Lupus Autoantibodies to Native DNA Cross-React with the A and D SnRNP Polypeptides

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Abstract

Antibodies to native DNA (nDNA) in sera from patients with systemic lupus erythematosus have been found to frequently correlate with antibodies to the A and D SnRNP proteins measured in Western blot assays. 40 of 54 SLE (74.1%) sera with anti-nDNA bound to A and D proteins, while 9 of 113 sera (8%) without anti-nDNA bound the A and D proteins, $P < 10^{-8}$ by Fisher's exact test. Antibodies to nDNA correlated closely with anti-A and anti-D in seven of eight patients followed sequentially, $r = 0.7865$. Nine human polyclonal anti-nDNA populations were isolated from DNA cellulose columns. Seven reacted equally with A and D, and two reacted predominantly with D. Two of three murine monoclonal anti-DNA antibodies isolated from NZB/NZW F_1 hybrid mice bound A and D equally in Western blot with a titer >1/40,000. These reactions were directed to the unfolded A and D proteins measurable in Western blot since these monoclonals (and several of the human anti-nDNA populations) failed to react with native U_1RNP in ELISA or in RNA immunoprecipitation experiments. These newly recognized cross reactions of anti-nDNA may amplify the immune response to DNA and be part of the original immunogenic drive. (*J. Clin. Invest.* 1994. 93:443–449.) Key words: autoimmunity • cross-reactions • anti-DNA • anti-SnRNP•NZB/NZW F_1 mice

Introduction

Autoantibodies to native DNA (nDNA)[1] are a serological hallmark of SLE and constitute strong support for the clinical diagnosis. In addition, anti-nDNA correlates positively with disease activity (especially nephritis) and remissions are usually associated with declining anti-DNA levels (1–4). Antibodies to nDNA have been shown to be enriched in serum cryoglobulins (5), as well as in acid eluates of glomeruli from patients with lupus nephritis (6–9).

The origin of antibodies to DNA represents an area of continued speculation since no method that renders DNA immunogenic with the production of antibodies to nDNA had been found until recently in animals. Krishnan and Marion immunized normal mice with mammalian DNA linked to an arginine-rich fusion protein, Fus-1, and induced IgG antibodies to nDNA, suggesting a critical role for nucleic acid binding proteins in induction of these antibodies (10). While the pathway of immunization for anti-nDNA is yet to be identified, antibodies to nDNA regularly appear in human SLE patients and in several mouse models of SLE, most notably the NZB/NZW F_1 hybrids and MRL *lpr/lpr* mouse strains and probably other mouse models of SLE. This report describes data that defines a cross-reaction of affinity-purified polyclonal human anti-nDNA, as well as monoclonal anti-nDNA derived from NZB/NZW F_1 female hybrid mice, which strongly cross-react with the unfolded A and D proteins of the SnRNP particles. This provides a mechanism for the mutual amplification of these responses and also raises questions about the original immunogenic stimulus for the production of antibodies to nDNA.

Methods

All patients' sera containing anti-nDNA that numbered 54 in this study were from SLE patients who satisfied American College of Rheumatology (formerly American Rheumatism Association) revised criteria for the classification of SLE (11). The sera from patients without anti-nDNA numbered 113 and were from several groups. 20 patients without precipitins all satisfied American Rheumatism Association criteria for SLE (11). Sera from patients with either anti-Ro/SSA precipitins alone or those with both anti-Ro/SSA and anti-La/SSB had either SLE, subacute cutaneous lupus erythematosus, or Sjögren's syndrome, and numbered 49, while there were 34 patients with anti-U_1RNP precipitins who either had SLE, scleroderma, polymyositis, or in a few instances, an overlap of two of these diseases. Multiple samples were obtained on most patients and were immunologically characterized. Anti-nDNA was measured by the Crithidia assay (12). Precipitating antibodies to Ro/SSA, La/SSB, U_1RNP, and Sm were assayed by gel diffusion using bovine spleen or calf thymus extracts (13, 14). Solid-phase assays for anti-U_1RNP and anti-Sm, as well as affinity purification of U_1RNP and Sm, were performed as previously described (15).

Western blotting with Molt 4 extract and affinity purification of antibody from nitrocellulose strips were performed as described (16). RNA immunoprecipitation was performed by the method of Forman et al. (17). Inhibition of Western blot reactivity against the A and D proteins was accomplished by preincubating appropriate dilutions of the human sera for 1 h at room temperature with calf thymus DNA (Sigma Chemical Co., St. Louis, MO) at final concentrations of 5 and 50 μg/ml. DNA concentrations were assessed by optical density at 260 nm using an extinction value of 1.0 at 50 μg/ml. Antibodies to nDNA were purified with DNA cellulose (contains double-stranded DNA)

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1. *Abbreviation used in this paper:* nDNA, native DNA.

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purchased from Sigma Chemical Co. The DNA cellulose was equilibrated with a buffer containing 0.02 M Tris, 0.145 M NaCl, pH 7.2. Sera were dialyzed against this buffer and then applied to the column. Effluents were collected until the OD at 280 nm fell below 0.01 and then reconstituted to the original serum volume by concentration by the Centriprep method (Amicon Corp., Danvers, MA). Specific antibody was eluted with 3 M $MgCl_2$, dialyzed against the Tris-NaCl buffer, and assayed for anti-nDNA by the Crithidia assay. These eluates were also concentrated to the volume of the serum from which they were prepared. Gamma globulin concentration was measured using an OD value of 1.5/mg protein per 280 nm.

Monoclonal antibodies to nDNA were prepared from NZB/NZW F_1 female mice and were characterized structurally and with respect to their ability to accelerate nephritis in young NZB/NZW F_1 mice and to cause it in healthy BALB/c mice (18). Concentration of immunoglobulin in the ascitic fluids was determined by single radial diffusion using a mouse IgG kit (Miles Inc., Diagnostic Division, Kanakee, IL).

Results

Studies of sera from SLE patients reveal the presence of antibodies to nDNA that characteristically occur in 70–90% of active untreated patients (19). A Western blot survey of numerous anti-nDNA SLE sera revealed a striking correlation between antibodies to the A and D proteins of the SnRNP particles and antibodies to nDNA. These data are listed in Table I for the reactivities to the major SnRNP peptides.

As seen, 40 of 54 (74.1%) SLE sera with anti-nDNA had antibodies that bound the A and D proteins of the SnRNP particles, while only 9 of 113 (8.0%) sera from patients with SLE and related diseases without anti-nDNA had antibodies that bound the A and D proteins. This was true regardless of the presence or absence of the presence of precipitins to the SnRNP particles. Correlation analyses of reactivity with the major SnRNP polypeptides are listed in Table II and significant associations are seen with A, D, BB′, and the combination of A and D with anti-nDNA. However, if we examine only the sera with anti-U_1RNP and/or Sm, then the only reactivities that correlate significantly with anti-nDNA are the A, D, and A and D reactivities as seen in Table III. When the reactivities of 10 SLE sera with Molt 4 extract containing both anti-nDNA and anti-Ro/SSA precipitins (lane 1–10) were studied as seen in Fig. 1, the intensity of the reactivity of the sera with the A and D proteins was very similar, and sera 1–9 all have anti-A and anti-D activity. This equal intensity of the A and D bands was seen with most but not all the sera with anti-nDNA activity. To assess the relationship of the anti-A and anti-D reactivities, we performed affinity isolation studies with serum 8 in Fig. 1. This experiment showed that eluted anti-A reacted with both the A and D bands, while eluted anti-D reacted with both the A and D bands. No other bands were reactive and elution from a region without reactivity was appropriately negative. These data indicate a shared epitope(s) between the A and D proteins and are consistent with their almost invariably paired reactivity. The identity of the reactants as the A and D proteins was assured by showing that affinity purified human U_1RNP gave an identical pattern of reactivity with such sera as did Molt 4 extract.

To show that the anti-A and anti-D bands are independent of their anti-Ro/SSA status, we have studied an additional 10 sera with anti-Ro/SSA precipitins that contained neither anti-nDNA nor anti-denatured DNA. As seen in Fig. 2, eight of these sera have antibodies to 52-kD Ro/SSA and five have anti-60-kD Ro/SSA. SLE sera with the A and D bands are dependent on their content of anti-nDNA, not their content of anti-Ro/SSA.

Finally, we have studied the anti-Ro/SSA sera in Fig. 1 with respect to their ability to bind affinity purified U_1RNP and Sm in ELISA, as well as their ability to immunoprecipitate

Table I. Frequency of Antibodies to SnRNP Proteins in Sera with and without Anti-nDNA

	Number of sera positive for SnRNP proteins in Western blot				
	A	D	BB′	70 kD	A and D
Sera with anti-nDNA (n = 54)					
Anti-RoSSA sera (n = 11)	10	10	6	1	10
Anti-U_1RNP and/or anti-Sm sera (n = 16)	12	12	13	7	12
SLE sera with no precipitins (n = 27)	18	21	6	3	18
Totals	40	43	25	11	40
Sera without anti-nDNA (n = 113)					
Anti-Ro/SSA sera (n = 20)	5	5	3	4	4
Anti-U_1RNP (n = 34)	15	0	20	8	0
Anti-Ro/SSA and anti-La/SSB sera (n = 29)	2	0	3	3	0
SLE sera with no precipitins (n = 20)	0	0	0	6	0
Totals	22	5	26	21	4

Table II. Comparison of Anti-nDNA Positive Versus Anti-nDNA Negative Sera for Reactivity with SnRNP Polypeptides in Western Blot

SnRNP Protein	Percent anti-DNA positive	Percent anti-DNA negative	P
A	74.1	19.5	$<10^{-8}$
D	79.6	4.4	$<10^{-8}$
BB′	46.3	20.4	6.2×10^{-4}
70 kD	20.4	18.6	0.47
A + D	74.1	3.5	$<10^{-8}$

P was calculated by Fisher's exact test.

Table III. Comparison of Anti-nDNA Positivity Versus Anti-nDNA Negativity for Anti-U_1RNP Sera (n = 50) in Western Blot

SnRNP	Percent anti-DNA positive	Percent anti-DNA negative	P
A	75.0	44.1	0.04
D	75.0	0	1.5×10^{-8}
BB′	81.8	57.1	0.11
70 kD	43.8	23.5	0.13
A + D	75.0	0	1.5×10^{-8}

P was calculated by Fisher's exact test.

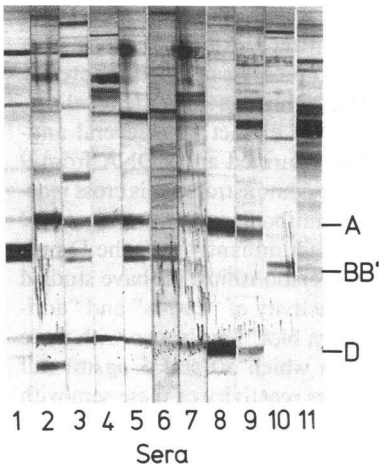

Figure 1. Western blot in 15% SDS PAGE of 10 SLE sera (1–10) with anti-Ro/SSA precipitins and anti-nDNA with Molt 4 extract. Serum 11 is an SLE serum with anti-Ro/SSA and anti-La/SSB precipitins. Sera were all applied at a 1/100 dilution.

U RNAs from Molt 4 extract. These properties are compared to the Western blot data in Table IV. As seen, all sera immunoprecipitated the Ro/SSA HYRNAs, but only four sera clearly immunoprecipitated U_1RNP, all four of which were also positive in ELISA for both U_1RNP and Sm. No sera bound the RNAs characteristic of the anti-Sm specificity, U_2, U_4, U_5, and U_6. Interestingly, of five sera positive only for the A and D bands in Western blot, four were negative for RNA immunoprecipitation, two were negative in U_1RNP ELISA, and the other three weakly positive for the U_1RNP ELISA. In contrast, all five sera that bind BB' in Western blot bound U_1RNP and Sm in ELISA. These data indicate that the major antigenic specificity of the anti-nDNA autoantibodies is to the denatured A and D proteins existing in the Western blot assay. Considering that the ELISA is 10–100 times more sensitive than immunoblotting, and that for several of these sera the reactivity with A and D is very much stronger in Western blot than ELISA (or RNA immunoprecipitation), the conclusion is clear that the reactivity of the anti-nDNA is to some structure(s) in the denatured A and D proteins.

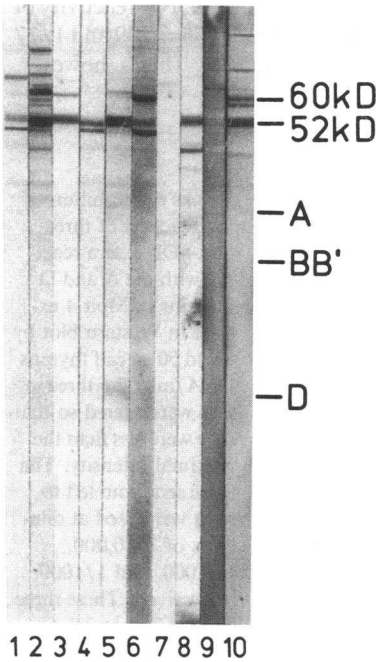

Figure 2. Western blot in 15% SDS-PAGE with Molt 4 extract with sera with anti-Ro/SSA precipitins without antibodies to either native or denatured DNA. Sera were all applied at a 1/100 dilution.

Table IV. Relationship of ELISA, RNA Gels, and Western Blot for Sera with Anti-Ro/SSA Precipitins and Anti-nDNA

Patient serum	RNA gels: Human y or Ro/SSA RNAs	RNA gels: U_1RNA	Western blot: A	Western blot: BB'	Western blot: D	ELISA O.D.: U_1RNP	ELISA O.D.: Sm
1	+++	+++	1+	4+	1+	>1.0	0.724
2	+++	0	4+	0	4+	0.336	0.336
3	+	0	2+	2+	2+	0.565	0.701
4	+++	0	3+	0	3+	0.499	0.545
5	+++	++++	3+	3+	3+	1.045	0.494
6	+	++++	0	2+	0	>1.0	0.694
7	+++	0	2+	0	2+	0.423	0.316
8	+++	0	4+	0	4+	0.295	0.328
9	+++	++	3+	0	3+	0.433	0.490
10	+++	0	0	3+	0	0.742	0.450

These are the data derived from the 10 SLE sera in Fig. 1. Intensity of the RNA bands and the protein bands (from Western blot) were scored by inspection. ELISA values are abnormal if >0.400 OD units which is the mean±2 SD for normal sera. All sera were assayed at a 1/100 dilution in the ELISA.

Among individual patients, the variation of anti-nDNA titer and the capacity to bind the A and D proteins in Western blot correlated very tightly in seven of eight patients studied at different time points. These data are graphically illustrated in Fig. 3, in which the strong relationship between the two different specificities is seen. A correlation coefficient was calculated comparing the ranks of the Crithidia titer and the anti-D intensity scores and was 0.7865. The program used for this calculation was JMP (SAS Institute, Inc., Cary, NC). In Fig. 4, we

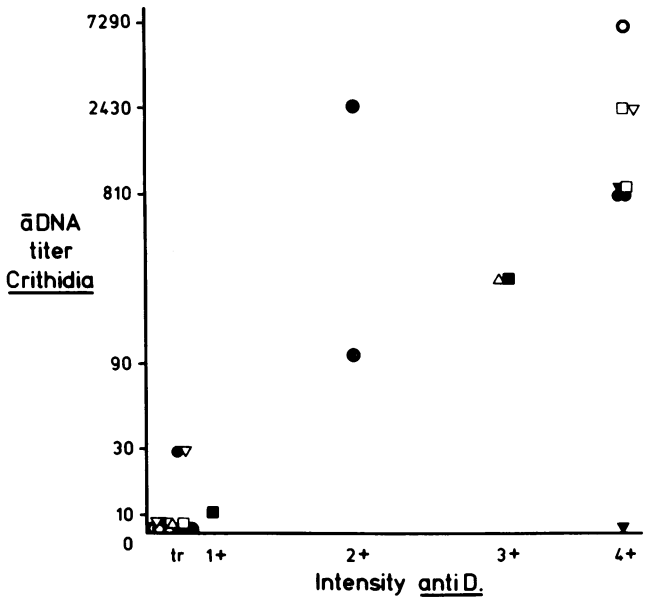

Figure 3. Diagram relating anti-DNA titer (by Crithidia) to the intensity of the anti-D band in Western blot with Molt 4 extract. Individual sera are designated by symbols. Note that serum ▼ had a strong anti-D band whether the anti-nDNA was positive or negative. All sera assayed at a 1/100 dilution.

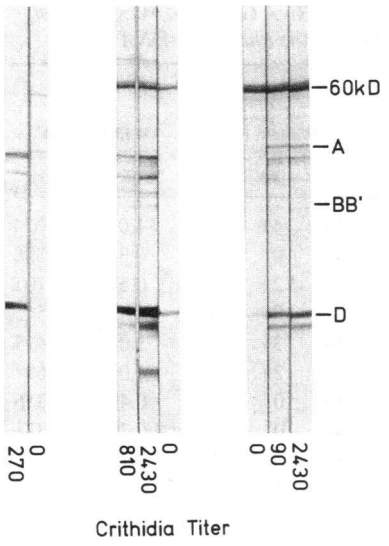

Figure 4. Serial sera studied by Western blot with Molt 4 extract in 15% SDS PAGE from three patients showing changes of anti-A and anti-D intensity with change in Crithidia titer. Note that with the sera on the far right that anti-A and anti-D vary but the anti-60 kD Ro/SSA does not vary with the anti-nDNA titer. All sera assayed at 1/100 dilution for the Western blot experiments.

show three representative patients' sera in which the concordant variation in anti-A and anti-D titers with the anti-nDNA titer is seen. We further investigated this relationship by direct isolation of polyclonal anti-nDNA on DNA cellulose columns and studied the eluates, the effluents, and the original sera on Western blot. The results from two representative sera are seen in Fig. 5. The only reactivities partially depleted in the effluent of patient L.B.'s serum are antibodies to the A and D bands, while antibodies to the 70-kD U_1RNP protein, the 60-kD Ro/SSA, and the BB' bands are undiminished in intensity. The eluate has only anti-A and anti-D reactivity. Similar results are seen on the right with serum P.C., which contains antibodies to 60-kD Ro/SSA, a 70-kD protein, and the A and D proteins in Molt 4 extract. Note the relative depletion of anti-A and anti-D in the effluent but not anti–60-kD Ro/SSA or the antibody to the 70-kD protein. The eluate has only anti-A and anti-D reactivity. While the extra bands seen in the A and D regions are not identified, they could be posttranslationally modified or partially degraded A and D proteins. These experiments directly demonstrate cross-reactivity of anti-nDNA with the A and D proteins, respectively, of Molt 4 extract. We have confirmed the identity of the A and D proteins by electrophoresing affinity-purified human U_1RNP, which gave an identical pattern of reactivity as whole Molt 4 extract with several anti-nDNA eluates. We have affinity-purified anti-nDNA from 9 SLE sera and have in seven cases demonstrated this cross reaction in the eluted anti-nDNA antibodies with the A and D proteins. Two eluates reacted predominantly with the D protein. To further illustrate these relationships, we have studied the ability of nDNA to block reactivity of "anti-A" and "anti-D" with their antigens in Western blot. This is seen with three representative sera in Fig. 6, in which 50 and 5 μg/ml calf thymus nDNA greatly diminishes reactivity of these sera with the A and D proteins, while the same final concentrations of transfer RNA have no effect on the reactivity with A and D (the RNA experiments are not shown). The first lane in each case has no added nDNA. Note also that with the serum on the far right, the 60-kD Ro/SSA band is not affected by the added DNA. As an additional control, calf thymus DNA was subjected to SDS-PAGE and Western blot at a concentration of 100 μg/ml and developed with a serum containing anti-A and anti-D activity, but no anti-nDNA activity, to rule out contaminating A and D proteins. The DNA preparation was not found to be contaminated with A and D proteins by this method.

Finally, we have studied three monoclonal anti-DNA antibodies derived from NZB/NZW F_1 mice that have been previously characterized by one of us (B. H. Hahn). Their reactivities in antinuclease antibody tests and on the crithidia substrate (anti-nDNA) are seen in Table V.

The reactivities of these monoclonal antibodies to DNA with Molt 4 extract in Western blot are seen in Fig. 7. In lanes 1 and 2, we see BWds1 and BWds3, while 5DG5 is seen in lane *3*. Note that the monoclonals in BWds1 and BWds3 react strongly with the A and D proteins, while 5DG5 does not (17). Interestingly, some monoclonal anti-DNA antibodies do not bind A and D. As shown in Table I, 26% of human polyclonal anti-nDNA containing SLE sera also fail to react with the A and D proteins. The failure of 5DG5 to bind the A and D proteins could be because of the lower anti-nDNA reactivity of this monoclonal antibody that has crithidia titers 1/9 and 1/27 as great as BWds3 and BWds1, respectively. This is, however,

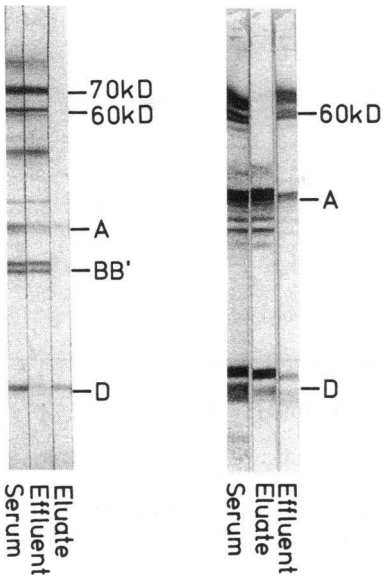

Figure 5. Comparison of serum, effluent, and eluate in Western blot with Molt 4 extract in 15% SDS-PAGE at 1/100 dilution for patient L.B. on the left and patient P.C. on the right. The IgG concentration of the L.B. eluate was 0.7 or 0.007 mg/ml at a 1/100 dilution. The eluate for patient P.C. on the right was applied at a 1/10 dilution while the serum and effluent were at a 1/100 dilution. The IgG concentration of the P.C. eluate was 0.151 or 0.015 mg/ml at a 1/10 dilution.

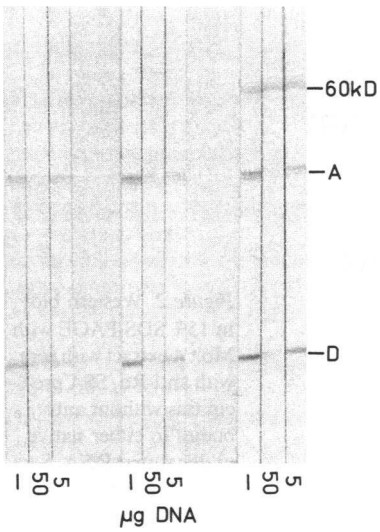

Figure 6. Inhibition of the reaction of three anti-nDNA sera reacting with the A and D proteins of Molt 4 extract in Western blot by 5 and 50 μg calf thymus DNA/ml. The three sera were titered so that they were less than the maximal intensity. The three sera from left to right were used at dilutions of 1/10,000, 1/5,000, and 1/1000, respectively. These three sera are 2, 4, and 8, respectively, from Fig. 1.

Table V. Antinuclease Antibody and Anti-nDNA Titers and IgG Content of Murine Ascitic Fluids with Monoclonal Anti-nDNA Antibodies

Murine monoclonal	Antinuclease antibody titer on Hep2 cells	Crithidia titer	IgG content
			mg/ml
BWds1	3,240	2,430	3.3
BWds3	9,720	810	5.4
5DG5	120	90	15.0

unlikely since while these blots were performed at a 1/100 dilution of ascitic fluid, both BWds1 and BWds3 had titers in Western blot with A and D greater than 1/40,000. This represents final Ig concentrations in the Western blot assay of 0.083 and 0.140 μg/ml for the BWds1 and BWds3, respectively. Thus, 5DG5, whose anti-DNA titer is 1/9 and 1/27 as great as that of BWds3 and BWds1 (Table V), has ≥400 times less activity for anti-A and anti-D in Western blot. 5DG5 ascitic fluid has an IgG concentration that is 15.0 mg/ml compared to 3.3 and 5.4 mg/ml for BWds1 and BWds3, respectively, so that the lesser reactivity of the 5DG5 is not caused by a lower Ig concentration. The equal reactivity of the monoclonal anti-DNAs with the A and D proteins is very similar to that of the polyclonal human anti-nDNA as is the finding that a shared epitope is recognized on A and D, implied by the monoclonality of the murine anti-DNA. In addition, we have performed competition experiments with human polyclonal anti-DNA eluates and BWds1. In such experiments, a 1/50 dilution of the BWds1 ascitic fluid (0.066 mg/ml) completely inhibits the reactivity of a 1/30 dilution (0.0051 mg/ml) of the human eluate P.C. (seen in Fig. 5) with both the A and D proteins, suggesting that the murine and human anti-nDNA antibodies are reacting with a similar or identical epitope(s) on the A and D proteins.

This specificity of the monoclonal antibodies with A and D is exclusively reactive with the form present in Western blot (presumably denatured) since while the titer in Western blot is greater than 1/40,000, these monoclonal antibodies fail to bind affinity purified U_1RNP or Sm particles in quantitative ELISA (presumably the native form of these particles) at ascitic fluid dilutions of 1/100. In addition, all the ascitic fluids containing very high levels of antibody activity fail to immunoprecipitate U RNAs from Molt 4 extract (data not shown). Thus, the behavior of both the murine monoclonal anti-nDNA and the polyclonal human anti-nDNA exhibit this almost exclusive preference for reacting with the form (presumably denatured) of the A and D proteins present in Western blot experiments.

Discussion

Demonstration of a cross-reaction between DNA and the two proteins A and D with autoantibodies in SLE patients brings into focus several questions about the specificity and origin of these autoantibodies. Specificity issues are amenable to immunochemical analysis and will be discussed first. As pointed out in Results, the cross reaction appears to be between nDNA and a specific epitope(s) shared by the denatured forms of the A and D SnRNP proteins. This conclusion is based on the fact that several of the SLE sera, as well as the mouse monoclonals, react strongly in Western blot with titers ranging from 1/5,000 to 1/40,000, while at titers of 1/100, they do not react with native, affinity-purified U_1RNP or Sm particles in ELISA assays or in RNA immunoprecipitation studies. It is clear that much effort will be expended toward identifying the reactive epitopes on the A and D proteins. There is little amino acid sequence homology between the A and D proteins, and indeed, not a single tetrapeptide amino acid sequence is shared between the two proteins. It is conceivable that tripeptide sequence homology or some conformational epitope acquired after refolding on the nitrocellulose controls the structure of the cross-reactive epitope. The D polypeptide also shares an epitope with the B'/B polypeptide defined by a murine monoclonal anti-Sm (Y12) as well as many anti-Sm containing human SLE sera. Similarly to the A and D proteins, D and B'/B share little sequence homology. This cross-reaction is also demonstrable in the Western blot assay and recent epitope analysis suggests that this epitope(s) is conformational (20).

An overlap in the anti-Sm and anti-DNA responses has been recently described in a large scale study of 41 hybridomas selected for Sm binding derived from MRL-*lpr/lpr* mice (21, 22). Of these 41 Sm binding hybridomas, 25 bound denatured or single-stranded DNA and of these 25, 14 also bound nDNA. Many of the hybridomas bound the D peptide but few bound the A peptide. Some of the hybridomas bound the B peptide and others bound the 70-kD and C peptides of the U_1RNP particle. From the amount of data presented, at least six hybridomas that bound D did not bind nDNA, but all three hybrid-

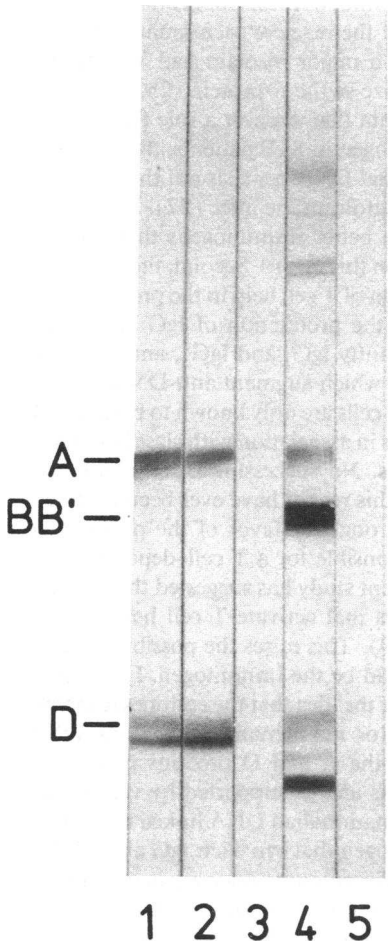

Figure 7. Western immunoblotting in 15% SDS PAGE of mouse monoclonal anti-nDNA and control mouse sera reacting with Molt 4 extract. Lane *1*, BWds1; lane *2*, BwdS3; lane *3*, 5DG5; lane *4*, serum from Palmerston North mouse; and lane *5*, normal mouse serum. All ascitic fluids and sera diluted 1/100. IgG concentrations of the three fluids determined by single radial diffusion were BWds1, 3.3 mg/ml; BWds3, 5.4 mg/ml; and 5DG5, 15.0 mg/ml. Unpublished work by Drs. Morris Reichlin and Barry Handwerger has shown that Palmerston North mouse sera frequently have antibodies to the A, BB', and D proteins as seen in lane *4*.

omas that bound both A and D polypeptides bound nDNA. Thus, binding to D protein alone in MRL/*lpr*/*lpr* derived anti-Sm hybridomas is not associated with binding to nDNA, while binding to both A and D proteins is associated with binding to nDNA. This finding is in accord with what we have demonstrated with polyclonal human anti-nDNA and monoclonal murine anti-nDNA derived from NZB/NZW F_1 mice. This association of the anti-A and anti-D autoantibody response in human SLE is previously unrecognized and somewhat surprising, since anti-A autoantibody has been considered part of the specific response to the U_1RNP particle, while antibodies to D are the most consistent and characteristic part of the autoimmune Sm specificity (19).

A more difficult issue is the identification of the immunogen that gives rise to and/or perpetuates the anti-nDNA response that cross reacts with the A and D SnRNP proteins. This cross-reaction can be added to the numerous non-nucleic acid molecules such as cardiolipin, vimentin, and platelet membranes that comprise the polyspecificity of human monoclonal anti-DNA antibodies described and discussed by Schwartz and Stollar (23). They have also pointed out the strong structural relationship of autoantibodies to DNA and some antibacterial antibodies (23). This raises the possibility that some antibodies to DNA derive from germline encoded antibacterial antibodies and/or such genes which have undergone somatic mutation as has been described in a well-defined murine system (24).

In two previous publications, murine monoclonal anti-nDNA antibodies have been shown to react with a set of membrane proteins. While in neither of these publications were the molecular nature of any of the reactive membrane molecules identified, in both studies a major reactant had a molecular mass of 34 kD, the same size as the A protein (25, 26).

While there are now data that support a role for bacterial DNA as a possible immunogen in SLE patients, there are several reasons for believing that DNA per se is not the immunogen in SLE patients or in autoimmune mice (27). Firstly, in a generic sense, proteins are better immunogens than nDNA, which is notoriously weak in this regard. Second, there are data that suggest the participation of T cell help in the production of anti-DNA. These include the production of IgG antibodies, which are often predominantly IgG_1 and IgG_3, and the isolation of T cell helper clones which augment anti-DNA production (28, 29). Yet, T helper cells are only known to be geared to respond to peptide-epitopes in association with class II antigens on antigen presenting cells. No successful experiments with oligonucleotides active in this regard have ever been reported. All of these facts weigh strongly in favor of the polypeptide nature of the epitope responsible for a T cell–dependent autoimmune response. A recent study has suggested that nucleosomes provide the peptides that activate T cell help for IgG anti-nDNA production (30). This raises the possibility that a DNA protein complex could be the immunogen. From these arguments, one would favor the idea that the epitopes responsible for the induction and/or maintenance of the anti-DNA response would reside on the A and D proteins perhaps in association with DNA. This idea is supported by very recent experiments showing that mammalian DNA linked to a fusion protein Fus-1 is an immunogen that can elicit IgG anti-nDNA in normal mice (10). Experiments are underway in our laboratory to see if animals immunized with isolated A and D proteins can lead to the production of antibodies to nDNA. Whatever the initiating immunogen for the anti-nDNA response, the presence of this cross-reaction provides multiple antigenic stimuli for the perpetuation and amplification of the anti-DNA response.

Finally, in other studies that will be reported elsewhere, in vitro cell culture experiments show that both monoclonal murine and polyclonal human antibodies to DNA bind and penetrate live tissue culture cell lines leading to injury. In all cases studied thus far, only those anti-nDNA antibodies that cross-react with the A and D proteins have the property of binding to and penetrating cells in culture. These are the same monoclonal antibodies found to accelerate nephritis in young NZB/NZW mice (18). Others have also recently reported the penetration of renal cells in vivo by murine monoclonal antibodies to nDNA (31). Thus, this cross-reaction may define those antibodies that have the property of mediating direct injury to cells. Further studies of these phenomena should greatly expand our understanding of the relationships between the specificity of the cross-reactions of anti-nDNA and pathogenicity.

Note added in proof. Another novel method has come to our attention for inducing normal animals (rabbits) to produce anti-dsDNA strongly reactive with autologous dsDNA. This method is to infect rabbits with human polyoma BK virus (Flaegstad, T., K. Fredriksen, B. Dahl, T. Traavik, and O. P. Rekvig. 1988. *Proc. Natl. Acad. Sci.* 85:8171–8175; Rekvig, O. P., K. Fredriksen, B. Dahl, B. Braunsether, V. Moens, A. Sundsfjord, and T. Traavik. 1992. *Scand. J. Immunol.* 36:487–495).

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