INTERACTION BETWEEN ANTI-DNA AND ANTI-DNA-BINDING PROTEIN AUTOANTIBODIES IN CRYOGLOBULINS FROM SERA OF PATIENTS WITH SYSTEMIC LUPUS ERYTHEMATOSUS

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Sera of patients with SLE almost invariably contain antinuclear antibodies (1), and frequently contain circulating immune complexes as well (2–5). Cryoglobulins are one type of immune complex (6) that can be isolated from these sera. In some instances, the cryoglobulins have been found to contain antinuclear antibodies (7–9). For example, anti-DNA antibodies are selectively concentrated in cryoglobulins isolated from some lupus patients' sera (9), and are also sometimes found in patients' glomerular immune deposits (7, 8, 10). The presence of the corresponding cellular antigens (e. g., DNA) in cryoglobulins or glomerular eluates remains controversial, however (4, 5, 9, 11–14). Although in certain situations classical antigens have been described (9, 13, 14), in many instances the only detectable components of these immune complexes have been self-associating Ig (4, 5, 12). Immune complexes consisting of Ig bound to rheumatoid factor, anti-$F(ab')_2$, and antiidiotype have been shown previously in SLE, as well as in other disorders (11, 15–19). The suggestion that certain autoantibodies might arise through an antiidiotypic route (20) led us to examine the possibility that immune complexes consisting of antinuclear antibodies and their antiidiotypes might be formed in SLE. We previously saw (21) that autoantibodies to a cellular DNA-binding protein complex (p70/p80) and to DNA frequently coexist in sera of patients with SLE. In the present study, we analyze the composition of the cryoglobulins found in many of these sera. The results suggest that anti-p70/p80 and anti-DNA antibodies can interact with one another, possibly by an idiotype-antiidiotype–like mechanism.

Materials and Methods

Patients. Sera were obtained from patients diagnosed and treated at The Rockefeller University Hospital. Patients met accepted criteria for the classification of SLE, mixed connective tissue disease (MCTD),[1] or progressive systemic sclerosis (PSS) (22–24).

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[1] *Abbreviations used in this paper:* dsDNA, double-stranded DNA; MCTD, mixed connective tissue disorder; PSS, progressive systemic sclerosis; RNP, ribonucleoprotein; ssDNA, single-stranded DNA.

Serologic Evaluation. Antibodies to p70/p80 were quantitated by ELISA, and their presence was confirmed by immunoprecipitation of p70 and p80 (see below and reference 21). Antibodies to single- and double-stranded DNA (ssDNA and dsDNA) were quantitated by RIA (25) and ELISA (26). Rheumatoid factor was measured by latex fixation and ELISA using rabbit gamma globulin as coating antigen (27).

Isolation of Cryoglobulins. Cryoprecipitates were isolated by centrifugation of patients' ice cold sera at 8,740 *g* for 15 min; the pellets were washed with ice-cold PBS, pH 7.5, and then with distilled water, as described (9). Each cryoprecipitate was resolubilized in 0.5 ml of PBS with agitation for 1 h at 37°C, and was centrifuged at 8,740 *g* for 5 min to remove insoluble material. Purity of the isolated cryoglobulins was monitored by electrophoresis on 12.5% SDS-polyacrylamide gels (28) using purified myeloma proteins as standards.

Immunoprecipitation of Radiolabeled p70/p80. K562 cells were metabolically labeled with [^{35}S]methionine as previously described (21). Washed cells were lysed by sonication in ice-cold 150 mM NaCl, 50 mM Tris pH 7.5, 2 mM EDTA, 0.5 mM PMSF, 5 mM iodoacetamide, and were then centrifuged at 8,740 *g* for 5 min. Aliquots of the cleared extract were immunoprecipitated for 1 h at 37°C with either 10 μl of cryoglobulin-depleted serum (see above), or 10 μl of the isolated, resolubilized cryoprecipitate. The immunoprecipitates were adsorbed to protein A–Sepharose 4B (Pharmacia Fine Chemicals, Piscataway, NJ) for 90 min at 37°C, and radioactive proteins were eluted from the beads by boiling in sample buffer, followed by analysis on 12.5% SDS-polyacrylamide gels (21) and fluorography (29).

Detection of Autoantibodies in Cryoprecipitates. Isolated cryoprecipitates were resolubilized as above, and tested for reactivity with various antigens by ELISA at 37°C. Antibodies to ss and dsDNA were measured by ELISA using microtiter plates (Costar, Cambridge, MA) coated with ssDNA or dsDNA from calf thymus (Sigma Chemical Company, St. Louis, MO), respectively (26). Anti-p70/p80 antibodies in the isolated cryoprecipitates were quantitated by ELISA using microtiter plates coated with anti-p70/p80 mAb 162-11 (21). We obtained crude p70/p80 antigen by adsorbing K562 cell lysate on DEAE cellulose, and eluting with 0.3 M NaCl; the eluted p70/p80 proteins were then immunopurified by binding to the mAb-coated wells for 2 h at 22°C. We removed cellular DNA bound to p70/p80 by washing the antigen-coated wells for 30 min with 0.5 M NaCl, 20 mM Tris (pH 7.5), 2 mM EDTA (30). Diluted test samples were added to the wells for 2 h at 37°C, followed by peroxidase-conjugated goat anti–human γ or μ chain antibodies (Tago Inc., Burlingame, CA) for 1 h at 37°C. Substrate was added and OD was read at 492 nm.

Assay for p70/p80 Antigen in Cryoprecipitates. p70/p80 antigen in the isolated, resolubilized cryoprecipitates was detected by ELISA (21). Microtiter plates were coated with 162-11 antibodies as above, followed by the addition of diluted cryoprecipitate for 2 h at 37°C. The binding of alkaline phosphatase–conjugated 111-12 (an mAb that binds to a different epitope of p70/p80 than does 162-11) was then measured.

Adsorption of Sera on DNA-Cellulose. 0.5 ml of patient CK serum (containing both anti-p70/p80 and anti-DNA antibodies) was dialyzed against PBS (pH 7.2) and adsorbed on a column of dsDNA cellulose (1.0 g dry powder from Sigma Chemical Co. in PBS) for 1 h. Unbound Ig was collected, and the column was washed extensively with PBS. Some of the bound anti-DNA antibodies were eluted with 0.5 M NaCl, 20 mM sodium phosphate buffer (pH 7.2). The eluate was dialyzed against PBS, and both eluate and effluent fractions were tested by ELISA for anti-p70/p80 antibodies, anti-dsDNA, and anti-ssDNA antibodies, and total Ig (31). The specific activity of either anti-p70/p80 or anti-DNA in the fractions was calculated as follows: (specific antibody in fraction × total antibody in serum)/(total antibody in fraction × specific antibody in serum).

Inhibition of Anti-p70/p80 Binding by anti-DNA mAbs. Microtiter plates were coated with either 162-11 or 111-12 purified mAbs at 10 μg/ml, incubated with p70/p80 antigen, and washed with 0.5 M NaCl to remove bound DNA as above. Sera containing anti-p70/p80 antibodies were diluted 1:10,000 in culture medium containing ~1 μg/ml of human IgM anti-DNA mAb or human IgM mAb without DNA reactivity. Anti-DNA

TABLE I
Serologic Characteristics of Patients

Patient	Diagnosis	Anti-p70/p80*	Anti-ssDNA‡	Anti-dsDNA‡	Cryo-globulin	Rheu-matoid factor§
			%	%		
CK	SLE	>15,000,000	96.9	94.0	+	−
DJ	SLE	156,250	77.3	66.5	+	−
AT	SLE	250	58.5	64.8	+	−
LG	SLE	250	97.6	100.0	+	−
JM	MCTD	3,906,250	40.7	23.7	+	−
JK	PSS	156,250	3.8	2.6	−	−

* Reciprocal titer (ELISA).
‡ RIA.
§ By latex fixation and ELISA.

mAbs tested included W1, W4, W8, W18, W48, W64, and W94 (obtained by immortalization of peripheral blood B cells from a patient with SLE, high levels of serum anti-DNA antibodies, and trace amounts of serum anti-p70/p80 antibodies) (32); J2, J3, J7, and J8 (from a patient with MCTD, low levels of anti-DNA antibodies, and 1:6,250 titer of anti-p70/p80 antibodies, reference 31); M3 (from a normal volunteer with a family history of SLE); and 15/14, 16/6, and 18/2 (a generous gift from Drs. Blair Ardman and Robert Schwartz, Hematology/Oncology Division, Department of Medicine, Tufts New England Medical Center, Boston, MA) (33). Irrelevant control antibodies were D3A (IgM), AC (IgM), EG5.1 (IgE, IgM), and U266 (IgE). Each mAb was preincubated with diluted serum for 30 min at 22°C before adding 100 μl to each antigen-coated well for 2 h at 22°C. The wells were washed and then incubated for 90 min with 100 μl of 1:1,500 peroxidase-conjugated goat anti–human L chain antibodies (κ and λ specificities, from Tago Inc.). After another washing, aminoantipyrine-phenol substrate was added and absorbance read at 492 nm. Binding was compared with a standard curve generated by different amounts of serum diluted in blocking medium.

We performed similar inhibition experiments using alkaline phosphatase–conjugated 162-11 or 111-12 mAbs; for each conjugated mAb, the microtiter plate was coated with antigen using the other (unconjugated) mAb as described (21). The conjugated mAbs were used at a final dilution of 1:100 in culture medium alone or culture medium containing ~1 μg/ml of human IgM mAb exactly as described above. The wells were washed, *p*-nitrophenyl phosphate substrate was added, and absorbance measured at 405 nm.

Results

Serologic Evaluation. We analyzed sera from six patients with connective tissue disorders that contained anti-p70/p80 antibodies (Table I). By standard clinical criteria (22–24), four of these patients had SLE, one had MCTD, and one had PSS. The titers of anti-p70/p80 antibodies in the patients' sera ranged from 1:250 to >1:15,000,000 as measured by enzyme immunoassay. Sera from each patient with SLE and from the patient with MCTD contained high levels of antibodies to ssDNA and dsDNA, as well as cryoglobulins. In contrast, serum from patient JK with PSS contained anti-p70/p80 antibodies at a titer of 1:156,000, but no anti-DNA antibodies or cryoglobulins. None of the sera contained IgM or IgG rheumatoid factors, as determined by latex fixation or ELISA (Table I).

Isolation of Cryoglobulins. Since cryoglobulins represent a type of circulating

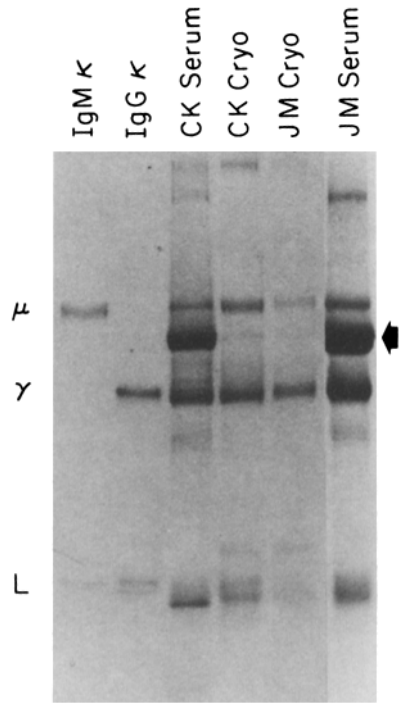

FIGURE 1. Gel analysis of isolated cryoglobulins. Cryoglobulins were isolated from patient CK and JM sera; 0.1 μl of serum after removal of the cryoprecipitate (*CK Serum* and *JM Serum*, respectively), 5.0 μl of CK cryoprecipitate in PBS (*CK Cryo*) and 10 μl of JM cryoprecipitate in PBS (*JM Cryo*) were analyzed by electrophoresis on a 12.5% SDS-polyacrylamide gel. Proteins were visualized by staining the gel with Coomassie Blue R-250. Purified myeloma proteins (6.0 μg/lane of IgM κ or IgG κ, respectively) were also analyzed on the same gel for comparison. The positions of μ and γ heavy chains and L chain are indicated. Arrow indicates serum albumin.

immune complex (6, 9), it was of interest to examine the composition of the cryoglobulins found in these sera. Cryoglobulins were isolated from four of the sera shown in Table I (patients CK, AT, LG, and JM). Because cryoglobulins can contain trapped extraneous serum proteins, the purity of the isolated, redissolved cryoglobulins was determined by SDS-PAGE under reducing conditions (Fig. 1). The relative amounts of human serum albumin in each isolated cryoglobulin, compared with those of cryoglobulin-depleted serum, were taken as an index of purity (i.e., lack of nonspecific trapping). After removal of the cryoprecipitate, serum from patient CK (*CK Serum*) contained a prominent band corresponding to the mobility of human serum albumin (*arrow*), as well as less prominent bands comigrating with μ and γ H chains and κ L chains. The isolated cryoglobulin (*CK Cryo*) contained μ and γ H chains in approximately equal amounts, as well as L chains, but only traces of serum albumin. p70/p80 antigen was not detectable in the cryoprecipitates by Coomassie blue staining. We obtained similar results using the cryoglobulin-depleted serum (*JM Serum*) and isolated cryoglobulin (*JM Cryo*) from patient JM. Cryoglobulins from patients AT and LG were of comparable purity and also contained mainly IgG and IgM

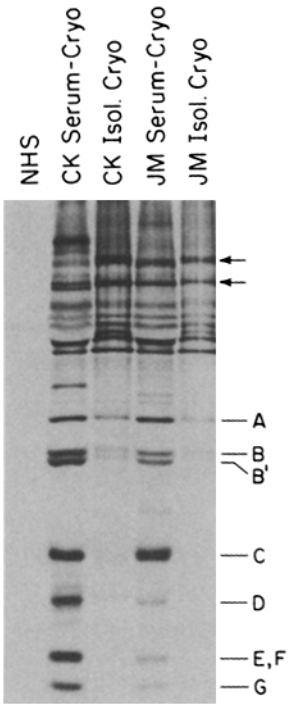

FIGURE 2. Immunoprecipitation of [^{35}S]methionine-labeled cell lysate using isolated cryoglobulins or serum depleted of cryoprecipitate. K562 erythroleukemia cells were labeled for 12 h with [^{35}S]methionine and a cell lysate was immunoprecipitated at 37°C with 10 μl of normal human serum (*NHS*), CK serum after removal of the cryoprecipitate (*CK Serum-Cryo*), CK-isolated cryoprecipitate (*CK Isol. Cryo*), JM serum after removal of the cryoprecipitate (*JM Serum-Cryo*), or JM isolated cryoprecipitate (*JM Isol. Cryo*). Immunoprecipitates were adsorbed to protein A–Sepharose, eluted by boiling in sample buffer, and analyzed on a 12.5% SDS-polyacrylamide gel. Immunoprecipitated proteins were detected by fluorography. Positions of proteins A, B, B′, C, D, E, F, and G characteristically immunoprecipitated by anti-Sm or RNP antibodies are indicated. The positions of p70 and p80 are indicated by arrows.

(not shown). The pattern obtained with purified myeloma proteins is shown for comparison (lane *1*, IgM κ; lane *2*, IgG κ). By comparing the amount of serum albumin trapped in the isolated cryoglobulins with that in serial dilutions of cryoglobulin-depleted serum, the concentration of serum albumin in the serum was found to be at least 1,250 times that contained in each of the four isolated cryoglobulins (i.e., that the extent of nonspecific trapping of serum proteins in the isolated cryoprecipitate was <0.1%).

Composition of the Isolated Cryoglobulins. To examine the specificities of the Ig constituents of the cryoprecipitates, the isolated warmed cryoglobulins were used to immunoprecipitate radioactively labeled cellular proteins (Fig. 2). We saw a strikingly different pattern of immunoprecipitated [^{35}S]methionine-labeled proteins with patients CK and JM cryoglobulin-depleted sera than that seen with their isolated cryoglobulins. CK cryoglobulin-depleted serum (*CK Serum-Cryo*) immunoprecipitated prominent bands A, B, B′, C, D, E, F, and G, consistent with the presence of anti-Sm and antiribonucleoprotein (RNP) antibodies in this serum, but very little p70/p80. In contrast, the isolated cryoglobulin (*CK Cryo*)

TABLE II
Relative Amounts of Antibodies in Isolated Cryoglobulins

Patient	Anti-p70/p80		Anti-dsDNA		Anti-ssDNA		p70/p80 antigen*
	IgG	IgM	IgG	IgM	IgG	IgM	
CK	5	125	25	45	ND‡	40	0.10
JM	2	5	ND	ND	ND	ND	0.02
AT	ND	50	ND	ND	ND	19	0.30
LG	10	250	1	192	1	83	0.07

* p70/p80 antigen (ELISA OD units).
‡ Quantity of antibody too small to permit calculation.

immunoprecipitated prominent bands of M_r ~70,000 and 80,000 (*arrows*), which typify anti-p70/p80 reactivity. Notably, the isolated cryoglobulin immunoprecipitated only traces of the small nuclear ribonuclear proteins A, B, B′, C, D, E, F, and G (compare *CK Isol. Cryo* with *CK Serum-Cryo*). Similar results were obtained with the cryoglobulin-depleted serum and the isolated cryoglobulin of patient JM. In this case, the cryoglobulin-depleted serum (*JM Serum-Cryo*) immunoprecipitated prominent bands A–G because of the presence of $>1:10^6$ titer of anti-RNP antibodies in this serum; p70/p80 also appeared in this immunoprecipitate. In contrast, the isolated cryoglobulin (*JM Isol. Cryo*) immunoprecipitated prominent p70/p80 bands, but only traces of bands A–G. The pattern obtained using normal human serum (*NHS*) is shown for comparison.

The amounts of serum autoantibodies in the isolated cryoglobulins were also assessed by ELISA (Table II). For each serum, we determined the enrichment of specific autoantibodies relative to the amount of nonspecific trapping of albumin. Anti-p70/p80 antibodies were enriched in each of the four isolated cryoglobulins, and anti-DNA antibodies were enriched in three of the four. Because of low levels of anti-DNA antibodies in JM serum (Table I), it could not accurately be determined whether these antibodies were enriched in the cryoglobulin. IgM anti-p70/p80 and anti-DNA antibodies predominated over IgG in each cryoprecipitate. Anti-Sm and RNP antibodies (patient CK) and anti-RNP antibodies (patient JM) were not enriched in the isolated cryoglobulins (data not shown). Using a highly sensitive and specific ELISA, p70/p80 antigen was detectable in the cryoprecipitate from patient AT, and in lesser quantities in the other three isolated cryoglobulins.

Anti-p70/p80 and Anti-DNA Antibodies Are Distinct Immunoglobulin Populations. Since certain anti-DNA antibodies also can bind nuclear proteins such as the Sm antigen (34), we tested the possibility that the anti-DNA and anti-p70/p80 reactivities in these patients' sera and cryoprecipitates might be due to the same antibodies. We warmed CK serum containing cryoglobulin to 37°C and adsorbed it on dsDNA-cellulose; the effluent and 0.5 M NaCl eluate were collected separately (Table III). The column effluent contained anti-p70/p80 activity, but only traces of anti-DNA activity. The eluate contained anti-DNA antibodies at ~100-fold increased specific activity, but no anti-p70/p80 antibodies. Thus, the anti-p70/p80 and anti-DNA antibodies in this serum are distinct Ig populations.

Binding of Anti-p70/p80 Antibodies to Antigen Is Blocked by Anti-DNA mAbs. The above experiments suggested that the cryoglobulins present in these sera contain

TABLE III

Anti-p70/p80 Antibodies Can Be Separated from Anti-DNA Antibodies by Adsorption on dsDNA-cellulose

Antibody	Serum before adsorption (A_{492})*	Effluent		Eluate‡	
		A_{492}*	Sp act§	A_{492}*	Sp act§
Anti-p70/p80	0.93	0.47	0.63	0.01‖	0
Anti-ssDNA	>2.0	0.05	0.11	0.25	110
Anti-dsDNA	0.89	0.04‖	0.0	0.08	63
Total IgG	0.97	0.92	—	0.10	

* Absorbance at 492 nm (ELISA).

‡ Eluate obtained with 500 mM NaCl.

§ Calculated as: (specific antibody in fraction × total antibody in serum)/(total antibody in fraction × specific antibody in serum).

‖ Background activity.

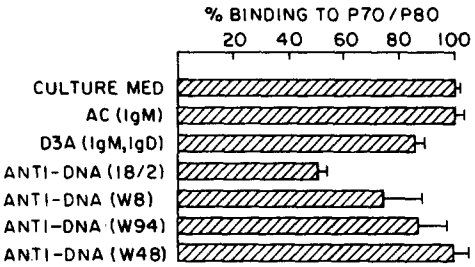

FIGURE 3. Inhibition of JM serum binding to p70/p80 by human anti-DNA mAbs. Microtiter plates were coated with p70/p80 antigen by adsorption to mAb 162-11; the binding of JM anti-p70/p80 antibodies to antigen was measured by ELISA in the presence or absence of human IgM mAbs at a final concentration of ~1 μg/ml. Binding in the presence of inhibitors is expressed as percent of control binding to p70/p80 as determined by comparison with a standard curve of JM serum binding to p70/p80 in the absence of inhibitors. Control mAbs without anti-DNA reactivity were AC (IgM), and D3A (IgM plus IgD); IgM anti-DNA antibodies tested included 18/2, W8, W94, and W48. SE is indicated.

at least three components: anti-p70/p80 antibodies, anti-DNA antibodies, and small amounts of p70/p80 antigen (in at least some instances). Although both anti-p70/p80 and anti-DNA antibodies would bind to the complex of p70/p80 and DNA, the antigen was only a minor component of the cryoglobulin, as determined by SDS-PAGE (Fig. 1) and ELISA (Table II). We therefore examined the possibility that the Ig components of the cryoglobulins might self-associate. Since rheumatoid factor activity was undetectable by ELISA and latex fixation assays in either the isolated cryoglobulins or the sera, we examined the possibility that anti-DNA and anti-p70/p80 antibodies might interact at their antigen-combining sites. Because of the relatively small amounts of purified cryoprecipitate obtainable from the patients' sera, we used an inhibition assay to determine whether human anti-DNA mAbs (32, 33) could inhibit the binding of anti-p70/p80 antibodies to p70/p80 antigen. 1 of 15 anti-DNA mAbs tested inhibited binding of JM anti-p70/p80 antibodies to p70/p80 antigen by ~50% (Fig. 3, anti-DNA 18/2). Lesser inhibition was displayed by others (e. g., anti-DNA W8), while others showed no inhibition. IgM mAbs that were not reactive with DNA (AC and D3A) displayed no substantial inhibition of binding. The anti-DNA

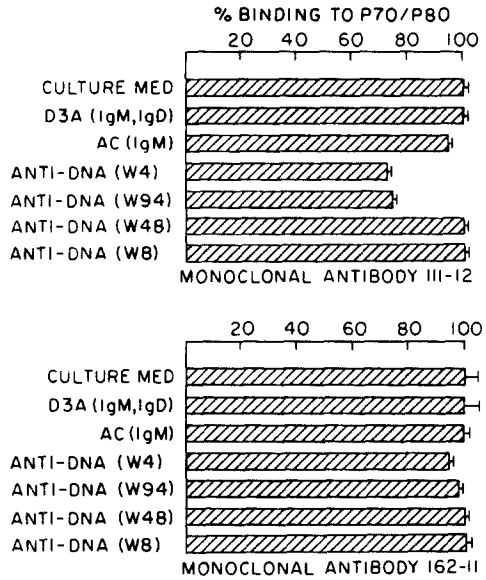

FIGURE 4. Inhibition of binding of murine mAbs to p70/p80 by human anti-DNA mAbs. For inhibition of 111-12 binding (*top*), microtiter plates were coated with 162-11 mAb followed by p70/p80 antigen; for inhibition of 162-11 binding (*bottom*) plates were coated with 111-12 followed by p70/p80 antigen. Antigen binding of alkaline phosphatase–conjugated mAbs 111-12 or 162-11, respectively, in the presence or absence of human IgM mAbs at a concentration of ~1 μg/ml was determined by ELISA. Binding in the presence of inhibitors is expressed as percent control binding to p70/p80 as determined by comparison with standard curves of alkaline phosphatase–conjugated 111-12 or 162-11 binding to p70/p80 in the absence of inhibitors. Control mAbs without anti-DNA reactivity were AC (IgM) and D3A (IgM plus IgD); IgM anti-DNA antibodies tested included W4, W94, W48, and W8. SE is indicated.

mAbs alone displayed no binding to the p70/p80 antigen-coated wells, showing that cellular DNA fragments bound to p70/p80 had been removed by the 0.5 M NaCl wash (see Materials and Methods), and that the monoclonal antibodies had no cross-reactivity with p70/p80.

Murine monoclonal anti-p70/p80 antibodies were also tested in the inhibition ELISA. Monoclonal antibodies 111-12 and 162-11 were previously shown to bind to distinct antigenic sites of p70/p80 (21). The binding of 111-12 to p70/p80 was inhibited substantially by several monoclonal anti-DNA antibodies including W4 and W94 (Fig. 4, top). Other anti-DNA mAbs inhibited less strongly or not at all (Fig. 4, *top, W48*), while irrelevant IgM monoclonal antibodies AC and D3A did not inhibit significantly. Neither the control IgM mAbs nor any of the 15 anti-DNA mAbs inhibited 162-11 binding to p70/p80 (Fig. 4, bottom).

Discussion

We have previously described autoantibodies that react with a DNA-binding protein complex, consisting of ~70,000 and ~80,000 dalton subunits, in sera from patients with SLE, MCTD, and PSS (21). Recently, exchange of sera has shown that this antigen is identical to the Ku antigen (35, 36). The present study extends our previous observation that anti-p70/p80 antibodies are frequently associated with high levels of serum anti-DNA antibodies in patients with SLE,

and in some patients with MCTD, suggesting that in these patients, a macromolecular complex consisting of the p70 and p80 proteins bound to DNA may be a major target of autoimmunity. Similarly, autoantibodies to several components of macromolecular complexes involved in mRNA splicing frequently occur together (37–40). The reasons for the linked production of autoantibodies to different components of the same macromolecular assembly are not well understood. One possibility is that a single autoantibody crossreacts with more than one component of the complex. For instance, an mAb reactive with both the Sm protein antigen and DNA has been described recently (34). This mechanism cannot explain the linkage of anti-p70/p80 and anti-DNA antibodies in our patients' sera, however, since the two autoantibody specificities can be separated by adsorption on DNA-cellulose (Table III). A second possibility is that antibodies to a normally nonimmunogenic molecule, such as DNA, can arise due to an association with an immunogenic protein. This mechanism has been proposed as an explanation for the observation that anti-DNA antibodies are produced during the course of immunizing animals with RNA polymerase, another DNA-binding protein (41). We cannot rule out a similar mechanism to explain the strong association of anti-DNA antibodies with anti-p70/p80 antibodies. However, another possibility is that certain linked autoantibodies are part of immune networks (20, 42). For example, immunization of animals with BisQ, an acetylcholine analog, results in the production of anti-acetylcholine antibodies as well as anti-antiacetylcholine antibodies, some of which react with the acetylcholine receptor and induce a myasthenia gravis–like syndrome (43). Autoantibodies to other cellular receptors can be induced as part of an antiidiotypic response to antihormone antibodies (44, 45) or anti-viral antibodies (46). By analogy, DNA might be considered to be a receptor for DNA-binding proteins such as p70/p80; our present study supports the notion that certain anti-p70/p80 antibodies might be linked to anti-DNA antibodies through an autoimmune network.

In this study, the analysis of cryoglobulins (i. e., immune complexes, Table I) found in the sera of SLE patients with anti-p70/p80 antibodies revealed a specific enrichment in anti-p70/p80 and anti-DNA antibodies, but not other autoantibodies such as anti-Sm or anti-RNP (Fig. 2, Table II). Although small amounts of p70/p80 antigen were detectable in several of the isolated cryoprecipitates (Table I), the predominant components of the cryoglobulins were IgG and IgM (Fig. 1), suggesting, as has been found with other isolated cryoglobulins (11, 47), that the Ig may self-associate. This possibility was supported by the finding that preincubation of the patients' anti-p70/p80 antibodies with human anti-DNA mAbs inhibited their ability to bind antigen (Figs. 3 and 4). An interaction between anti-p70/p80 and anti-DNA antibodies in the cryoglobulins is not likely to be due to rheumatoid factor–like activity, since both the sera and the isolated cryoprecipitates had no rheumatoid factor activity detectable by either latex fixation or by ELISA (Table I). In addition, the selectivity of antibodies in the cryoprecipitates argues against a rheumatoid factor–like interaction. Since the anti-DNA mAbs inhibited binding of anti-p70/p80 antibodies to p70/p80 antigen (Fig. 3), and since the anti-DNA mAbs do not bind to other Ig (32) or to p70/p80 antigen, the anti-DNA antibodies most likely interact with the antigen-combining site of anti-p70/p80 antibodies. However, we have not yet determined if the

p70/p80 antibodies bind to the antigen-combining site or a noncombining site V region determinant of anti-DNA antibodies, i.e., whether the two species of autoantibodies are homobodies or epibodies (48). Antibodies to hormones and their antiidiotypes that react with hormone receptors (42, 48) are thought to be examples of homobodies or internal images. In contrast to epibodies, homobodies appear to share certain topographical features with antigen, and are thus frequently not restricted to a specific individual or animal species (49). It is noteworthy, therefore, that human anti-DNA antibodies inhibited antigen binding of other patients' anti-p70/p80 antibodies as well as a murine anti-p70/p80 mAb (Fig. 4). If certain anti-p70/p80 and anti-DNA antibodies are, in fact, homobodies, then it would be predicted that a population of autoantibodies reactive with the DNA-binding site of p70/p80 exists in the sera and isolated cryoglobulins of these patients. Preliminary studies suggest that this may be the case (unpublished observation).

The association of anti-DNA and anti-p70/p80 autoantibodies in these patients' sera may be relevant to their immune complex disease. Immune complex formation is a central feature of the network theory (50), and deposition of immune complexes is thought to be an important aspect of immune injury in SLE (2, 7, 8). The present studies suggest that immune complexes consisting of anti-DNA antibodies bound to the V regions of anti-p70/p80 antibodies occur in the sera of some patients with SLE. Other anti-DNA antibodies that bind to the Fc portion of Ig have also been described (51). The production of anti-DNA antibodies as antiimmunoglobulins (reactive with either variable or constant regions) might be one way of generating autoantibodies to a normally nonimmunogenic substance, such as DNA. However, we cannot tell from these studies how frequently such antibodies are encountered, or whether they are significant in the pathogenesis of immune complex disease. In regard to the latter issue, preliminary studies in our laboratory indicate that in one patient with SLE, a sharp rise in anti-p70/p80 antibodies occurred just before a parallel rise in serum anti-DNA antibodies and cryoglobulins, and a decline in complement levels and worsening of lupus nephritis (52). It seems plausible that immune complexes consisting of anti-p70/p80 and anti-DNA (idiotype-antiidiotype) might have played a role in the patient's renal deterioration. Thus, the significance of anti-DNA antibodies in SLE might be analogous, in some respects, to that of rheumatoid factors in rheumatoid vasculitis, where immune complexes consisting of Ig and rheumatoid factor are thought to play a significant pathogenic role (53). In contrast to the patients with SLE, a patient with PSS and high serum levels of anti-p70/p80 antibodies did not produce anti-DNA antibodies or cryoglobulins (Table I, patient JK), and maintained nearly constant levels of anti-p70/p80 antibodies over time (unpublished observation). This is of particular interest, since whereas American patients with anti-p70/p80 antibodies have presented with a relatively broad spectrum of autoimmune disease, including SLE, MCTD, and PSS (21, 36), Japanese patients have presented mainly with PSS-polymyositis overlap syndrome (35). It will be of interest to see whether the different clinical presentation of the latter group of patients might reflect an inability to generate a population of antibodies with dual Ig-DNA reactivity.

Summary

We have previously shown that sera from some patients with SLE and related disorders contain autoantibodies to a DNA-binding protein complex designated p70/p80. The present study shows that anti-p70/p80 autoantibodies are frequently accompanied by anti-DNA antibodies and cryoglobulins. When the cryoglobulins were isolated, they were found to be specifically enriched in both anti-p70/p80 and anti-DNA activities. The anti-p70/p80 and anti-DNA antibodies were found to be distinct populations of autoantibodies rather than a single crossreactive species, since they could be separated from one another by chromatography on DNA-cellulose. Certain human anti-DNA mAbs could inhibit the binding of autoimmune polyclonal anti-p70/p80 antibodies to p70/p80, suggesting that anti-DNA antibodies might also associate with the variable regions of some anti-p70/p80 antibodies in the cryoglobulins. Binding of one murine anti-p70/p80 mAb (111-12) also was inhibited by certain human anti-DNA mAbs, but the binding of another murine mAb (162-11) to a different epitope of p70/p80 was not. These studies suggest that certain anti-DNA antibodies may interact with the variable regions of a population of anti-p70/p80 antibodies. The cryoglobulins found in the sera containing both anti-p70/p80 and anti-DNA antibodies may represent immune complexes consisting, in part, of idiotype and antiidiotype.

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