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Research Article

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Characterization of a High Molecular Weight Acidic Nuclear Protein Recognized by Autoantibodies in Sera from Patients with Polymyositis-Scleroderma Overlap

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A B S T R A C T Autoantibodies in the serum from a patient with connective tissue disease have been used to define a high molecular weight acidic nuclear protein antigen. The antigen tentatively termed Ku, after the first two letters of patient's name, has distinct physicochemical properties and immunological specificities that distinguish it from previously reported antigens. The Ku antigen has an apparent 300,000 mol wt as determined by gel filtration and sucrose density gradient ultracentrifugation techniques. The antigen is destroyed by trypsin, mild heating, and pH variations >10 and <5. Treatment with ribonuclease or deoxyribonuclease did not affect the antigenic reactivity. The Ku antigen was demonstrated in the soluble extracts of human, calf, and rabbit, but not of rat tissues. Purified antibody localized the Ku antigen within the nuclei of human liver where a "reticular" pattern of immunofluorescence was seen. Of 330 patients with various connective tissue diseases, 9 had precipitating antibodies to the Ku antigen. Preliminary results of clinical analysis indicated that antibody to the Ku antigen might become a useful marker for a group of patients with clinical characteristics of both polymyositis and scleroderma with a good prognosis.

INTRODUCTION

The immunological abnormalities of patients with diffuse connective tissue diseases are characterized by autoantibodies reactive with cellular constituents (1). Recent studies strongly indicate that an association exists between the presence of specific antibodies and the pattern of clinical expression of the connective tissue diseases. In addition to antibodies to DNA, which are associated with immunologically mediated renal disease of systemic lupus erythematosus (SLE)[1] (2), new antigen-antibody systems have been described that serve as markers for specific connective tissue diseases or for subsets within a particular disease entity. Antibodies reactive with nuclear ribonucleoprotein (RNP) antigen are associated with mixed connective tissue disease (MCTD) or SLE patients with a lower frequency of severe nephritis (3-7). Antibodies to the Sm and Scl-70 have been shown to serve as specific markers for SLE and scleroderma, respectively (8-11). Autoantibodies directed against the PM-1 and Jo-1 antigens were reported to be specific for polymyositis (12-14). Antibodies reactive to SS-B (La, Ha) antigen are associated with Sjögren's syndrome not associated with other connective tissue diseases (sicca syndrome alone) and a subset of SLE with sicca syndrome (15-19). Therefore, clarification of these biological reactions with known specificities, not only have clinical usefulness but might also elucidate immunopathological mechanisms involved in the connective tissue diseases.

This report describes an acidic nulcear protein with a high molecular weight that can be recognized by specific autoantibodies. The antigen was termed Ku after the first two letters of the original patient's name whose serum was used as a prototype. The physicochemical properties of this antigen are compared with other cellular antigens to which patients with con-

[1] *Abbreviations used in this paper*: CTE, calf thymus extract; CTNE, calf thymus nuclear extract; DNase, deoxyribonuclease; FANA, fluorescent antinuclear antibody; MCTD, mixed connective tissue disease; PBS, phosphate buffered saline; RNase, ribonuclease; RNP, nuclear ribonucleoprotein; SLE, systemic lupus erythematosus.

nective tissue disease produce autoantibodies. The preliminary results of clinical studies of patients with anti-Ku antibodies are also reported.

METHODS

Patients. Serum samples of 330 Japanese patients with connective tissue diseases were obtained from the serum bank of the Division of Connective Tissue Disease, Department of Medicine, Keio University Hospital. 150 patients with SLE had more than four items of the preliminary criteria for the classification of SLE as defined by the American Rheumatism Association (20). 45 patients with scleroderma and 30 patients with polymyositis met the definite category as defined by Medsger (21, 22). 54 patients with rheumatoid arthritis had definite or classical disease according to the American Rheumatism Association criteria (23). 26 patients with over-lap syndrome each fulfilled two or more of the above sets of criteria (3, SLE-scleroderma-polymyositis; 7, SLE-sclero-derma; 5, SLE-polymyositis; 11, scleroderma-polymyositis). 25 patients with mixed connective tissue disease (MCTD) were defined by both clinical and serological characteristics described by Sharp et al. (3-5). Although the overlap syndrome was defined solely on clinical criteria, only two patients were simultaneously classified in the MCTD group: of 26 patients with overlap syndrome, 13 were negative for anti-RNP antibodies and among 13 with anti-RNP antibodies, 11 had additional precipitating antibodies. 56 control sera were obtained from healthy volunteers.

Reference sera. The standard reference serum used to identify the Ku antigen was obtained by plasmapheresis from a patient with overlap syndrome of scleroderma and poly-myositis. The Ku serum showed two precipitating lines against the calf thymus extract (CTE) in immunodiffusion; the major system, designated Ku, had a precipitin titer of 1:128, and the minor system, identified as the PM-1 system, had a titer of 1:4. It gave a weak reaction (1:4) on cryostat section of rat liver by standard fluorescent antinuclear antibody (FANA) test and was negative for anti-DNA antibodies ([3H] DNA binding assay [24]) and rheumatoid factor. The specificities of standard sera containing antibodies to the Sm, RNP, SS-B (La, Ha) antigens were confirmed at the symposium held by the Kroc Foundation (25). The following systems were identified by the reference sera provided from the laboratories where the original studies were performed: SS-A (Ro), Dr. Miyachi and Dr. Tan, Denver, Col.: Dr. Nishikai and Dr. Reichlin, Buffalo, N. Y.: PM-1, Drs. Wolfe, Takano, and Sharp, Columbia, Mo.: Jo-1 and Mi, Dr. Nishikai and Dr. Reichlin: Scl-70, Dr. Miyachi and Dr. Tan (10–14, 18, 26–28).

Tissue extracts

Whole tissue extracts. Whole tissue extract was prepared by homogenizing and extracting calf thymus glands in an equal weight volume of saline at 4°C. The homogenate was centrifuged at 10,000 g for 60 min followed by centrifugation at 76,000 g for 90 min (type 30 rotor in a Beckman model L5-50 ultracentrifuge, Beckman Instruments Inc., Palo Alto, Calif.). The supernate was dialyzed against saline, lyophilized, and stored at −20°C until use. This product was designated as the CTE and used for the characterization of the Ku antigen. To study the species and organ distribution of the Ku antigen, extracts of liver and spleen from rat, rabbit, and human organs obtained at necropsy were prepared similarly. The rabbit thymus extract was prepared by extracting the rabbit thymus acetone powder (Pel Freeze Biologicals, Inc., Rogers, Ark.)

with 0.01 M phosphate-buffered saline, pH 7.2 (PBS) containing 0.02% Na_3N (wt/vol) for 8 h at 4°C while stirring (1.5 g acetone powder/10 ml PBS). The extract was centrifuged at 2,000 g for 15 min and the supernate was lyophilized.

Nuclear extracts. The soluble nuclear extracts of calf thymus (CTNE), rat, and human liver were prepared according to the method described previously (16).

Cytoplasmic fractions. The cytoplasmic fractions of calf thymus gland, rat and human liver were prepared according to the method of Clark and Reichlin (26).

Immunological techniques

Immunodiffusion. For the demonstration and identification of precipitating antibodies reactive with antigens in the CTE, the Ouchterlony double immunodiffusion method was used (29). 22.5 ml of 0.6% agarose (SeaKem Agarose, Marine Colloids, Inc., Springfield, N. J.) in PBS containing 0.02% Na_3N and 0.02% trypan blue were pipetted into a 100 × 15-mm plastic petri dish (Eiken Kizai Co., Tokyo, Japan). Wells of 7 mm Diam placed 3 mm apart were cut by a gel puncher (Auto-Gel, Grafar Corp., Detroit, Mich.). For most of the study, the CTE was used as a source of antigen at a concentration of 100 mg/ml (wt/vol). The optimal dilutions of standard sera were determined and used for the identification of the precipitating antibodies. Precipitin lines were developed at 22°C and examined at 24, 48, and 72 h.

Immunoelectrophoresis. The anti-Ku antibodies in serum and the Ku antigen in the CTE were studied by standard immunoelectrophoresis (30). 1.0% agarose plates in veronal buffer (μ^m = 0.06, pH 8.6) were cooled to 4°C during electro-phoresis (3 mA for each centimeter width of plate) (Multiphor, LKB-Produkter AB, Bromma, Sweden).

Immunofluorescent test. The standard FANA test was performed with 4-μm cryostat sections of rat or human liver (31). The IgG fraction of rabbit antiserum to human IgG conjugated with fluorescein isothiocyanate (F/P ratio: 1.7) (32) and a Nikon fluorescent microscope (Fluophot, Nippon Kogaku K. K., Tokyo, Japan) were used.

Purification of specific antibodies. To determine the intracellular location of the Ku antigen, specific antibodies were prepared from immune precipitates. Partially purified Ku antigen was obtained from CTE by ammonium sulfate fractionation (30–60% saturation) and DEAE-cellulose chromatography (NaCl 0.15 M–0.35 M in 0.05 M Tris/HCl buffer, pH 7.4) and showed a single precipitin line against the serum Ku in double diffusion. This material was reacted at equivalence with IgG obtained from serum Ku in the presence of 3.5% polyethylene glycol (final concentration in the mixture, Sigma Chemical Co., St. Louis, Mo.). After 72 h incubation, the mixture was centrifuged for 15 min at 2,000 g, washed three times with cold PBS, and resuspended in 0.5 M glycine, 0.15 M NaCl buffer, pH 3.0. The clarified solution was centrifuged for 30 min at 19,000 g and the supernate was concentrated by ultrafiltration (Minicon B-15, Amicon Corp., Lexington, Mass.), and neutralized by dialyzing against PBS. The final product was tested by immunoelectrophoresis and immunodiffusion for its purity.

Column chromatography

Gel filtration. The CTE was fractionated through the calibrated Bio-Gel A–0.5 m, A–1.5 m (Bio-Rad Laboratories, Richmond, Calif.) and Sephacryl S-200 superfine (Pharmacia Fine Chemicals AB, Uppsala, Sweden) column (2.5 × 90 cm). The fraction of eluates were ~10 times concentrated by ultra-filtration (Minicon B-15, Amicon Corp. Lexington, Mass.)

and tested for their antigenic activities by immunodiffusion.

Ion exchange chromatography. IgG was prepared by ion exchange chromatography from the patient's serum using DEAE-cellulose (DE-52, Whatman Ltd., Springfield, Mill Maidstone, Kent, England) (33).

Ion exchange chromatography of the CTE was performed according to the method described by Clark et al. (24) with some modification. Ion exchanger (DE-52, Whatman Ltd.) was equilibrated with an initial buffer (0.05 M Tris/HCl, 0.05 M NaCl, pH 7.4, containing 1 mM dithiothreitol [Sigma Chemical Co.]) and the CTE dialyzed against the initial buffer was eluted with a linear gradient using 0.05 M Tris/HCl, 0.4 M NaCl, pH 7.4, containing 1 mM dithiothreitol as a limiting buffer. The antigenic activities in the fractions were tested in a manner similar to that used in gel filtration.

Zone centrifugation. Sucrose density gradient ultracentrifugation of the CTE was performed by the method of Kunkel (34). 0.2 ml of the CTE (partially purified by ammonium sulfate fractionation and DEAE-cellulose chromatography) was layered over 5 ml of 10–40% sucrose gradient and centrifuged for 17 h at 140,000 g in a SW-50 rotor. Protein concentration of fractions collected from the bottom was determined by Bio-Rad Protein Assay (Bio-Rad Laboratories) using bovine serum albumin as a standard and antigenic activities were tested by immunodiffusion (35).

Physicochemical characterization of the Ku antigen

Enzymatic treatment. Enzymatic digestion of the CTE was performed according to Holman and Deicher (36). The protein, DNA, and RNA contents of the CTE were determined chemically (35–37), and the CTE was treated with trypsin, ribonuclease (RNase) and deoxyribonuclease (DNase) I (Worthington Biochemical Corp., Freehold, N. J.) using various enzyme-to-substrate ratios at 22°C for 2 h. The treated extract was titered out against the reference sera in double diffusion and compared with controls.

Sensitivity to heat and pH variations. The sensitivity of the Ku antigen to heating and pH changes was examined by the method of Mattioli and Reichlin (6). The effect of pH variations on the antigenic activity was studied by dialyzing the CTE against buffers of various pH at 4°C; glycine/HCl (pH 3.0), acetate (pH 4.0–5.0), phosphate (pH 6.0–8.0), and glycine/NaOH (pH 9.0–11.0) (39). The residual activity was tested by immunodiffusion.

RESULTS

Specificity of the Ku system. The immunological specificity of the Ku system was first demonstrated by immunodiffusion. Fig. 1A illustrates that the precipitin lines formed between the serum Ku and the CTE crossed over the lines of the RNP, Sm, SS-A, and SS-B systems. In Fig. 1B, the antigenic difference of the Ku from the PM-1 and Jo-1 systems was clearly shown by immunological nonidentity of precipitin lines. The Mi and Scl-70 systems were barely seen when whole CTE was used. The immunological independence of the Ku system from the Mi-1 and Mi-2 systems was demonstrated by double immunodiffusion using CTNE as antigen. Because the Ku system could not be seen at low concentration of the CTNE, we added partially purified Ku antigen by ammonium sulfate fractionation
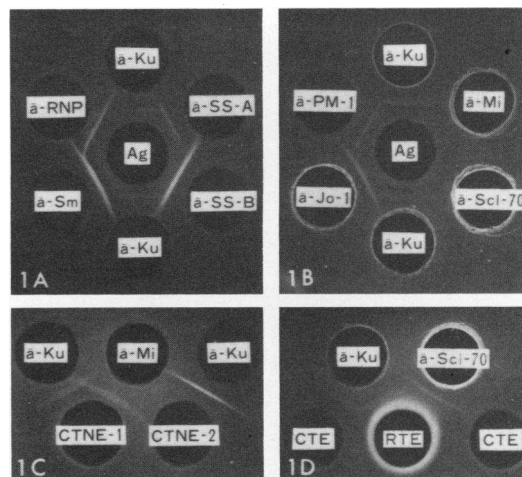

FIGURE 1 Demonstration of the immunological independence of the Ku system from the previously described precipitating systems in the connective tissue diseases by double immunodiffusion. The precipitin lines formed between the serum Ku (diluted 1:20 with PBS) and CTE (100 mg/ml, Ag well) go through the lines of the RNP (1:10), Sm (1:1), SS-A (1:20), and SS-B (1:20) systems (A). The Ku system has different specificity from the PM-1 (1:1) and Jo-1 (1:4) systems (B). The Mi and Scl-70 systems were not visualized in Fig. 1B. The Mi system could be demonstrated by using an extract from isolated calf thymocyte nuclei (Mi, 1:1, CTNE-1 100 mg/ml, CTNE-2 10 mg/ml plus partially purified Ku antigen, (C). The clear line between the serum Mi (1:1) against CTNE-1 was designated as the Mi-2 system and the faint line against CTNE-2 as the Mi-1 system. The immunological difference of the Ku antigen from the Scl-70 antigen (1:10) systems was demonstrated by using rabbit thymus extract (RTE 40 mg/ml as the source of antigens (D).

and DEAE-cellulose (see Results) in the antigen well (well Ag of Fig. 1C). The Ku lines crossed over the precipitin lines of both the Mi-1 system (against CTNE-2) and Mi-2 system (against CTNE-1). The rabbit thymus extract revealed the different specificity of the Ku from the Scl-70 system (Fig. 1D).

The immunoglobulin class of precipitating antibodies reactive with the Ku antigen was studied. Serum Ku and a normal human serum were electrophoresed and reacted against rabbit anti-whole human serum and the CTE. The specific arc of the Ku system was observed in the gammaglobulin region with the characteristic location and curvature of IgG (Fig. 2A). IgG purified from the serum Ku showed the identical specificity as the original serum in gel diffusion (Fig. 2B). Four other sera with precipitating antibodies to the Ku antigen showed the same results.

Characterization of the Ku antigen

Sensitivities to enzymes, heating, and pH. Sensitivity of the Ku antigen to enzymatic digestion was studied by titering out the CTE treated with trypsin,
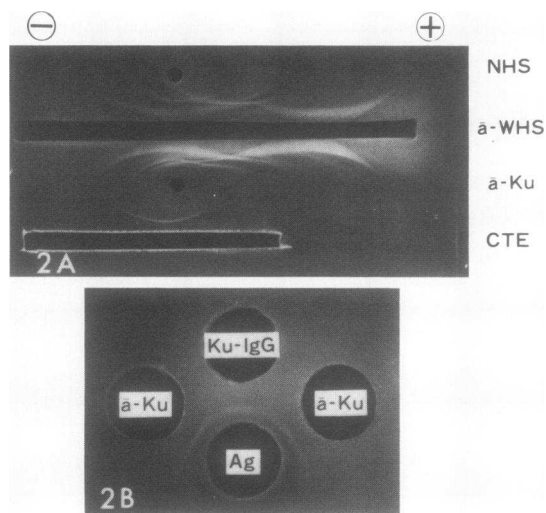
FIGURE 2 The isotype analysis of precipitating antibodies to the Ku antigen by immunoelectrophoresis and immunodiffusion. The precipitin arc developed against the CTE has the characteristics of IgG in immunoelectrophoresis (A). IgG purified from serum Ku (1 mg/ml) and the original serum showed identical specificity (B).

RNase, or DNase against the standard reference serum (Fig. 3). The reactivity of the Ku antigen was completely destroyed by trypsin at an enzyme to substrate ratio of 1:50 (2 mg/ml). The Jo-1 and RNP antigens, but not the Sm antigen, were affected by trypsin (Fig. 3A). The Ku, like Jo-1 and Sm antigens, was not affected by RNase treatment. The effectiveness of RNase digestion was confirmed by loss of antigenic activity of RNP (Fig. 3B). The DNase treatment did not affect the Ku nor the Jo-1, Sm, and RNP reactivities. The activity of DNase was checked by a separate experiment according to Kunitz (40).

The Ku antigen was extremely sensitive to heating (Fig. 4). Complete loss of activity was observed by incubating the CTE for 30 min at 37°C and 15 min at 56°C. The Jo-1 was not affected at 37°C, but its activity was abolished at 56°C after 30 min. Addition of the potent protease inhibitor phenylmethylsulfonylfluoride at a final concentration of 1 mM did not protect the Ku antigen from heat inactivation at 37°C.

Fig. 5 shows the sensitivity of the Ku antigen to pH variations. After the CTE was dialyzed against buffers ranging from pH 3 to 11 for 17 h at 4°C, it was neutralized by dialysis against PBS and the residual activities were examined by immunodiffusion. The activity of the Ku antigen was destroyed below pH 5 or above pH 10, which contrasted with the more stable RNP, Jo-1, and Sm antigens.

Characterization by salt fractionation, gel filtration, ion exchange chromatography and zone centrifugation. The majority of the Ku antigen was precipitated

by ammonium sulfate 30–60% saturation at 4°C. Fig. 6 depicts the elution profile of the CTE from a DEAE-cellulose column (2.5 × 25 cm) using a linear NaCl gradient at 22°C. The Ku activity determined by immunodiffusion using concentrated fractions was eluted between 28.5 and 42.0 mmho, which correspond to NaCl concentrations of 0.17 and 0.29 M, respectively. Approximately 10 times enrichment of the activity could be achieved by ammonium sulfate fractionation followed by stepwise DEAE-cellulose chromatography. This fraction showed a single precipitin line against serum Ku and was used for immunoelectrophoresis, zone centrifugation, and purification of specific antibodies. However, it contains other antigens such as RNP, Sm, and SS-A.

In gel filtration, the Ku antigen was excluded from a Sephacryl S-200 and included by a Bio-Gel A–0.5-m column. Fig. 7 shows the elution curve of the CTE from a Bio-gel A–1.5-m column (2.5 × 90 cm) moni-
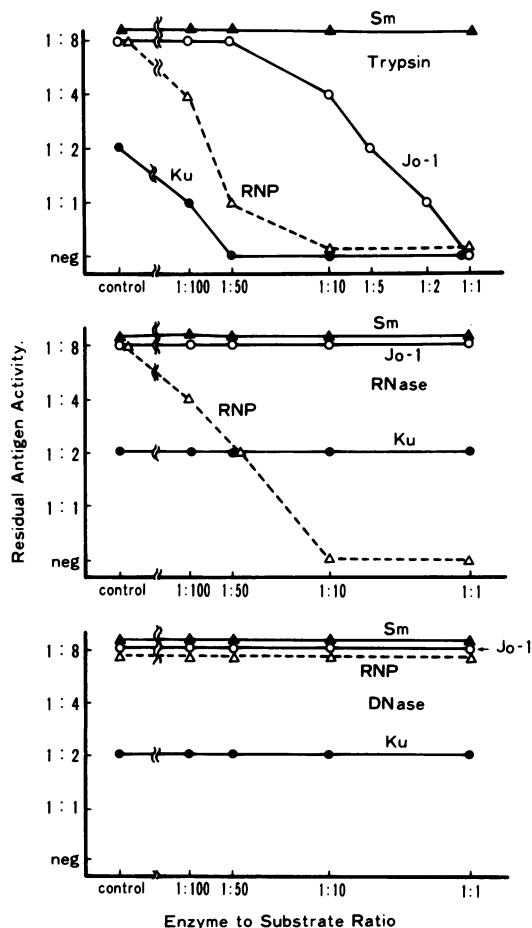

FIGURE 3 The effects of enzymatic digestion on the Ku antigen reactivity. CTE treated by enzymes was titered against the reference sera. The sensitivity of the Ku antigen to trypsin but not to RNase or DNase was demonstrated.

tored continuously at 280 nm. The activity of the Ku antigen was eluted between horse ferritin (440,000 mol wt) and human IgG (150,000 mol wt) markers. The approximate molecular weight of the Ku antigen was calculated to be 300,000 daltons, which is larger than other known antigens in the CTE. These results were confirmed by sucrose gradient ultracentrifugation studies using the partially purified Ku antigen by ammonium sulfate fractionation and DEAE-cellulose (Fig. 8). The Ku antigen was found in fractions between IgM and IgG.

Immunoelectrophoresis. The electrophoretic mobility of the Ku antigen was compared with that of normal human serum components. The partially purified CTE (20 µl) and normal human serum (5 µl) were electrophoresed for 120 min on a 1.0% agarose plate cooled to 4°C. The Ku antigen detected by serum Ku showed a rapid anodal mobility and migrated in the albumin region (Fig. 9).

Intracellular location, organ, and species distribution. The indirect immunofluorescence technique using specifically purified antibodies was used to study the intracellular location of the Ku antigen. The elution of specific antibodies from immune precipitates formed by the patient's IgG and partially purified CTE yielded ~15-fold purification of the immunoglobulin demon-

FIGURE 5 The stability of the Ku antigen of pH changes. CTE was dialyzed against buffers of varying pH, neutralized, and the antigenic activities were tested by double diffusion. Like the RNP, the Ku antigen was stable over a more narrow range of pH than the Jo-1 and Sm antigens. The reactivity of the Ku antigen was abolished < pH 5 and > pH 10.

strating a clear precipitin line in agar at 25 µg/ml. When the purified anti-Ku antibody was examined by immunofluorescence, there was a striking difference among the sources of tissue sections. Bright nuclear staining on human liver sections, but not on rat liver sections, was seen (Fig. 10A and B). On high power view, the nuclei were stained in a reticular pattern sparing the nucleoli. This pattern can be distinguished from speckled patterns produced by anti-Sm, RNP, or SS-B (Ha) antibodies.
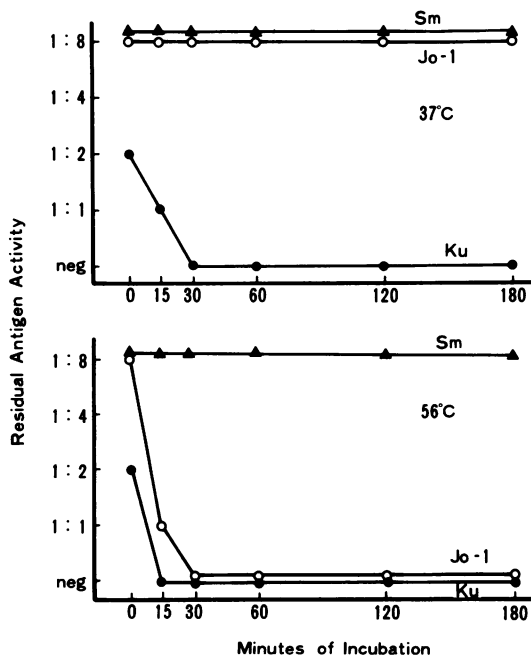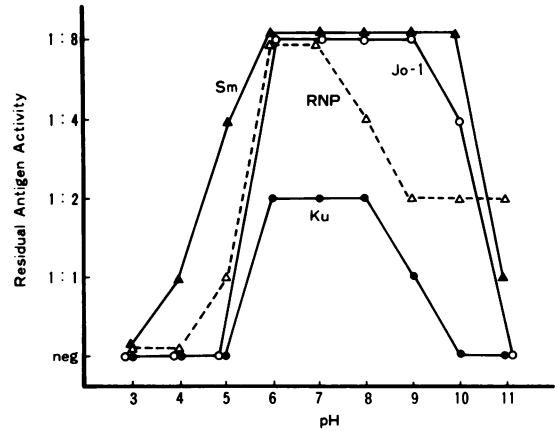
FIGURE 4 The sensitivity of the Ku antigen to heating. CTE was incubated in the water bath at 37 or 56°C and the residual activity was tested by immunodiffusion. The Ku antigen activity was abolished by incubating for 30 min at 37°C or 15 min at 56°C. The Jo-1 antigen was stable at 37°C but destroyed by 30 min incubation at 56°C. In contrast, the Sm antigen was not affected under these conditions.
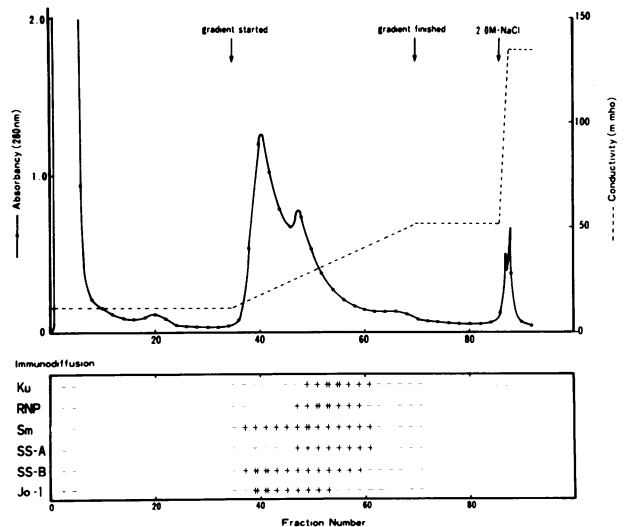
FIGURE 6 DEAE cellulose chromatography of CTE. The antigenic activity of the Ku antigen examined by reference sera using the concentrated eluates recovered from the fractions with NaCl 0.17–0.29 M in 0.05 M Tris/HCl buffer, pH 7.4 at 22°C.
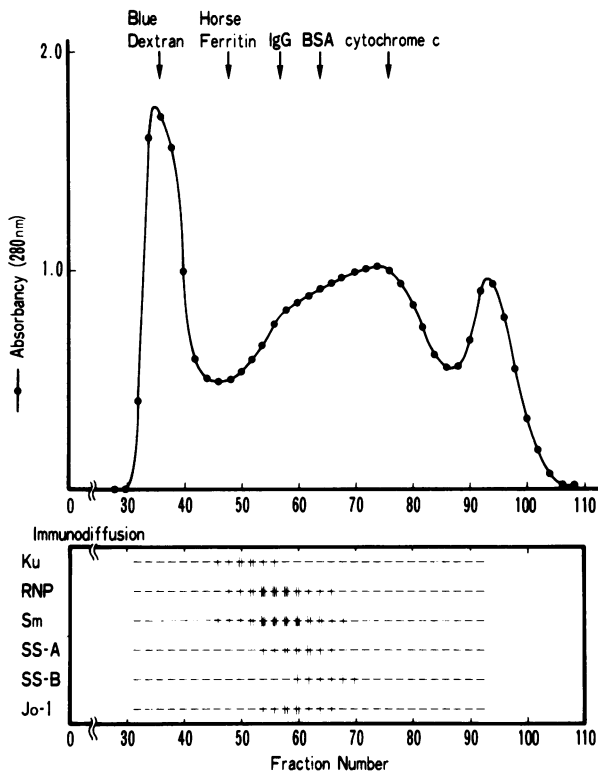
FIGURE 7 Gel filtration of CTE through a Bio-Gel A–1.5-m column (2.5 × 90 cm). The Ku antigen has an estimated molecular weight of 300,000.
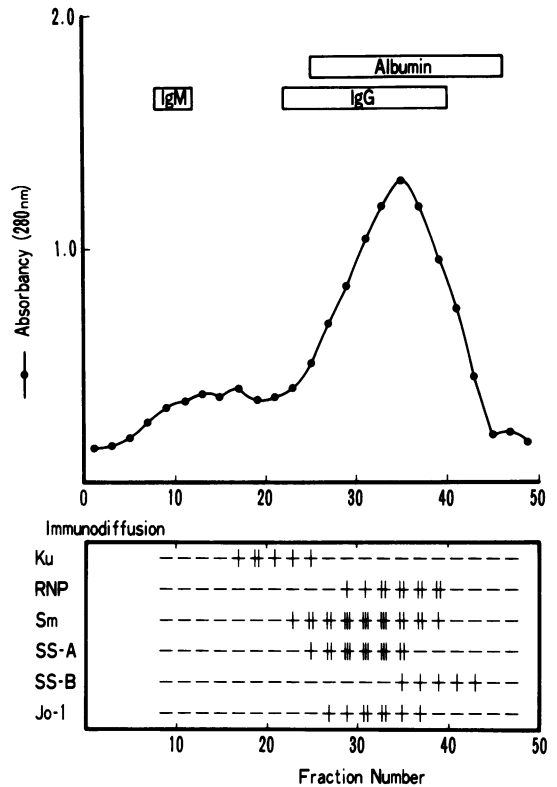

FIGURE 8 Sucrose density gradient ultracentrifugation of the CTE. The Ku antigen sedimented in fractions between IgM and IgG.

The nuclear origin of the Ku antigen was confirmed by cell fractionation technique. The minimum protein concentrations that showed definite precipitin line against serum Ku were ~2.5 and 50 mg/ml for extracts of human liver nuclear and cytoplasmic fractions, respectively. The presence of Ku antigen in whole tissue or nuclear extracts prepared from human (thymus, liver, and spleen) and rabbit organs (thymus, liver, and spleen) was confirmed by double diffusion. However, several experiments designed to demonstrate the antigen in rat tissues were not successful.

Incidence and disease specificity. Incidence of antibodies to the Ku antigen in various connective tissue diseases is shown in Table I. Undiluted and 1:8 diluted serum samples were tested against CTE in agarose plates (whole CTE and 30–60% ammonium sulfate cut CTE, both at 100 mg/ml (wt/vol)). The specificities of precipitating antibodies were determined at optimal serum dilution with standard reference sera. 9 out of 330 patients were positive for anti-Ku antibodies (1 patient with SLE, 1 with scleroderma, and 7 with overlap syndrome [1: SLE-scleroderma-polymyositis, 6: scleroderma-polymyositis]). Serial determinations in four patients over 3-yr period revealed the persistence of anti-Ku antibodies in their sera. Precip-

itating antibodies of other specificities are also shown for these nine patients (Table II). In addition to the anti-Ku antibodies, these patients had other precipitating antibodies that included the PM-1, RNP, SS-A, and unidentified systems. FANA titers in these patients are invariably higher on human than rat liver nuclei, and in two patients FANA were negative on rat liver sections.
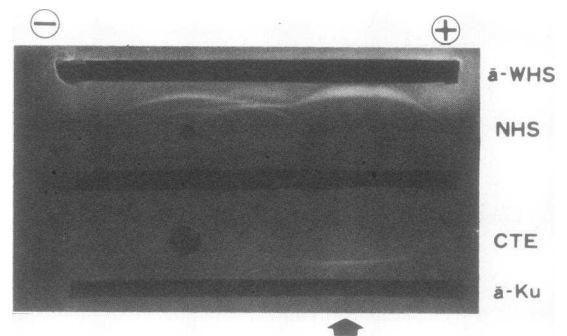

FIGURE 9 Immunoelectrophoresis of CTE. The acidic nature of the Ku antigen that migrated into the albumin region (arrow) was demonstrated. CTE was electrophoresed for 120 min at 4°C and the precipitin arc developed by the serum Ku was compared with normal human serum.

616   *T. Mimori, M. Akizuki, H. Yamagata, S. Inada, S. Yoshida, and M. Homma*

TABLE I

Frequency of Precipitating Antibodies to the Ku Antigen
in Connective Tissue Diseases

Disease	Number tested	Number positive	Percent positive
SLE	150	1	1
Scleroderma	45	1	2
Polymyositis	30	0	0
Rheumatoid arthritis	54	0	0
MCTD	25	0	0
Overlap syndrome	26	7	27
SLE-scleroderma-polymyositis	3	1	33
SLE-scleroderma	7	0	0
SLE-polymyositis	5	0	0
Scleroderma-polymyositis	11	6	55
Normal controls	56	0	0

Antibodies to the Ku antigen were not frequently found. However, these nine patients had unique clinical characteristics in common (Table III). Patients 1–7 had clinical characteristics of both polymyositis and scleroderma, fulfilling criteria for these diseases. Though patients 8 and 9 were categorized as SLE and scleroderma by strict criteria, both had clinical features suggestive of scleroderma and polymyositis (8: Raynaud's phenomenon, sclerodactyly, proximal muscle weakness, elevated serum levels of creatine phosphokinase and aldolase, 9: elevated myogenic enzymes). Systemic manifestations, such as fever and inflammatory signs other than myositis, are infrequent. Sclerodermatous skin changes were confined to face and/or extremities and did not extend to the trunk. Flexion contractures of the joints, gangrenes, or advanced internal organ involvements characteristic of scleroderma were not observed. All patients showed good response to corticosteroid therapy and none died during the average follow-up period of 5.4 yr (1–16 yr).
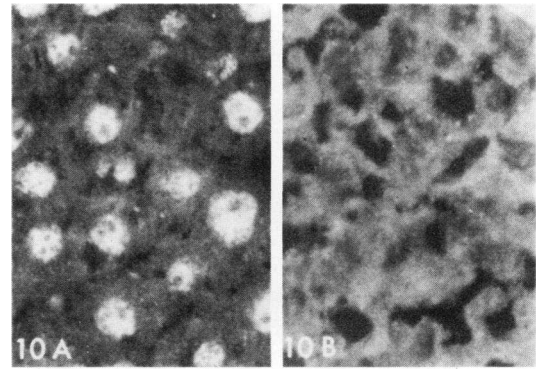

FIGURE 10   Demonstration of the intracellular localization of the Ku antigen by standard indirect immunofluorescent technique. Purified anti-Ku antibodies prepared by acid elution of immune-precipitates showed reticular nuclear immunofluorescence sparing the nucleoli on human liver section (A). No specific staining was observed on rat liver nuclei (B).

DISCUSSION

The present report describes an antigen-antibody system that may serve as a serological marker for a subset of patients with polymyositis-scleroderma overlap syndrome. The active antigen in the soluble extract of calf thymus defined by the autoantibodies in the serum of a patient with polymyositis-scleroderma overlap was termed Ku after the first two letters of her name. The immunological independence of the Ku antigen from the Sm, RNP, SS-A (Ro), and SS-B (La, Ha) antigens was easily demonstrated by double immunodiffusion technique. There are four antigen systems reported with specificities for polymyositis, dermatomyositis, or scleroderma (10–14, 27). The recent studies by Nishikai and Reichlin (28) revealed that Mi consists of two precipitin systems that can be

TABLE II

Fluorescent Antinuclear Antibodies and Precipitating Antibodies to
Tissue Antigens in Nine Patients with Anti-Ku Antibodies

Patient	FANA titer		Antibodies to				
	Rat liver	Human liver	Ku*	RNP	PM-1	SS-A	Others‡
1 K.T.	1:4	1:2048	1:128	−	+	−	−
2 K.K.	1:64	1:2048	1:128	−	+	−	−
3 K.K.	1:64	1:1024	1:64	−	−	−	+2
4 T.T.	1:64	1:1024	1:64	−	+	+	−
5 H.T.	Negative	1:1024	1:64	−	−	+	−
6 N.S.	1:4	1:4096	1:256	−	−	−	+2
7 A.T.	Negative	1:4	1:1	−	−	−	+1
8 K.T.	1:256	1:4096	1:128	+	−	+	−
9 K.T.	1:16	1:2048	1:128	−	−	+	−

* Titer of precipitating antibodies to the Ku antigen.
‡ Number of unidentified precipitin systems.

TABLE III
Clinical Characteristics of Nine Patients with Precipitating Antibodies to the Ku Antigen

Patient	Diagnostic classification	Fever (≥38°C)	Raynaud's phenomenon	Sclerodermatous skin change	Muscle weakness	Elevated* enzymes	Arthritis
1 K.T.	Overlap syndrome	−	+	+	+	+	−
2 K.K.	Overlap syndrome	−	+	+	+	+	−
3 K.K.	Overlap syndrome	−	+	+	+	+	−
4 T.T.	Overlap syndrome	+	+	+	+	+	+
5 H.T.	Overlap syndrome	−	+	+	+	+	−
6 N.S.	Overlap syndrome	−	+	+	+	+	−
7 A.T.	Overlap syndrome	−	+	+	+	+	−
8 K.T.	SLE	+	+	+	+	+	+
9 K.T.	Scleroderma	−	+	+	−	+	−

* Elevated creatine phosphokinase (≥80 IU/liter) and/or aldolase (≥6 IU/liter).

demonstrated in agar using extracts from calf thymus nuclei. Wolfe and Sharp (12) described that antibodies to the PM-1 antigen occurred in 60, 17, and 87% of polymyositis, dermatomyositis, and polymyositis-scleroderma overlap, respectively. Tan and his associates (10, 11) described the Scl-70 (a basic nuclear protein with 70,000 mol wt, whose antibodies are found in ~20% of patients with scleroderma. More recently, Nishikai and Reichlin (13, 14) reported the existence of a reaction termed the Jo-1 system with high specificity for polymyositis. The physicochemical and immunological characteristics of the Ku antigen were compared and found to be different from the antigens previously mentioned.

Based on the results of the present work, the Ku antigen is considered to be a labile acidic nuclear protein. The acidic nature was demonstrated by immunoelectrophoresis and by its behavior in anion exchange chromatography that required high salt concentration for elution. Susceptibility to mild heating, pH variations, and proteolytic enzyme, but not nuclease treatment, suggests that the Ku antigen is a protein. The estimated molecular weight of the Ku antigen as determined by gel filtration and sucrose density gradient ultracentrifugation is 300,000, which is considerably larger than other defined soluble antigens from tissue extract reactive with sera of patients with connective tissue diseases.

The immunofluorescence technique using the purified anti-Ku antibody localized the Ku antigen within the nuclei of human liver. The reticular nuclear fluorescence sparing the nucleoli can clearly be distinguished from the speckled pattern observed with antibodies to the soluble acidic nuclear antigens such as Sm, RNP, or SS-B (6, 16, 41). One of the interesting characteristics of the Ku is its species distribution. Our original serum Ku has a weak immunofluorescent reaction on rat liver (a titer of 1:4 compared with a high titer in immunodiffusion (1:128). The tissue extracts from human, calf, and rabbit organs contained the Ku antigen, but not rat liver extract. Indeed, in repeated experiments, purified antibodies did not stain the rat liver nuclei. Presumably, the positive FANA test on rat liver, by serum containing anti-Ku antibodies are due to antibodies with other specificities.

Patients with antibodies to the Ku antigen are characterized clinically by overlapping of scleroderma and polymyositis. Seven of nine patients with anti-Ku antibodies met the criteria and two patients (8, 9) developed clinical features of these diseases during the follow-up. One SLE patient (8) with butterfly rash, Raynaud's phenomenon, leucopenia, and LE cells later developed inflammatory myositis (elevation of myogenic enzymes and proximal muscle weakness) and sclerodermatous skin changes suggesting the presence of both polymyositis and scleroderma. Another patient (9), with scleroderma, showed elevation of myogenic enzymes while under observation. Additional features common in these patients were scarcity of severe systemic manifestations, extensive sclerodermatous skin changes, and severe internal organ involvements. Furthermore, treatment with corticosteroid hormone was effective for inflammatory myositis and these patients are all still living and seem thus far to have a good prognosis. Three patients with antibodies to the Ku antigen had also anti-PM-1 antibodies. However, differences in above mentioned characteristics of scleroderma and polymyositis were not observed in the presence or absence of anti-PM-1 antibodies. The clinical manifestations suggestive of Sjögren's syndrome were not found in four patients who had antibodies to the SS-A. In the present series, 14 of 26 patients with overlap syndrome had both scleroderma and polymyositis (3: SLE-scleroderma-polymyositis, 11: scleroderma-polymyositis). Among these patients, seven were negative for anti-Ku antibodies. These patients, in contrast to patients with anti-Ku antibodies, frequently had systemic manifestations such as

high fever, overt inflammatory signs such as arthritis, and other multiple autoantibodies (anti-DNA, RNP, and Sm antibodies). Therefore, patients with antibodies to the Ku antigen seem to constitute a relatively homogeneous group with overlapping features of primarily scleroderma and polymyositis.

The complexities of clinical expressions of diffuse connective tissue diseases are most prominent in patients with overlapping features. The results of the present study indicate that identification of serological reactions with high specificities for a particular disease entity or a subset within a disease might serve to divide a heterogeneous group of patients into more homogeneous subgroups in regard to prognosis and treatment.

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