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Research Article

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Association of Autoantibodies to Different Nuclear Antigens with Clinical Patterns of Rheumatic Disease and Responsiveness to Therapy

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A B S T R A C T Using a hemagglutination test which can detect antibodies to (a) native and denatured deoxyribonucleic acid (DNA) and (b) an extractable nuclear antigen (ENA), a comparative study of patterns of autoantibody formation has been done in systemic lupus erythematosus (SLE) and related rheumatic diseases. Antibody to native DNA was present in the serum in 96% of patients with active SLE and disappeared during remissions. Antibody to ENA was found in 86% of those patients with SLE nephritis who responded to treatment but in only 8% of those who did not. The highest titers of antibody to ENA were found in patients having a mixed connective tissue disease syndrome with features of SLE, scleroderma, and myositis. The latter syndrome was notable for the absence of renal disease and for a striking responsiveness to corticosteroid therapy. Hemagglutination testing of 277 sera from normal persons and patients with a wide variety of acute diseases other than SLE revealed the presence of antibody to native DNA in only 1.4% and antibody to ENA in only 0.4%.

These results yield significant correlations among the pattern of autoimmune reactivity, the clinical form of the rheumatic disease, and responsiveness to treatment. They implicate the qualitative nature of the patient's immune response as a conditioning factor in the type of disease. Together with other correlations they may allow classification of rheumatic diseases into more biologically meaningful groups and lead to more selective methods of therapy.

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INTRODUCTION

Autoantibodies are commonly found in many diseases; they are most abundant in rheumatic diseases. In systemic lupus erythematosus (SLE) autoantibodies reacting with many different cytoplasmic and nuclear constituents may arise (1-13). Circumstances leading to the appearance of these autoantibodies have not been clarified, and it has not been possible to incriminate a direct reaction of autoantibody with fixed tissue antigen as the cause of the principal pathological changes.

In general, knowledge of the variety and amounts of autoantibodies which are formed has developed proportionately to the breadth of the search and the sensitivity of the methods used to detect the antibodies. This has led to continuous change in descriptive details. Generalizations about relationships of particular autoantibody patterns to particular disease states have been difficult to establish.

A number of factors indicate that important correlations exist. For example, experiments have recently suggested that different types of circulating immune complexes can damage the glomerular basement membrane (14), provoke granulomas (15), or lyse platelets (16). Appreciable evidence exists that soluble circulating complexes of deoxyribonucleic acid (DNA) and antibody to DNA participate in the induction of the renal lesion in patients with SLE (6, 17, 18) and in the analogous lesion in New Zealand hybrid mice (19). Conversely, there is also substantial evidence in other experimental systems involving autoimmunity and transplantation that antitissue antibodies can protect certain tissues from subsequent potential immunological damage (20-23). This raises the possibility that at least some autoantibodies in man may be protective rather than harmful or neutral.

In a recent study (24) antibody to native DNA was detected by immunodiffusion in 67% of patients with active SLE nephritis and by complement fixation in 76%. These techniques are relatively insensitive. The fluorescent antibody technique is more sensitive and permits correlation of a specific pattern of nuclear fluorescence with a particular antinuclear antibody. However, it has the disadvantage that the presence of one antinuclear antibody may be obscured by another when the serum contains several (25). In order to avoid these problems, a hemagglutination method has been developed which has sensitivity comparable with the fluorescent antibody technique and which can identify specific antinuclear antibodies. It detects antibody to DNA and to the extractable nuclear antigen (ENA). The latter antigen is abundant in cell nuclei, may exist in intimate association with deoxyribonucleohistone, and is neither DNA nor histone (10). The hemagglutination method has revealed relationships of certain autoantibodies to the clinical forms of the disease and to the effectiveness of therapy. Specifically, autoantibody to native DNA is almost always present in patients with SLE who have active renal disease and disappears during remissions. Autoantibody to ENA is more commonly present in those patients with renal disease of SLE who remit than in those who do not, and the highest titers of antibody to ENA were found in patients having a syndrome characterized by the absence of renal disease and an unusual responsiveness to corticosteroid therapy.

METHODS

Patients

Serum specimens were collected at frequent intervals from 71 patients with SLE and 25 patients with a mixed connective tissue disease with features of SLE, scleroderma, and myositis. Active SLE was present in 47 patients. They had typical involvement of many systems and, at some point in the course of their disease, had positive LE cell preparations, and/or significant antinuclear antibody titers detected by complement fixation. Active disease was defined as arthritis, fever not due to infection, serositis, myocarditis, rash, neuropathy, anemia, leukopenia, and/or active renal disease. The latter was indicated by significant hematuria and increasing proteinuria with or without a diminished creatinine clearance, and was sometimes confirmed by renal biopsy. The 24 remaining patients were designated as inactive SLE because they did not have signs of disease activity at the time of study.

Preparation and characterization of antigens

DNA. Calf thymus DNA was prepared following the method of Kay, Simmons, and Dounce (26). The thymus glands were obtained promptly after slaughter of the animals and were transported to the laboratory packed in ice. The tissue was then frozen at -20°C , in one case for 24 hr and in another for 3 wk before processing. Aliquots of the DNA were removed at various stages in the Kay procedure and tested for activity in the hemagglutination test.

Material removed after step 7 of the Kay procedure was antigenically active. The more highly purified DNA carried through step 10 of the Kay method, and a commercial preparation (Worthington Biochemical Corp., Freehold, N. J.) have been inactive in hemagglutination testing. Denaturation of native DNA was accomplished by the following two methods: (a) heat denaturation to single-stranded DNA (HSS-DNA) by placing the DNA in a boiling water bath for 10 min and then in ice, and (b) alkali denaturation to single-stranded DNA (ALK-SS-DNA) by exposure to pH 12 for 20 min followed by return to pH 7 with hydrochloric acid. Fig. 1 shows the absorption spectrum of our step 7 heat-denatured (HSS-DNA) and double-stranded DNA (DS-DNA). The viscosity of the product was measured with an Oswald viscometer in a water bath at 30°C . The commercial DNA had a viscosity $4.11 \times$ water, and the calf thymus DNA which we prepared was $4.08 \times$ water, while HSS-DNA and ALK-SS-DNA were respectively 1.38 and $1.18 \times$ water. The hyperchromic shift after denaturation and the viscosity values indicate that the step 7 DNA we prepared was in the double-stranded form and comparable with a commercial preparation of DNA, and that subsequent denaturation was quite complete. Protein determinations by the method of Lowry, Rosenbrough, Farr, and Randall (27) on different preparations of the DNA showed 2-4% protein after step 7 and 0.7% protein after step 10 of the Kay procedure. This is probably a valid estimation of the amount of protein because a standard curve with histone was practically identical with that of the bovine serum albumin used as a standard in the Lowry test. Step 7 DNA was brought into solution by stirring in low molarity buffer for 48 hr, then made isotonic, and stored at -20°C for use in the hemagglutination test. There was a slight diminution in reactivity after storage for 3-4 months.

ENA. The extractable nuclear antigen (ENA) was prepared as described by Holman (10). The lyophilized antigen was stored in the cold and dissolved in isotonic buffer for use in the hemagglutination test.

Absorption of sera. Absorption of SLE sera with varying amounts of purified step 10 or commercial DNA was carried out at 37°C for 30 min and then at 4°C for 12-24 hr to confirm the DNA specificity of the hemagglutination reaction obtained with cells coated with step 7 DNA.

Enzyme treatments. DNA was digested with crystalline preparations of pancreatic deoxyribonuclease (DNase), pancreatic trypsin, and yeast ribonuclease (RNase) obtained from the Worthington Biochemical Corp. Digestions were conducted using the conditions described by Holman and Deicher (8) after the antigens were on the red cell surface.

Hemagglutination

A modification of the Stavitsky method for tanned red cell agglutination was used (28). The test was performed at pH 7.4 using a veronal buffer system. Sheep red blood cells were treated with a 1:25,000 tannic acid solution for 30 min at room temperature by gently adding a 4% suspension of red cells to the tannic acid solution. 1 mg of either native or denatured calf thymus DNA in 1 ml of veronal buffer or 5 mg of ENA was used to coat 1 ml of a 33% suspension of tanned sheep red blood cells. The red cells were then incubated for 30 min at room temperature and washed in a 1:150 dilution of heat-inactivated, normal rabbit serum (absorbed with sheep red blood cells) in veronal buffer. The suspensions of antigen-coated red cells were made up to give a reading of 39% transmission on the Coleman Junior spectrophotometer, model 6/20, at 650 m μ . Use

of a more concentrated suspension of red cells resulted in decreased sensitivity of the test, while use of a more dilute suspension led to nonspecific agglutination. The sera to be tested were inactivated for 30 min at 56°C, absorbed twice for 30 min at 37°C with a half volume of sheep red blood cells, and diluted with 1:150 normal rabbit serum in veronal buffer using a clean pipet for each dilution. To 0.25 ml of serum dilution was added 0.25 ml of red cell suspension, and the tubes were shaken and allowed to settle overnight at 4°C. When cells were allowed to settle at 25°C or were brought to 25°C after settling at 4°C, they sometimes reacted less well with antibody to DNA. Titers were expressed as the highest dilution of antiserum that resulted in a definite carpet of agglutinated cells covering the bottom of the tube, which would be between a 1+ and 2+ reading on the Stavitsky scale (28). Parallel controls at each dilution received tanned red cells without antigen. Because of the great tendency for nonspecific agglutination at low dilutions, a titer of 1:10 was required for a serum to be labeled as positive.

Complement fixation

Complement fixation testing for antinuclear antibody (ANA) and anticytoplasmic antibody (ACA) was carried out as described by Robbins, Holman, Deicher, and Kunkel (29). The antigens used were whole calf thymus nuclei and liver cytoplasm from human or rabbit.

Immunodiffusion

Immunodiffusion using 0.5% agarose as described by Tan and Kunkel (11) was used to detect both free DNA and antibody to DNA in sera.

Serum C3 determinations

The concentration of C3 protein in serum was determined by the radial immunodiffusion technique of Mancini, Carbonara, and Heremans (30).

RESULTS

Incidence of hemagglutinating antibody to DNA and to the extractable nuclear antigen (ENA) in SLE. Table I shows the incidence of hemagglutinating antibody to DNA and ENA in 47 patients with active SLE and in 24 patients with inactive SLE. 45 of the 47 patients with active SLE had antibody to native or double-stranded DNA (DS-DNA), and 32 of the 47 patients had antibody to alkali-denatured single-stranded DNA (ALK-SS-DNA). All 47 patients with active disease had one or both of these antibodies.

The difference in specificity of the antibodies reacting with single-stranded and double-stranded DNA is shown by the fact that in 17 of the 47 patients only one of the two antibodies was present. 40% of the anti-DNA reactions detected by the hemagglutination test were missed by the immunodiffusion technique, 20% of the sera with hemagglutinating antibody against DNA could not induce LE cell formation, and 42% of sera with antibody to DNA had a negative complement fixation test for anti-nuclear antibody. The titers of antibody to DNA usually ranged from 1:10 to 1:1000 and only rarely reached a higher level. All patients with active renal disease from

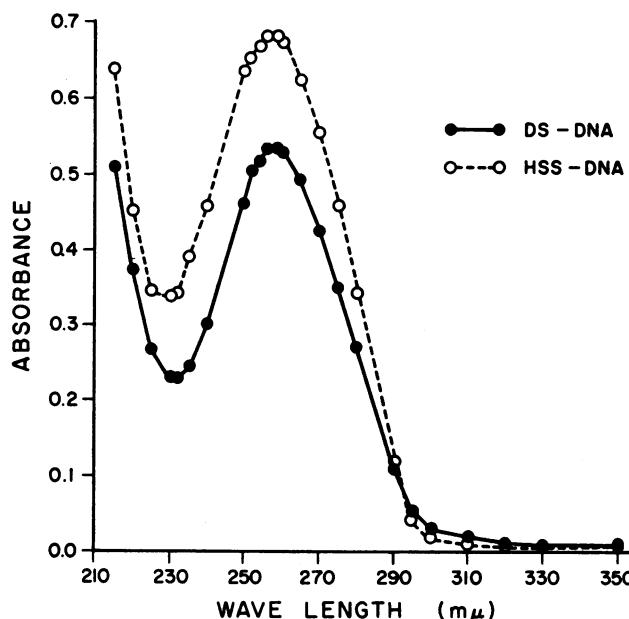


FIGURE 1 UV absorption spectrum of native calf thymus DNA (DS-DNA) (●—●) which has been processed through step 7 of the method of Kay et al. (26) (see text), and of the same specimen after heat denaturation (HSS-DNA) (○---○).

SLE had before treatment, circulating autoantibody to double-stranded DNA. Antibody to double-stranded DNA was uniformly absent in the 24 patients with inactive SLE, even though 54% of these patients previously had active renal disease.

In contrast with the strong correlation between antibody to native DNA and SLE disease activity, antibody to the extractable nuclear antigen (ENA) was present with about the same frequency in patients with inactive disease (50%) as in those with active SLE (55%) (Table I). Only 41% of the patients with active renal disease had antibody to ENA, while 78% of those with active SLE but without renal involvement had this antibody. The titers of antibody to ENA were in the range of 1:10 to 1:10,000 in both the active and inactive disease groups.

Absorption of 10 positive sera with 10 $\mu\text{g}/\text{ml}$ to 1.0 mg/ml of our highly purified native DNA or the commercial DNA removed anti-DNA hemagglutinating activity in every case without changing the anti-ENA antibody titer. After coating tanned red blood cells with DNA, treatment with DNase, but not with RNase or trypsin, abolished reactivity with antibody to DNA.

Correlation of the presence of free DNA and antibody to native DNA with disease activity and response to immunosuppressive therapy. 34 of the 47 patients with active SLE were followed for a sufficient time (6 months-5 yr) to determine that 22 went into sustained remission while 12 were treatment failures having continued, progressive disease activity after treatment with high doses of corticosteroids (usually 1 mg of prednisone per kg) with or without alkylating agents (Table II). Nine of the latter group died: six from their renal disease, one from widespread vasculitis and massive gastrointestinal

TABLE I
Incidence of Hemagglutinating Antibody to DNA and to the Extractable Nuclear Antigen in SLE

	Hemagglutinating antibody to:		
	ALK-SS-DNA	DS-DNA	ENA
Active SLE	32/47*	45/47	26/47
Renal cases	20/29	29/29	12/29
Nonrenal cases	12/18	16/18	14/18
Inactive SLE	1/24	0/24	12/24
Renal cases	0/13	0/13	7/13
Nonrenal cases	1/11	0/11	5/11

ALK-SS-DNA = alkali-denatured (single strand) DNA; DS-DNA = native (double strand) DNA; ENA = extractable nuclear antigen.

* Number positive/number tested.

TABLE II

Correlation of the Presence of Antibody to DNA and to the Extractable Nuclear Antigen with Response to Immunosuppressive Therapy in SLE

	Hemagglutinating antibody to:	
	DS-DNA	ENA
Responders (14 with renal disease)		
Before therapy	22/22*	19/22
After therapy	0/22‡	8/22
Failures (11 with renal disease)		
Before therapy	12/12	1/12
After therapy	10/12§	0/12

DS-DNA = native (double strand) DNA; ENA = extractable nuclear antigen.

* Number positive/number tested.

‡ None with free DNA in the serum.

§ The two who were negative for antibody to DNA had free DNA in the serum.

bleeding, one from cerebral hemorrhage, and one from renal disease plus infection. When patients with SLE went into remission, there was a disappearance of free DNA and antibody to native DNA from the serum. Conversely, those patients who did not respond to therapy continued to have detectable free DNA or antibody to native DNA in their serum.

Numerous serum analyses of all patients with SLE revealed that 15 had free DNA detected by immunodiffusion at some time during the course of their disease. All but one had serious disease, and renal disease was present in 12. The course of one such patient is shown in Fig. 2. Initially, the patient presented with arthritis, anemia, leukopenia, and hematuria. Renal biopsy was typical of SLE showing cellular proliferation, fibrinoid necrosis of capillaries, and focal basement membrane thickening. She had a positive LE cell test and antibody to single-stranded and double-stranded DNA by hemagglutination but no antibody to ENA. On 50 mg of prednisone daily, the patient developed progressive edema, mental depression, and increasing hematuria at a time when the hemagglutinating antibody to DNA had disappeared. Immunodiffusion showed free DNA in the serum at this time. During subsequent treatment with prednisone, nitrogen mustard, and cyclophosphamide, the patient continued to worsen with hypertension, peripheral neuropathy, myalgias, severe abdominal pain, and a deteriorating urine sediment. During this 7 month period, free DNA was consistently present in the serum, and the serum C3 protein level remained low (50-80 mg/100 ml).

During serial studies of patients through remissions and exacerbations of their disease, four patients with

SLE, while well, suddenly developed hemagglutinating antibody to double-stranded DNA in their serum, and the serum C3 protein level fell. 1-3 months later, all developed recurrent active nephritis.

Correlation of the presence of antibody to ENA with SLE disease activity and response to immunosuppressive therapy. The two groups of patients with active SLE summarized in Table II initially appeared quite comparable clinically except that renal disease was evident in 91% of those who failed to respond to treatment and in 64% of the "responders" who went into remission on therapy. At the onset of treatment, there was no significant difference between the groups in terms of age, degree of proteinuria, urinary sediment abnormalities, or renal function. The responders to therapy had a median age of 29 and a mean creatinine clearance of 77 ± 20 cc/min, while the treatment failure group had a median age of 26 and a mean creatinine clearance of 68 ± 24 cc/min. All of the patients initially had antibody to native DNA in their serum, and the range and mean titers were the same in both groups. All except one patient received prednisone 1-1.5 mg/kg, and two-thirds of each group also received alkylating drugs. The striking difference between the two groups was that the patients who responded to treatment had, before therapy, a much higher frequency of antibody to ENA than did those patients who failed to respond to treatment. Of those who

went into remission, 86% had antibody to ENA whereas it was present in only 8% of the patients who were treatment failures. The titer of antibody to ENA ranged from 1:10 to 1:10,000. Of the patients who went into remission, one-third maintained a positive test for antibody to ENA after remission though antibody to DNA disappeared.

Recently we have followed a family in which one sibling with antibody to native DNA and no antibody to ENA died with fulminating central nervous system and renal SLE, while her brother who has a 1:100,000 hemagglutinin titer of antibody to ENA and no antibody to DNA has had very mild SLE with only intermittent skin and joint involvement for 6 yr.

Patients with mixed connective tissue disease and a high titer of hemagglutinating antibody to the extractable nuclear antigen (ENA). Over the past several years an interesting group of patients came to our attention because of a persistently high antinuclear antibody titer by complement fixation. The high titer persisted when the patients entered remission on corticosteroids to which they seemed very responsive. We have now followed 25 such patients, 21 of whom are females (Table III). Their disease pattern characteristically combined features of SLE, myositis, and scleroderma, and none has had evidence of renal disease. Almost all have had the following symptoms: Raynaud's phenomenon; swelling with

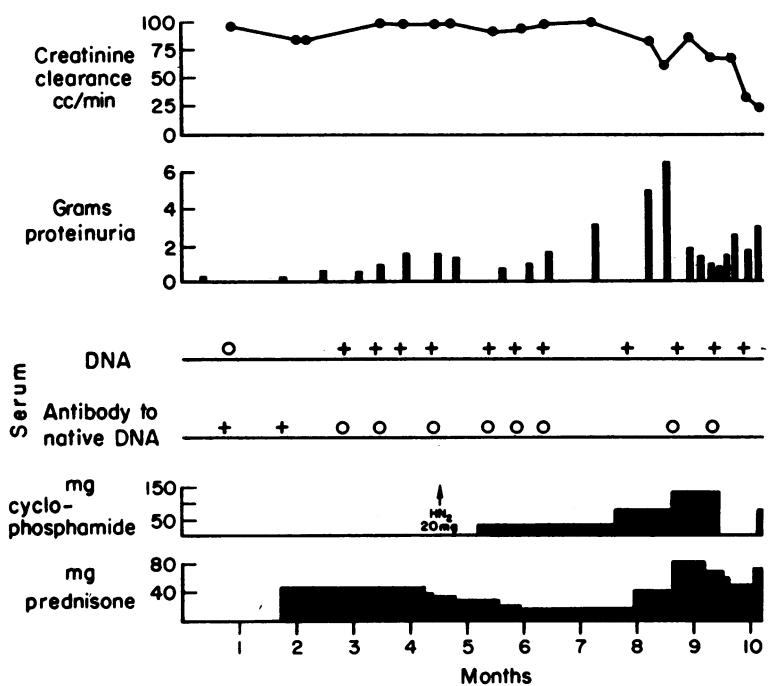


FIGURE 2 Persistent free serum DNA in a patient with SLE nephritis, unresponsive to treatment with prednisone, nitrogen mustard (HN_2), and cyclophosphamide.

TABLE III

Patients with Mixed Connective Tissue Disease with High Titer of Hemagglutinating Antibody to the Extractable Nuclear Antigen

Patient	Complement fixation titer of:			Hemagglutination titer of antibody to:			Steroid response
	ANA	ACA	LE preparation	ALK-SS-DNA	DS-DNA	ENA	
1	>1:512	0	0	0	0	1:400,000	+
2	>1:512	0	0	1:10	0	1:800,000	+
3	>1:512	0	0	1:10	0	1:200,000	+
4	>1:256	0	0	1:10	1:20	1:4000	+
5	>1:256	0	0	0	1:10	1:100,000	+
6	1:128	0	+	0	0	1:1000	+
7	>1:256	0	0	0	0	1:800,000	-*
8	>1:256	0	0	0	1:20	1:1000	+
9	>1:256	0	0	0	0	1:200,000	+
10	>1:256	0	+	0	0	1:80,000	+
11	<1:256	0	0	1:10	0	1:200,000	+
12	>1:256	0	0	0	0	1:80,000	+
13	1:2	0	0	0	0	1:1000	-*
14	1:16	1:2	0	0	0	1:100,000	+
15	>1:256	0	0	0	0	1:80,000	+
16	1:4	1:4	0	0	0	1:1000	+
17	1:16	1:4	0	0	0	1:4000	+
18	>1:256	1:4	0	0	0	1:200,000	+
19	>1:256	1:2	+	0	0	1:400,000	+
20	1:4	0	0	0	0	1:8000	-*
21	1:4	1:4	0	0	0	1:4000	-*
22	>1:256	1:128	+	0	0	1:40,000	+
23	>1:256	0	0	0	0	1:400,000	+
24	1:32	0	0	0	0	1:4000	+
25	1:64	0	+	0	0	1:80,000	+

ALK-SS-DNA = alkali-denatured (single strand) DNA; DS-DNA = native (double strand) DNA; ENA = extractable nuclear antigen; ANA = antinuclear antibody; ACA = anticytoplasmic antibody.

* Not treated with steroids.

tightening and thickening of the skin over the hands (which appeared typical of scleroderma grossly, and microscopically in 60% of those whose skin was biopsied); roentgenographic evidence of abnormal esophageal motility identical with that found in scleroderma; muscle tenderness and weakness with abnormal electromyograms, elevated muscle enzymes in the serum, and when performed, muscle biopsies typical of polymyositis; and arthralgias and/or arthritis. In addition, many have had serositis, lymphadenopathy, hepatomegaly, splenomegaly, fever, leukopenia, and anemia. Most of them had hypergammaglobulinemia. A detailed description of these patients will be the subject of another report.

The striking serological finding is that all of these patients had a very high titer (1:1000 to 1:1,000,000) of antibody to ENA throughout their course, during both remission and disease activity. Only five have had positive LE preparations. Over the period of 1-8 yr observation, antibody to DNA has been infrequent and of low

titer. Fluorescent antibody studies performed by Dr. Eng Tan on sera from these patients have shown the speckled pattern of antinuclear fluorescence in every case. In addition, Dr. Tan found that these sera do not contain antibody to the Sm antigen (11).

21 of these patients have been treated with corticosteroids and have been very responsive to this treatment. The hand swelling with skin tightening disappeared or markedly improved, often within a few days of initiation of therapy. This response is in marked contrast with the unresponsiveness of skin in typical progressive systemic sclerosis treated with corticosteroids. The myositis, serositis, arthritis, lymphadenopathy, fever, anemia, leukopenia, and hepatosplenomegaly have also responded to treatment, with the result that most of these patients are in remission or significantly improved. Insufficient studies have been performed to comment on the reversibility of the esophageal changes.

TABLE IV
Incidence of Antibody to DNA and to the Extractable Nuclear Antigen in Control Groups

Group	Total tested	Number positive for antibody to:		
		ALK-SS-DNA	DS-DNA	ENA
Normals				
College students	69	2	1	0
Blood bank donors	29	0	0	0
Congenital cataract family	12	0	0	0
Other	12	0	0	0
Rheumatoid arthritis	20	0	0	0
Scleroderma	10	0	0	0
Other rheumatic diseases	28	0	0	0
Hypergammaglobulinemia	11	0	0	0
Lymphoma	18	0	1	0
Myocardial infarction	16	0	0	0
Toxoplasmosis	20	2	2	1
Tuberculosis	17	0	0	0
Other renal disease	10	0	0	0
Miscellaneous	5	0	0	0
	277	4	4	1
		1.4%	1.4%	0.4%

ALK-SS-DNA = alkali-denatured (single strand) DNA; DS-DNA = native (double strand) DNA; ENA = extractable nuclear antigen.

Incidence of antibody to DNA and to the extractable nuclear antigen (ENA) in control groups. Not only do the DNA and ENA hemagglutination tests have a high sensitivity but they also seem to have a great specificity for SLE and related syndromes. Hemagglutination testing of 277 sera from normal persons and patients with a wide variety of acute diseases other than SLE revealed the presence of antibody to native DNA in only 1.4% and antibody to ENA in only 0.4% (Table IV). It is particularly noteworthy that other patients with classical scleroderma or polymyositis did not have antibody to ENA in contrast with those patients with the above mentioned mixed connective tissue disease who had very high titers of this antibody.

DISCUSSION

In our studies there was a close correlation between activity of SLE and the presence of antibody to native DNA. Before treatment all patients with SLE and active renal disease possessed circulating antibody to double-stranded DNA as detected by the hemagglutination test. These findings are similar to those of Schur and Sandson (24) except that we found a higher incidence of antibody to native DNA in patients with active SLE. This difference is probably due to the greater sensitivity of the hemagglutination assay when compared with the agar gel diffusion and complement fixation assays. The sensitivity appears similar to that of the recently reported

methods for detecting antibody to DNA by ammonium sulfate precipitation of bound radioactive DNA (31-33). As was the case in the report of Koffler, Carr, Agnello, Fiezi, and Kunkel (34), antibody to native DNA occurred almost exclusively in sera of patients with active SLE.

Antibody reacting with alkali-denatured DNA was detected somewhat less frequently than antibody to native DNA in active SLE and was found only rarely in other disease states. In contrast with this specificity, our preliminary hemagglutination evidence agrees with other reports which indicate that antibody reacting with heat-denatured DNA is less specific for SLE, being found in sera of patients with a variety of diseases and of some normal individuals (34-36).

During serial studies of patients with SLE through remissions and exacerbations of their disease, four patients, while well, suddenly developed hemagglutinating antibody to double-stranded DNA in their serum and their serum complement fell. Within 1-3 months they developed recurrent active nephritis. Schur and Sandson also reported patients in whom the reappearance of antibody to native DNA and fall of serum complement were harbingers of disease exacerbation (24). Presumably the reappearance of antibody leads to formation of immune complexes and consequently glomerular disease. Possibly frequent determinations of these immunologic factors

will be useful in predicting potential disease and thus permit earlier application of treatment.

When the hemagglutinating antibody to DNA disappeared from the serum of patients whose disease persisted despite steroid and/or alkylating drug therapy, circulating free DNA was usually found by agar gel diffusion. In our 15 cases, as well as those reported by Tan, Schur, Carr, and Kunkel (6), the finding of free DNA in the serum was almost always associated with disease activity and especially with renal involvement. In our series, as well as others recently reported, there was only a 5-21% incidence of free DNA (6, 24). If circulating DNA is the antigenic stimulus for the production of DNA antibodies, it might be expected to be more frequent in the sera of SLE patients before they develop antibody to DNA. Determination of the true incidence of circulating DNA may be dependent upon the availability of more sensitive detection methods. The fluorometric method of LePecq and Paoletti (37) and the ammonium sulfate method described by Wold, Young, Tan, and Farr (31) may be useful in this regard.

The nature of ENA has not been established. It does not appear to be either DNA or histone and is present in soluble form within the cell nucleus. It is intimately bound to isolated deoxyribonucleohistone (10). Perhaps it is a constituent of chromosomes, or perhaps the association is a consequence of contamination during isolation of the nucleohistone. The finding that sera from patients with the mixed connective tissue disease with antibody to ENA were negative for antibody to the Sm nuclear antigen described by Tan and Kunkel (11), indicates that these soluble nuclear antigens are not identical.

The sera of our patients with a high titer of antibody to ENA have the speckled pattern of antinuclear fluorescence in high titer. Whereas sera of patients with many rheumatic diseases have shown this speckled pattern of antinuclear fluorescence (12), the hemagglutinating antibody to ENA has been found by us almost exclusively in SLE and in the mixed connective tissue disease described. Therefore, antibody to ENA is probably only one of the antibodies which yield a speckled pattern.

There is now substantial evidence that various autoantibodies may possess biological activities which range from damage to protection of tissues. The correlation between active SLE and the presence in the serum of free DNA or antibody to native DNA, and the evidence incriminating soluble DNA-anti-DNA complexes in glomerular damage, are consistent with the former. In contrast, the highest titers of antibody to ENA appear in patients having a mixed connective tissue disease without renal disease. Furthermore, antibody to ENA is found in much greater frequency in those patients with SLE nephritis and antibody to DNA who respond to

treatment than in such patients who do not. These observations can be interpreted as indicating that either ENA or the antibody to ENA exerts a protective effect, perhaps by interfering with the formation or consequences of DNA-anti-DNA complexes. In preliminary experiments ENA itself inhibited *in vitro* precipitation and hemagglutination reactions between DNA and antibody to DNA. The concept that an autoantibody might protect against immunological damage has emerged from a number of other experimental studies including those with allergic encephalomyelitis (21), aspermatogenesis (22), and thyroiditis (23), and from a recent review of the regulatory effect of antibody on the immune response (20).

An alternative interpretation of our results might be that ENA antibody was absent in the serum of treatment failures because antibody had already been taken out of the circulation in the formation of immune complexes which had localized in the kidneys. However, there is some evidence against the hypothesis that ENA-anti-ENA complexes are active at the site of tissue injury. First, one might have expected during serial examinations extending over months or years that antibody to ENA would occasionally have been detected in the serum of these treatment failures; this was not the case. Second, as summarized in Table II, 19 patients who had ENA antibody before therapy went into remission regardless of whether ENA antibody became undetectable or persisted in the circulation. This is in contrast with the situation with antibody to native DNA in which persistence of DNA antibody in the circulation correlated with progressive SLE nephritis while its disappearance usually correlated with remission of the disease. ENA-anti-ENA is a nonprecipitating system, and at the time these patients were being studied, a test for circulating ENA had not been developed.

Finally, the different autoantibody patterns in patients with the mixed connective tissue disease might merely reflect qualitative differences in the underlying immunopathological processes which account for the clinical differences.

The treatment of SLE has been in the main based upon uncontrolled clinical experience. The reversal of renal lesions in some cases by prolonged treatment with high doses of corticosteroids has led to common use of this type of therapy for management of patients with severe disease (1, 38). However, the results are far from ideal. Some patients do not respond, and it has been impossible to predict beforehand who will. Some begin to respond and then worsen. A disturbingly large number succumb to complications of therapy. Until recently, it has not been possible to explain the variations in therapeutic responses. Now, if the hypotheses concerning the role of soluble immune complexes are applicable to SLE

nephritis, it may be possible to perceive how the disease progresses in some cases in spite of, or as a result of, treatment. In the studies of Dixon, Feldman, and Vazquez, experimental animals developed more severe chronic nephritis when their antibody response was modest, and continued antigen injections resulted in soluble immune complexes (14). In a recent study, Germuth, Valdes, Senterfit, and Pollack presented evidence that, because of its inhibitory effect on antibody production, cortisone accelerated the rate of occurrence of glomerulonephritis in chronic serum sickness (39). The cortisone-induced depression in antibody production may have enhanced the formation of circulating, soluble immune complexes in these animals. The patient described in this report, who had rapid progression of her disease resulting in impaired renal function while receiving moderate to high doses of corticosteroids and alkylating agents, may be an example of the possible harmful effects of partial immunosuppression.

The development of the hemagglutination test which can detect both antibody to DNA and ENA has allowed establishment of correlations among the pattern of autoimmune reactivity, the clinical form of rheumatic disease, and responsiveness to treatment that implicate the qualitative nature of the patient's immune response as a conditioning factor in the type of disease. Possibly the particular kind of circulating autoantibody determines the degree of damage or protection of a target organ. Such correlations may ultimately allow classification of rheumatic diseases into more biologically meaningful groups and lead to different methods of therapy. For example, the association of high titers of antibody to ENA with the mixed connective tissue disease syndrome permits identification of a group of patients who appear to respond favorably to corticosteroid treatment. This contrasts with the more classical scleroderma in which such therapy has little chance of success and probably should not be employed (40). Finally, it may now be possible to study the effect of treatment in a more meaningful way because the direct immunological impact of different therapeutic agents upon certain measurable parameters related to disease can be estimated, and the general clinical response of the patient will no longer be the sole means of assessing the value of different drugs.

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