Clin. exp. Immunol. (1989) **76**, 366–372

Antibodies against Ku protein in sera from patients with autoimmune diseases

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(Accepted for publication 23 March 1989)

SUMMARY

Immunoaffinity-purified Ku protein was used to screen sera from patients with systemic lupus erythematosus (SLE), scleroderma, myositis and Sjögren's syndrome for anti-Ku antibodies in a quantitative immunoblot assay. Sixteen percent of the 159 studied sera were reactive with the Ku protein; significantly increased frequencies of anti-Ku antibodies were found in SLE (19%) and scleroderma (14%) sera. Patients with myositis and Sjögren's syndrome showed similar frequencies. All positive sera had antibodies to the 86 kD subunit of Ku protein; only one serum did not react with 70 kD subunit. Frequencies of other autoantibodies were compared in anti-Ku positive and negative patients. Only anti-Sm antibodies, especially in the absence of anti-nRNP, appear to be associated with the presence of anti-Ku antibodies. A strong correlation between anti-Ku antibodies and the class II HLA antigen DQw1 (89% of the positive sera) was observed, suggesting participation of MHC genes in the mounting of the anti-Ku immune response.

Keywords anti-Ku antibodies autoimmune disease immunoblotting

INTRODUCTION

The Ku protein has been identified previously as a DNA-binding nuclear protein complex composed of two polypeptides of 86 and 70 kD in 1:1 ratio (Mimori *et al.*, 1981; Reeves, 1985; Yaneva *et al.*, 1985; Mimori, Hardin & Steitz, 1986). Autoantibodies to Ku protein were originally described in sera from patients with a polymyositis-scleroderma overlap syndrome (Mimori *et al.*, 1981). Although the frequency of these antibodies determined by immunodiffusion was low (only about 3% of the tested sera), patients with anti-Ku antibodies were felt to share unique clinical characteristics of scleroderma and polymositis overlap (Mimori *et al.*, 1981). In subsequent studies utilizing ELISA assays, it was shown that 39% of systemic lupus erythematosus (SLE), 55% of mixed connective tissue disease (MCTD), and 40% of scleroderma sera contained at least low levels of antibodies to the Ku protein (Reeves, 1985). Franceour *et al.* (1986), however, reported that by both immunoprecipitation and immunoblot analysis only 10% of SLE sera and none of 100 scleroderma sera tested were positive for anti-Ku antibodies. In other studies (van Venrooij *et al.*, 1985), 60% of sera from patients with diffuse scleroderma were positive by immunoblot analysis for an antigen of 86 kD molecular weight; later, however, it was found that this antigen was a breakdown product of topoisomerase I (Scl-70) (Maul *et al.*, 1986). It is possible that these variations in the reported frequencies of anti-Ku antibodies were due to the different methods employed for screening. With the immunoblot assay only linear (continuous) epitopes can be detected, while in ELISA and in immunoprecipitation conformational (discontinuous) epitopes will be detected as well. Case selection and clinical differences in patient populations may also account for some of the discrepancies. Currently, the frequencies of autoantibodies to Ku protein in various connective tissue diseases are unclear. Moreover, any relationships between anti-Ku and clinical disease features, other autoantibody systems, and major histocompatibility complex (MHC) genes have yet to be determined.

We report here prevalence data for anti-Ku antibodies in patients with SLE, scleroderma, myositis and Sjögren's syndrome. Attention was focused on the linear (continuous) or conformationally reversible epitopes detected only in immunoblot assays using immunoaffinity purified antigen. The results show that 26 (16%) of 159 totally screened patients were reactive with the Ku protein; 19% of SLE patients, 14% of scleroderma, 12% of myositis and 20% of Sjögren's were positive under testing conditions. Moreover, there appears to be a linkage between anti-Ku and anti-Sm autoantibodies, especially when anti-Sm is not accompanied by anti-nRNP (U1-RNP). An effect on the anti-Ku immune response by the MHC class II allele HLA-DQw1 is also suggested.

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PATIENTS AND METHODS

Patient selection

Stored sera from 159 patients with connective tissue diseases

were studied for autoantibodies to Ku protein using immunoblotting. The majority were followed by the Division of Rheumatology at the University of Texas at Houston. Fifteen of the scleroderma sera had been collected during a community-based study in southeastern Georgia (Arnett *et al.*, manuscript submitted), and an additional five were kindly provided by Dr M. Reichlin (University of Oklahoma at Oklahoma City). Selection was based only on certainty of diagnosis and availability of sera. We did not attempt to study disease activity in relation to Ku antibodies, although the majority of patients with SLE and myositis had active disease and were being treated with corticosteroids. In addition, all sera had been extensively studied for other autoantibodies (see below), and most patients had been typed for HLA antigens during the course of other studies.

The diagnosis in each patient was based on an evaluation by a rheumatologist. Criteria for selection included the 1982 revised ARA criteria for SLE (Tan *et al.*, 1978) the preliminary ARA criteria for diffuse scleroderma (Masi *et al.*, 1981), and the criteria proposed by Bohan & Peter for myositis (Bohan & Peter, 1975). The CREST syndrome (calcinosis, Raynaud's, esophageal dysmotility, sclerodactyly, and telangiectasia) was diagnosed on clinical grounds. Primary Sjögren's syndrome was defined as symptomatic dry eyes and/or dry mouth plus ophthalmologic confirmation of keratoconjunctivitis sicca and/or a positive minor salivary gland biopsy with a focus score of $\geqslant 2$ as suggested by Daniels (Daniels, 1984). Secondary Sjögren's syndrome required the definition above plus fulfillment of criteria for SLE, scleroderma, CREST, or myositis. Assays for anti-Ku antibodies were performed without knowledge of diagnosis.

Table 1. Reactivity of human autoimmune sera with purified 86/70 kD complex

Diagnosis	Serum (1:200 dilution)	ELISA (A405)*
SLE	AKR	0·575
	AF	1·071
Scleroderma/	Kat	1·129
/polymyositis	Oh	1·563
overlap syndrome	Ku	0·800

* Values after subtraction of the normal human serum value.

Table 2. Demographic, clinical, serologic and HLA features of 26 connective tissue disease patients with autoantibodies to Ku protein

Patient	Race	Sex	Diagnosis	Reactivity to Ku Protein in immunoblot (arbitrary units)			ANA pattern	Other antibodies	HLA-DR/DQ specificities
				86 kd	70 kd	86/70 ratio			
LP	H	F	CREST/Sj	18·2	5·6	3·22	Spindle	None	DR1,7,w53/DQw1,2
JE	B	M	Scl	2·8	2·9	0·97	Speckled	Scl-70	ND
DW	B	F	SLE	0·01	0·17	0·06	Speckled nucleolar	None	DR2,6,w52/DQw1
IW	W	F	Scl/Sj	9·5	7·1	1·34	Centromere	Sm	DR1,2/DQw1
BE	B	F	SLE	2·6	1·3	2·00	Speckled	dsDNA	DR1,2/DQw1
RR	W	F	SLE	15·0	7·6	1·98	Homogenous	nRNP dsDNA	DR2,4,w53/DQw1,3
SL	W	M	SLE	18·5	17·8	1·04	Speckled	None	DR6,8,w52/DQw1,3
LS	W	F	SLE	2·7	2·6	1·04	Speckled	SM	DR2,5,w52/DQw1,3
ED	W	F	Scl	3·3	3·4	0·97	Speckled	None	ND
CG	B	F	DM	64·3	56·5	1·14	Speckled	None	DR5,8,w52/DQw1,3
AS	W	F	Scl	1·2	2·6	0·46	Negative	None	ND
LM	H	M	DM	34·2	0·0	—	Negative	None	DR1,2/DQw1
JC	W	F	SLE	6·9	1·7	4·1	Speckled	LA	DR3,6,w52/DQw1,2
BV	B	F	SLE/Scl/PM	10·7	7·1	1·51	Speckled	Sm,nRNP	ND
JJ	B	M	SLE	2·7	1·7	1·59	Speckled	Sm	DR3,7,w52,w53/DQw1,2
DM	H	F	SLE	19·5	9·5	2·05	Homogenous	nRNP Ro,La	DR1,4,w53/DQw1,3
KG	W	F	SLE/Sj	37·9	20·3	1·87	Homogenous	Ro,La	DR3,8,w52,/DQw2,3
AM	B	F	SLE	30·6	46·7	0·65	Speckled	Sm,Ro	DR3,5,w52/DQw1,2
BS	W	F	SLE/Sj	9·9	2·5	3·9	Speckled	Ro	DR1,7,w53/DQw1,2
JS	B	F	SLE/Sj	2·0	2·2	0·91	Speckled	Ro,nRNP	DR2,6,w52/DQw1
BA	W	F	Sj	2·7	2·3	1·17	Speckled	None	DR2,5,w52/DQw1
ML	H	F	SLE	12·3	13·0	0·94	Speckled	Ro	ND
LM	H	F	Sj	9·1	9·3	0·98	Speckled	None	ND
RS	W	M	CREST	3·7	0·7	5·39	Centromere	None	DR5,8w52/DQw1
BW	W	F	CREST	5·3	2·1	2·52	Centromere	None	DR4,7,w53/DQw2,3
LC	W	F	Scl	10·0	7·0	1·43	Centromere	ND	ND

H hispanic; B black; W white; Scl diffuse scleroderma; Sj Sjögren's syndrome; DM dermatomyositis; PM polymyositis.

Healthy controls

Sera from 25 healthy adult volunteers who were university or hospital personnel were similarly studied for autoantibodies. Healthy control frequencies for HLA antigens included 175 whites and 49 blacks studied locally, as well as published frequencies from the 8th and 9th International Histocompatibility Testing Workshops (Histocompatibility Testing 1980; Histocompatibility Testing, 1984). Hispanic patients were included with Caucasians.

Purification of Ku protein

The Ku protein was purified from HeLa cell nuclei as described (Yaneva *et al.*, 1985). The last step of purification included chromatography on immunoaffinity column constructed with a monoclonal antibody specific for the 86 kD subunit of Ku protein.

Immunoblot assay

Affinity-purified antigen was electrophoresed on 7·5% SDS-polyacrylamide gel with 10 μg of antigen per well, and transferred to nitrocellulose membrane (Towbin, Staehelin & Gordon, 1979). After blocking with 5% chicken serum and 3% bovine serum albumin, nitrocellulose strips with the antigen blotted were reacted with human sera at a dilution of 1:200. Control strips were incubated with a 1:1 mixture of monoclonal antibodies specific for the 86- and 70-kD polypeptides in molar excess to the antigen applied. The antigen-antibody complexes were detected with second anti-human (or anti-mouse for the control monoclonal antibodies) antibodies, conjugated with alkaline phosphatase (Promega Corp., Madison, WI). The reaction was developed for 10 min at pH 9·0 using nitro-blue tetrazolium (NBT) and 5-bromo-4-chloro-3-indolyl phosphate (BCIP) (Promega). The immunoblots were photographed and scanned on a Visage 110 Image Analyser (BioImage, Ann Arbor, MI). The relative level of autoantibodies were evaluated by assigning the reactivity of healthy control serum as 0 (background) and the reactivity with monoclonal anti-Ku antibodies as 100 (or 100 arbitrary units). One arbitrary unit is 1% of the reactivity of 86 or 70 kD with the monoclonal antibodies, respectively.

ELISA

This assay was performed as described earlier (Engval, 1980), using peroxidase-conjugated goat anti-mouse IgG as a second antibody.

Other autoantibodies

All sera were tested for antinuclear antibodies (ANA) using indirect immunofluorescence of Hep-2 cells as antigen substrate. (Antibodies Incorp., Davis, CA). A titre ⩾1/80 was considered positive. Anti-centromere antibodies were defined by their typical ANA staining pattern (Moroi *et al.*, 1980), as were antibodies to the mitotic spindle apparatus (McCarty, Valencia & Fritzler, 1984). For indirect immunofluorescence on HeLa cells, fresh cells were centrifuged onto glass coverslips in Dulbeco's modified Eagle's medium (Gibco). Fixation of cells and staining with the autoimmune serum was as described (Yaneva *et al.*, 1985). Antibodies to Ro (SS-A), La (SS-B), Sm, and nRNP were studied in all by countercurrent immunoelectrophoresis (CIE) against extracts of calf thymus and human spleen using our modifications (Hamilton *et al.*, 1988) of the method of Johnson, Edmonds & Holborow (1973). All scleroderma sera were studied for antibodies to Scl-70 (topoisomerase I) by immunoblotting (Towbin, Staehelin & Gordon, 1979) (kindly performed by Dr E. Durban, Baylor College of Medicine). The majority of SLE patients had been tested previously in the clinical laboratory for the lupus anticoagulant (LA) using a thromboplastin inhibition assay.

Table 3. Frequencies of autoantibodies to Ku protein detected by immunoblotting in connective tissue diseases

Disease	Anti-Ku positive (%)	*P**
SLE	13/69 (19)	0·01
Scleroderma	9/57 (14)	0·04
Diffuse	5/38 (13)	0·07
Mild or CREST syndrome	3/18 (17)	0·06
Localized scleroderma	0/1	
Myositis	2/23 (9)	0·22
Dermatomyositis	2/12 (17)	0·09
Polymyositis	0/11 (0)	—
Primary Sjögren's syndrome	2/10 (20)	0·07
Healthy controls	0/25	—

* Compared with healthy control frequencies (Fishers' exact text, 2-tailed).

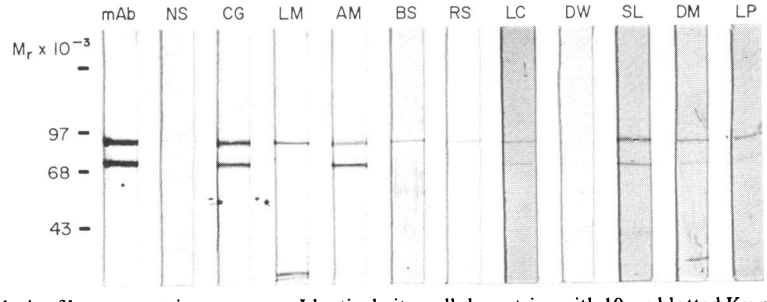

Fig. 1. Immunoblot analysis of human autoimmune sera. Identical nitrocellulose strips with 10-μg blotted Ku protein were reacted with 1:1 mixture of monoclonal antibodies to 86 and 70 kD (Lane mAb), normal human serum (NS) and patient sere (CG, LM, AM, BS, LC, RS, DW, SL, DM and LP as in Table 2) at 1:200 dilution. Immune complexes detected as described in Materials and Methods.

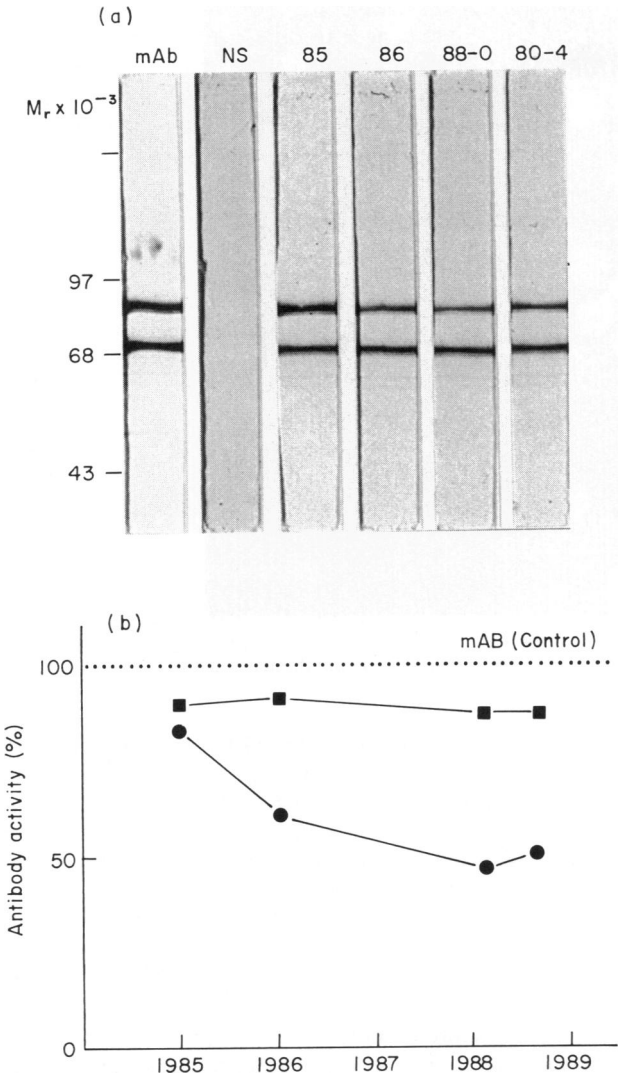

Fig. 2. Longitudinal anti-Ku antibodies profile of a dermatomyositis patient CG. (a) Immunoblot analysis; mAB monoclonal antibodies; NS normal serum; numbers above the lines indicate year of bleedings. (b) O.D. values of the scanned immunoblot in (a) as percent of monoclonal antibody O.D. values. ■ 86 kD; ● 70 kD.

HLA typing

HLA-A, B and C antigens were detected by a standard microcytotoxicity test using Terasaki trays. HLA-DR and DQ specificities were determined by a two-colour fluorescent method as previously described (Bias *et al.*, 1981). HLA-DR and DQ antisera standardized against those of the 8th and 9th International Histocompatibility Testing Workshops (Histocompatibility Testing, 1980; Histocompatibility Testing, 1984) were used to detect DR1-DRw10, DRw52, DRw53 and DQw1-DQw3.

Statistical analyses

All comparisons of autoantibody frequencies were tested for significance using a two-tailed Fishers' exact test. HLA antigen frequencies were compared using combined 2×2 tables for white and black patients and controls, respectively, analysed by the Mantel–Haenszel method.

RESULTS

Identification of the Ku protein

We have recently immunoaffinity purified and characterized a DNA-associated protein complex using a monoclonal antibody produced by immunization of mice with HeLa nucleoli (Zweig *et al.*, 1984; Yaneva *et al.*, 1985; Yaneva and Busch, 1986). To identify the purified antigen as the Ku protein, we tested its immunoreactivity with autoimmune sera from patients with SLE and scleroderma/polymyositis overlap syndrome specific for the Ku antigen. These sera (kindly provided by Drs E. Tan & J. Hardin) reacted with the antigen in both immunoblot and ELISA (Table 1). Moreover, one serum (Kat in Table 1) reacted with the fusion proteins expressed by cDNA clones specific for 86-kD polypeptide (Yaneva *et al.*, manuscript in preparation). On the basis of these reactivities and the similarity of the biochemical and physico-chemical properties reported (Mimori *et al.*, 1981; Reeves, 1985; Yaneva *et al.*, 1985; Mimori, Hardin, Steitz, 1986; Franceour *et al.*, 1986; Yaneva & Busch, 1986), we concluded that our purified antigen was the Ku protein.

Diagnosis of patient sera

Autoimmune sera from 159 patients with SLE, scleroderma, myositis or Sjögren's syndrome were screened for anti-Ku antibodies by immunoblot. Sixty-nine patients were included in the SLE group; 67 fulfilled ARA criteria for lupus (Tan *et al.*, 1978) and two had neurologic disease associated with LA. There were 57 patients with scleroderma, including 38 with diffuse disease, 18 with CREST syndrome and one with linear scleroderma. The myositis group had 12 patients with dermatomyositis and 10 with polymyositis. Ten patients had primary Sjögren's syndrome.

Immunoblot analysis

All sera were reacted at a 1:200 dilution with the two polypeptides of Ku protein blotted onto nitrocellulose. At this dilution none of the 25 control sera showed any reactivity with either of the Ku polypeptides. Of the 159 patients with a connective tissue disease, 26 (16%) showed some reactivity to Ku polypeptides (Table 2). Significantly increased frequencies of antibodies to protein Ku were found in both SLE (19%) and scleroderma (14%) sera (Table 3). Both the diffuse and mild forms of scleroderma were nearly equally represented among anti-Ku positive sera. In addition, two patients with dermatomyositis (17%) and two with primary Sjögren's syndrome (20%) were positive.

The immunoblot data with some of the positive sera are presented in Fig. 1. All sera were tested under identical conditions: constant amount of antigen, serum dilution 1:200, and time for developing of the phosphatase reaction. This allowed us to evaluate the relative amount of autoantibodies in each serum. The reactivity with the monoclonal antibodies showed that the antigen molecules on nitrocellulose accessible for interaction with antibodies were in excess with respect to autoantibodies at that dilution (Fig. 1, lane mAb). A wide range of autoantibody levels was observed in different patients. The highest level was in a dermatomyositis patient CG (64·3 units); the lowest detectable level was in patient DW, with SLE (0·01 units) (Table 2 and Fig. 1).

Only one patient, LM, with dermatomyositis, had antibodies to the 86-kD polypeptide alone. Interestingly, this was

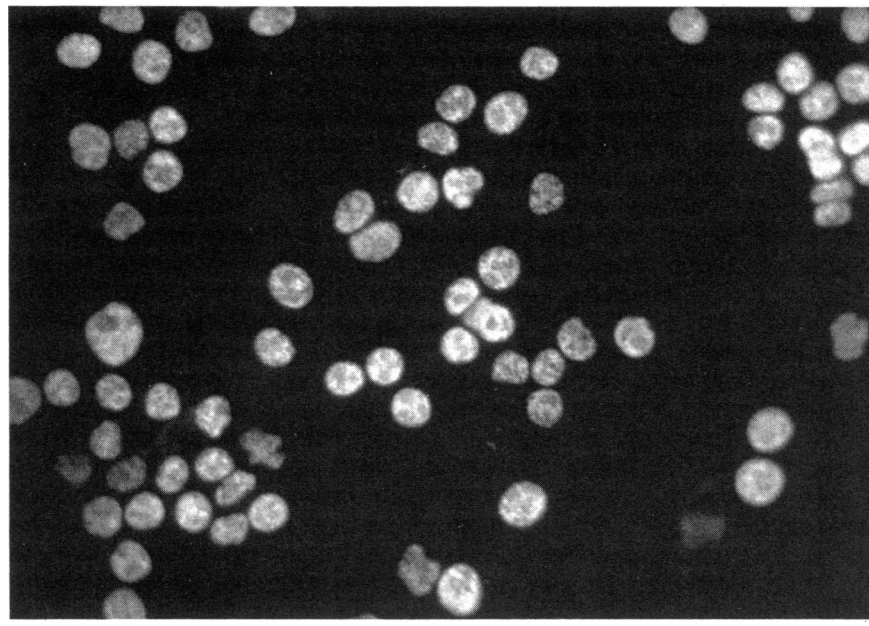

Fig. 3. Immunofluorescence pattern of staining of HeLa cells with dermatomyositis patient CG serum diluted 1:200.

Table 4. Relationships between anti-Ku and other autoantibodies

	Anti-Ku Positive* (%)	Anti-Ku Negative* (%)	*P*
Anti-Sm	5/25 (20)	5/129 (4)	0·01
Anti-Sm–SLE	4/13 (31)	4/56 (7)	0·03
Anti-nRNP	4/25 (16)	15/129 (12)	NS
Anti-nRNP–SLE	4/13 (31)	11/56 (20)	NS
Anti-Ro	6/25 (24)	26/129 (20)	NS
Anti-La	2/25 (8)	9/129 (7)	NS
Anticentromere scleroderma	3/8 (38)	13/48 (27)	NS
Anti-Scl 70 scleroderma	1/8 (13)	9/48 (19)	NS
Lupus anti-coagulant–SLE	1/13 (8)	7/56 (13)	NS

NS not significant.

* Five sera from scleroderma patients (patient LC in Table 2 and 4 anti-Ku negatives) were not tested for antibodies to Sm, nRNP, Ro and La.

the only myositis patient whose disease had been inactive for several years and who was taking no corticosteroids. The rest of the positive sera had antibodies to both polypeptides, but their ratios varied (Table 2). We could not distinguish clearly three specificities in anti-Ku antibodies as suggested by Franceour *et al.* (1986), but in general anti-86-kD antibodies were more frequent. Anti-Ku positive patients included whites of both hispanic and non-hispanic origin as well as blacks (Table 2). Among those with SLE, frequencies of nephritis, central nervous system disease, rash and arthritis did not differ significantly between anti-Ku positive and anti-Ku negative patients. Only one anti-Ku positive SLE patient (BV in Table 2) was considered to have an overlap syndrome and fulfilled criteria for SLE (Tan *et al.*, 1978), scleroderma (Masi *et al.*, 1981), and myositis (Bohan & Peter, 1975). Three SLE and two scleroderma patients with anti-Ku antibodies had documented secondary Sjögren's syndrome.

In several cases, sera collected at different periods during the disease were analysed for anti-Ku antibodies. Results from one such study of patients CG with dermatomyositis are shown in Fig. 2. Anti-86-kD antibody levels were highest at onset of disease and before corticosteroid therapy but decreased and plateaued over the next several years of disease suppression. The anti-70-kD antibody levels remained almost constant.

Immunofluorescence

The serum from patient CG did not react with any other protein in immunoblot with total nuclear proteins, suggesting that its antibodies were monospecific for Ku protein. The immunofluorescence pattern observed when this serum was used for staining of HeLa cells was similar to that observed when monoclonal antibodies specific for Ku protein were used for immunofluorescence staining (Yaneva *et al.*, 1985). Diffuse speckled nuclear staining was seen (Fig. 3). Absence of nucleolar staining can be explained with the confluent state of HeLa cells used for immunofluorescence, since nucleolar staining was observed only in the G1 phase (Zweig *et al.*, 1984).

Anti-Ku antibodies by CIE

Only two (1·3%) of the 159 sera were positive for anti-Ku using CIE (patients CG and LM in Table 2).

Other autoantibodies in the anti-Ku positive sera

Frequencies of other autoantibodies were compared in anti-Ku positive and negative patients (Table 4).

Anti-Sm antibodies, although infrequent, were significantly increased in anti-Ku positives overall (20% versus 4% in anti-Ku negatives), especially in those with SLE (31% versus 7% of other SLE patients). One additional non-SLE patient with anti-Sm and anti-Ku antibodies had diffuse scleroderma, secondary Sjögren's syndrome and anti-centromere antibodies. Interestingly, four of the five anti-Ku and Sm positive patients did not have anti-nRNP antibodies, while four of five anti-Ku negative, anti-Sm positive patients did have anti-nRNP. No other autoantibodies studied, including anti-nRNP, appeared to be associated with Ku antibodies. Interestingly, only one patient with scleroderma and anti-Scl 70 also had anti-Ku; however, this was not significantly different from anti-Ku negative scleroderma patients.

Because of the association between anti-Ku and anti-Sm autoantibodies observed (Table 4), five additional sera from patients with SLE, positive for anti-Sm antibodies but with no anti-U1 RNP antibodies were screened for the presence of anti-Ku antibodies. All five sera tested were positive to varying degree in the immunoblot assay.

HLA antigens had been determined in 19 of the 26 anti-Ku positive patients (Table 2). No HLA-A, B or C antigens appeared to be increased in these patients compared with race-matched healthy controls. The class II HLA antigen DQw1, however, appeared to be in excess. HLA-DQw1 was present in 17 (89%) of 19 anti-Ku positives: $P=0{\cdot}01$; RR = 5·8 compared with its frequency in local white (58%) and black (61%) controls, and $P=0{\cdot}01$, RR = 6·5 compared with healthy North American white (54·9%), and black (62·9%) controls typed by the 8th Histocompatibility Testing Workshop (Histocompatibility Testing, 1980) (Mantel–Haenszel analysis). Most HLA-DQw1 positive, especially white, patients also possessed HLA-DR1 ($P=0{\cdot}06$ compared with local white controls); however, other HLA-DQw1-associated alleles included HLA-DR2, DR5, and DRw6. Correction of the P value by multiplying the number of DQ antigens tested ($n=3$) did not negate the significant association of anti-Ku with HLA-DQw1 ($P=0{\cdot}03$).

DISCUSSION

Autoantibodies to the Ku protein, as detected by immunoblotting of human sera to affinity-purified Ku antigen, were found in 14–20% of patients with connective tissue diseases. The largest numbers of sera tested were from patients with SLE and scleroderma, although comparable frequences of anti-Ku antibodies were found in smaller numbers of patients with myositis, especially dermatomyositis, and primary Sjögren's syndrome. Within the SLE group, anti-Ku positive sera did not segregate with any clinical disease feature. Similarly, within the scleroderma spectrum, anti-Ku antibodies occurred nearly equally in patients with diffuse disease and those with the milder CREST variant. In this study, anti-Ku was not a marker for overlap syndromes.

The increased sensitivity of immunoblotting compared with immunodiffusion for detecting anti-Ku antibodies is also demonstrated in this study. Only two (1·3%) of these 159 patients demonstrated a precipitating antibody to Ku in CIE against calf thymus extract. This frequency is comparable with the 3% prevalence reported by Mimori *et al.* (1981), who used passive immunodiffusion to define anti-Ku antibodies. Neither of our two patients with anti-Ku precipitins, and the highest levels of antibodies by immunoblotting, had overlap syndromes. One had dermatomyositis and the other had primary Sjögren's syndrome with immune thrombocytopenic purpura.

In our series, all anti-Ku positive sera reacted with the 86-kD polypeptide, and all but one, a patient with inactive disease, were positive for the 70-kD specificity. Ratios of reactivity between the 86- and 70-kD peptides, however, varied widely among different sera, and no particular pattern appeared to discriminate specific disease entities. These variations suggest that the two polypeptides were recognized by at least two different antibody specificities, i.e., that there are different antigenic determinants on the polypeptides, although the possibility that there are common epitopes between the peptides cannot be ruled out at present.

Similar to clinical features, frequencies of autoantibodies to Ro, La, nRNP (U1-RNP), anti-phospholipid (LA), centromere, and Scl-70 (topoisomerase I) showed no associations with anti-Ku antibodies. However, anti-Sm antibodies as previously reported (Franceour *et al.*, 1986) were significantly correlated with anti-Ku, especially in SLE patients. Interestingly, patients with anti-Ku and anti-Sm infrequently had accompanying anti-nRNP (U1-RNP). Anti-Sm and anti-nRNP (U1-RNP) usually occur together in SLE patients, while anti-Sm without nRNP (U1-RNP) is uncommon (Hardin, 1986). In recent studies of autoantibody profiles in SLE patients, we found that only 1% of white and 5% of black patients had anti-Sm alone (Arnett *et al.*, 1988). The association of anti-Ku with anti-Sm alone and not with the anti-Sm, nRNP combination, or nRNP alone patterns may be noteworthy but at present is of unclear significance. It is possible that this is a new set of linked antibodies involved in SLE. Since these sets of antibodies are thought to be directed against individual nucleoproteins acting as direct immunogens for antibody production (Craft & Hardin, 1987), it is of interest to determine any relationship between Ku and Sm antigens. At present, there is no report of a possible co-localization of the two proteins in the cell nucleus. Although the two proteins probably have completely different biochemical functions, during the course of autoimmune disease they may be released in the form of different nucleoprotein complexes, but recognized by the immune system in a similar way.

Finally, patients with anti-Ku antibodies, across disease categories, appear to have an increased frequency of the MHC class II antigen HLA-DQw1 compared with race-matched healthy controls. HLA-DQw1 has previously been found to be increased in white patients with SLE, usually in the context of HLA-DR2 with which it is in linkage disequilibrium (reviewed by Goldstein & Arnett, 1987). HLA-DQw1 has not been found to be significantly increased in scleroderma, myositis or primary Sjögren's syndrome (Goldstein & Arnett, 1987). In our anti-Ku positive patients, HLA-DQw1 occurred variously with HLA-DR1, DR2 and DRw6 phenotypes, all of which may be linked to DQw1 (Histocompatibility Testing, 1984). Clearly, this MHC association with anti-Ku needs to be confirmed in another study. The number of patients studied here was small, and HLA-DQw1 is a common specificity in the healthy population. If it is correct, however, the anti-Ku response could be added to a growing list of autoantibodies which show stronger correlations with MHC products than their parent diseases (Goldstein & Arnett, 1987). Moreover, underlying genetic mechanisms

involving MHC-restricted T-lymphocyte responses would be suggested for the development of autoantibodies to the Ku protein.

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