected by immunoprecipitation (unpublished results), and the RNP antigen contains several polypeptides complexed with U1-RNA (8, 25).

Nevertheless, the autoimmune response to aminoacyl-tRNA synthetases still appears to be closely associated with myositis. We have suggested a mechanism whereby the infection of genetically predisposed individuals by a particular virus such as coxsackie might lead to both myositis and the generation of antibody to histidyl-tRNA synthetase (4). This hypothesis proposes that viral nucleic acid imitating $tRNA^{His}$ interacts with the enzyme and renders it immunogenic. An-

TABLE II

Summary of tRNA- and Myositis-related Autoantibodies

Antibody system	Number of examples known	Disease association (frequency)	Antigen	References
Jo-1	>50	Myositis (25–30%), myositis with interstitial lung disease (70%)	Histidyl-tRNA synthetase	4–7
PL-7	5	Myositis (3–4%), other connective tissue diseases (~0.1%)	Threonyl-tRNA synthetase	This paper
Mas	2	Myositis (~2%)	?tRNA	8
Fer	1	Myositis (~1%)	Protein (?EF-Tu)	Footnote 2
tRNA-1	5	SS with myositis, SLE with myositis, SLE, juvenile rheumatoid arthritis, healthy control	Alanyl-tRNA synthetase, $?tRNA^{Ala}$	21, 22, and footnote 2
tRNA-2	2	Myositis, SLE	Protein	21
Serum Re	1	Myositis	RNA	22

other virus or another strain of coxsackievirus might induce the antibody to threonyl-tRNA synthetase in like fashion. Although direct support for this specific hypothesis is still lacking, the data do fit well with the more general idea that autoantibodies result from the interaction of foreign or altered nucleic acid with host cell proteins. Interestingly, as we have discussed elsewhere (26), the data fit less well with the antiidiotype hypothesis, at least in the form advanced by Plotz (27), although they do not exclude it.

The rabbit and human antibodies have different specificities. Rabbit anti–threonyl-tRNA synthetase fails to precipitate tRNA with the enzyme, presumably because it binds in such a way as to obstruct the attachment of tRNA or binds to a form of the enzyme incapable of complexing with tRNA. It may be significant that the immunogen in this case was purified protein. On the other hand, the human autoantibody can recognize the enzyme in an RNA-bound state, and it is tempting to deduce, in support of our hypothesis (4), that the human autoimmune response is elicited by RNA-bound antigen.

Summary

An autoantibody known as PL-7 was found in the serum of four patients with myositis and one with a systemic lupus erythematosus–like syndrome. The PL-7 antigen is an 80,000 dalton polypeptide that coprecipitates with transfer RNA. In aminoacylation reactions, PL-7 IgG inhibited the charging of tRNA with threonine but had little or no effect on charging with other amino acids. Experimental antibodies raised against purified threonyl-tRNA synthetase recognized the same 80,000 dalton polypeptide, but tRNA was not coprecipitated. We conclude that PL-7 antibody is directed at threonyl-tRNA synthetase, and that different antigenic sites are recognized by the human and experimental autoantibodies. Our findings emphasize the link between myositis and autoimmunity to tRNA-related structures.

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