The Occurrence of Single-Stranded DNA in the Serum of Patients with Systemic Lupus Erythematosus and Other Diseases

DAVID KOFFLER, VINCENT AGNELLO, ROBERT WINCHESTER, and HENRY G. KUNKEL

From the Department of Pathology of Mount Sinai School of Medicine, New York 10029, and The Rockefeller University, New York 10021

ABSTRACT Single-stranded DNA (SDNA) occurs in high incidence and in greatest concentration in the sera of patients with systemic lupus erythematosus (SLE), where levels as high as 250 μg/ml were observed. SDNA appears to be an imunogen for anti-SDNA antibodies and forms complexes in vivo of both anti-SDNA-SDNA and anti-NDNA-SDNA types, which apparently play a role in the pathogenesis of the glomerulonephritis found in patients with SLE. SDNA is also found in high incidence but at lower levels in the sera of patients with rheumatoid arthritis. Lesser amounts of SDNA are found in several other diseases in which a low incidence of anti-SDNA antibodies is observed.

INTRODUCTION

Previous studies have indicated that single-stranded DNA (SDNA)[1] is a potential immunogen for antibodies reactive with polynucleotides in various diseases (1, 2). The high incidence of anti-SDNA antibodies, especially in certain diseases with active tissue destruction (1), suggests that SDNA antigen may be frequently released into the circulation. Anti-SDNA antibody has been observed to occur with greatest frequency in patients with systemic lupus erythematosus (SLE). A question of major interest is whether these antibodies play a role in the pathogenesis of tissue lesions by combining with SDNA and forming immune complexes. Studies of glomerular eluates obtained from SLE kidneys indicate that anti-SDNA antibodies are selectively concentrated in the glomeruli of these kidneys (3), and immunofluorescence studies have demonstrated that SDNA antigen is deposited in glomerular lesions of SLE kidneys in association with gamma globulin and complement deposits (4).

The present investigation was undertaken to assess the occurrence of SDNA antigen in sera of patients with SLE and other diseases. The relationship between the appearance of SDNA antigen and antibody during the course of the disease was studied. Direct evidence for the presence of circulating immune complexes was also sought by assaying for the presence of both antigen and antibody in the same serum specimens. The evidence obtained from these studies indicates that serological aspects of the SDNA-anti-SDNA system have similarities to those of the native DNA (NDNA)-anti-NDNA system. Anti-NDNA antibodies which also are found in high incidence in patients with clinically active SLE (1, 2) have been demonstrated to alternate with DNA antigen in serum (5, 6), and most probably participate in the formation of antigen-antibody complexes. Considerable evidence has been accumulated indicating that immune complexes comprised of SDNA antigen and antibody are also formed during the periods when patients with SLE have disease activity or progressive renal lesions.

MATERIALS AND METHODS

Human sera were obtained from patients with the following diseases: SLE (60), rheumatoid arthritis (54), chronic glomerulonephritis (40), leukemia (19), malignant tumors (20), random hospital diseases (60), and normal subjects (56). In addition, sera from serial studies of 18 patients

This work was supported by U. S. Public Health Service Grants AM 13721 and AM 04761.

Received for publication 27 July 1972 and in revised form 5 September 1972.

[1] *Abbreviations used in this paper:* anti-A, rabbit antisera prepared to adenosine; anti-T, rabbit antisera prepared to thymidine bovine serum albumin; NDNA, native DNA; SDNA, single-stranded DNA; SLE, systemic lupus erythematosus.

with SLE and from 10 patients with rheumatoid arthritis were assayed. The serial studies were comprised of 470 sera from SLE patients and 192 sera from rheumatoid arthritis patients.

Polynucleotides employed for immunological tests. Native calf thymus DNA was obtained from Worthington Biochemical Corp., Freehold, N. J.; poly A, from Miles Laboratories, Inc., Kankakee, Ill.; and poly dA, dT, dG, and dC, from Biopolymers, Inc., Chagrin Falls, Ohio. Denatured DNA was prepared by heating native DNA at 100°C for 10 min and transferring directly to an ice bath.

Three sera were used for the routine assay of SDNA in serum: rabbit antisera prepared to adenosine (anti-A) and to thymidine bovine serum albumin (anti-T) (7), and an SLE serum (Esp). In addition, selected sera were tested for SDNA with a rabbit antiserum prepared to SDNA complexed to methylated bovine serum albumin (8). The latter sera showed a sensitivity similar to that of Esp serum for detecting antigen. They showed no reactivity with native DNA when tested by direct hemagglutination or agar gel diffusion. An SLE serum containing antibodies reactive with NDNA (Ann) was also used in the hemagglutination inhibition test for determination of DNA.

The specificities and relative sensitivities of the antisera were determined by a hemagglutination inhibition test using a constant end-point dilution of antiserum and serial dilutions of antigen (Table I). The rabbit anti-A and anti-T sera showed restricted specificity for homologous polynucleotides, whereas the SLE serum showed reactivity with most polynucleotides tested, indicating a considerably greater heterogeneity of antibody directed against determinants of SDNA. The rabbit anti-SDNA serum showed more restricted specificity, reacting with both dG and dC.

Assay of sera for the presence of total DNA or SDNA was carried out by a hemagglutination inhibition test as previously described (2). A constant end-point dilution of an antiserum reactive with SDNA was used with serial dilutions of sera to be tested for SDNA. The maximal inhibition by anti-A, anti-T, or Esp serum of hemagglutination of red blood cells coated with SDNA was compared with the inhibitory activity of a known concentration of SDNA, and the approximate concentration was calculated. The antiserum with the greatest sensitivity (Esp) was capable of detecting 0.4 μg of SDNA in serum. The true incidence of NDNA antigen could not be directly ascertained by hemagglutination inhibition, inasmuch as insufficient amounts of sera with unique specificity for NDNA were available. Human serum (Ann) was therefore utilized to determine "total" DNA, and sera reactive with Ann but inactive with anti-T, anti-A and Esp were considered to have NDNA.

TABLE I

Minimal Concentration of Polynucleotide Antigens Inhibiting End-Point Dilution of Anti-SDNA Antisera

Antiserum	NDNA	SDNA	A	dA	dT	dG	dC
SLE patient Esp	—*	0.4‡	15	7.5	31	—	5
Rabbit anti-A (no. 441)	—	0.9	15	3.8	—	—	—
Rabbit anti-T (no. 440)	—	1.9	—	—	1.9	—	—
Rabbit anti-SDNA (no. 285)	—	0.4	—	—	—	1.2	0.6

* No inhibition.

‡ Concentration of antigen in micrograms per milliliter.

TABLE II

*SDNA Antigen in Human Sera**

	No. of sera	Incidence of SDNA antibody	Incidence of SDNA antigen	SDNA
		% positive	*% positive‡*	*μg/ml*
SLE	60	52	55	23.0§
Rheumatoid arthritis	54	24	49	18.6
Chronic glomerulonephritis	40	5	18	5.9
Leukemia	19	5	39	9.6
Malignant tumors	20	0	15	7.5
Hospital diseases	99	5	20	6.0
Normal human sera	56	4	4	5.6

* Hemagglutination inhibition assay.

‡ Incidence in sera without detectable antibody.

§ Mean concentration.

Agar gel diffusion was utilized as an ancillary test for detection of SDNA in serum (5). Rabbit anti-A and anti-T sera contained precipitating antibodies capable of detecting 5–10 μg of SDNA, although clear precipitin reactions were demonstrable only at higher concentrations. This technique was therefore used to confirm the presence of SDNA demonstrated by hemagglutination inhibition in selected sera and to ascertain the antigenic identity of SDNA prepared in vitro and that detected in human serum.

Enzyme treatment. Deoxyribonuclease and ribonuclease treatment of sera were performed as previously described (2). Diphenylamine determinations for DNA were performed as previously described (5).

RESULTS

Incidence of SDNA antigen in various diseases (Table II). Approximately one-half of SLE and rheumatoid arthritis sera negative for anti-SDNA antibody contained circulating SDNA antigen. A somewhat lower incidence of sera positive for SDNA antigen occurred in groups of diseases in which antibody was not detectable. SDNA appeared infrequently in normal sera. Sera from individual patients with SLE and rheumatoid arthritis manifested the highest levels of SDNA antigen. In contrast, all other groups of sera contained SDNA antigen in the range of 5–10 μg/ml.

DNA antigen in sera from serial studies of SLE patients. Sera from a group of 18 patients with SLE were studied for periods of 6–51 mo for the presence of anti-SDNA antibody and SDNA antigen (Table III). 17 of 18 patients manifested anti-SDNA antibodies during circumscribed periods of time, with a widely varying frequency of occurrence in sera obtained at different stages of disease. SDNA antigen was demonstrated in sera obtained from 13 of 18 patients. In contrast to the moderate levels of SDNA present in randomly selected SLE sera, the serial studies revealed sera from certain patients with extremely high levels of SDNA in the range of 125–250 μg/ml. The mean serum concentration of SDNA antigen

TABLE III

Incidence of SDNA Antigen and Antibody in Serial Studies of SLE Patients

Patient	Duration of study	No. of sera tested	% of sera positive for SDNA antigen	Mean concn of SDNA antigen in sera	% of sera positive for SDNA antibody
	mo			*μg/ml*	
Wol	24	22	0	0	23
Loo	6	11	0	0	46
Esp	25	15	0	0	54
Gam	9	21	0	0	66
Har	22	24	0	0	42
Vra	36	45	13	41.3	62
Bro	31	26	15	17.1	39
Hau	51	55	15	20.1	0
Ros	24	25	20	19.9	32
McK	16	19	21	15.2	37
For	24	24	25	19.1	54
Egl	31	34	28	22.2	21
Ber	16	28	29	18.2	32
Pea	17	24	33	25.9	83
Rus	31	40	48	65.4	32
Sil	13	15	60	111.8	27
Wil	34	22	64	125.4	27
Wei	10	20	90	52.6	20

in the serial studies was 53.0 μg/ml, more than twice that observed in the random SLE sera (Table IV).

Five serial studies are cited below which illustrate the main types of data derived from these studies with respect to SDNA antigen. The majority of patients showed levels of SDNA antigen alternating with anti-SDNA antibody (Fig. 1). One patient (Sil) showed high levels of antigen ranging from 100 to 250 μg/ml. In certain patients, antigen in serum persisted for long periods of time and was associated with the presence of renal disease. Patient Rus manifested a period of antigenemia associated with proteinuria followed by a quiescent period. A second episode of proteinuria was associated with high titers of anti-SDNA and anti-NDNA antibody and no detectable DNA antigen (Fig. 2). In contrast, certain patients demonstrated anti-SDNA antibody with only occasional sera containing detectable SDNA antigen. 7 of 23 sera obtained from a patient with active renal disease (Pea) contained antigen, whereas most sera demonstrated anti-SDNA antibody (Fig. 3). Five of the sera containing SDNA antigen were also shown to have antibody, indicating that imune complexes were present. Another patient (Vra) also demonstrated predominantly antibody activity in multiple sera (Fig. 4). Isolated sera containing antigen preceded or followed peaks of antibody activity. It should also be noted that several peaks of NDNA antigen were observed in the absence of SDNA in sera that had been stored at 4°C for several years.

No unique clinical or serological features were apparent in the patients in whom SDNA was not identified in any sera. Two of the five patients with no demon-

TABLE IV

Summary of Serial Studies

	SLE	Rheumatoid arthritis
Number of patients	18	10
Number of sera	470	192
Number of sera containing SDNA	118	71
Mean concentration of SDNA in positive sera (*μg/ml*)	53.0	13.6

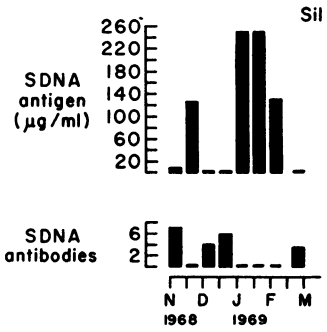

FIGURE 1 Sil. Patient with SLE and progressive renal disease. SDNA antigen and antibody alternate in appearance, with extremely high levels of SDNA present during periods of antigenemia.

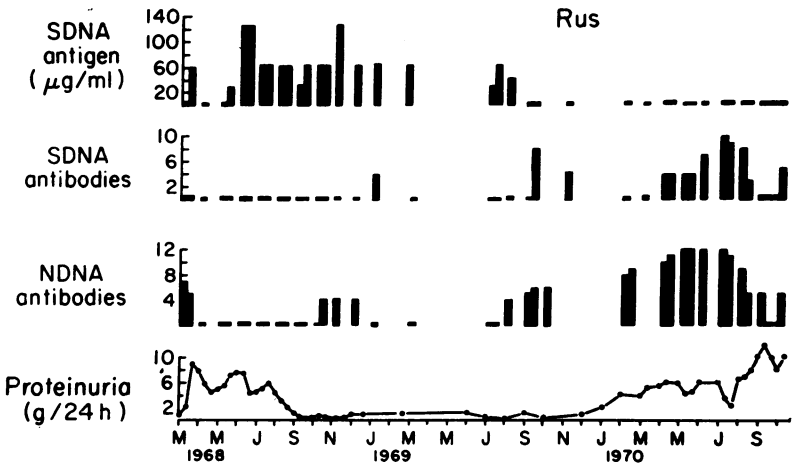

FIGURE 2 Rus. Patient with SLE followed for a period of 2½ yr during which two major episodes of glomerulonephritis occurred. The first exacerbation of renal disease was associated with a brief rise in titer of anti-NDNA antibodies and no demonstrable anti-SDNA antibodies. The second episode of renal disease was associated with high titers of both anti-SDNA and anti-NDNA antibodies. SDNA was detectable in sera during the first episode of renal disease, whereas none was observed during the second episode.

strable SDNA (Loo and Esp) showed multiple sera containing NDNA. Patient Hau, who has been followed for a period of 51 mo with no demonstrable anti-SDNA antibody, had a relatively low level and incidence of SDNA antigen (15% of 55 sera), although this patient had severe renal disease and active SLE.

Serial studies of sera from rheumatoid arthritis patients also showed a widely varying frequency of occurrence of SDNA antigen and antibody. 4 of the 10 studies had only one serum positive for SDNA (Table V). The levels of antigen observed in these were considerably lower than in the SLE serial studies, and the mean concentration of SDNA was 13.6 μg/ml (Table IV).

Agar gel diffusion. Sera containing demonstrable SDNA by hemagglutination inhibition were tested by agar gel diffusion. Sera with higher concentrations of SDNA gave clear precipitin reactions with rabbit anti-A or anti-T sera. The precipitin reactions between the rabbit antisera and the SLE sera showed lines of identity when in vitro prepared SDNA was placed in a well adjacent to the SLE sera. Sera with lower concentrations of SDNA, i.e. less than 15 μg/ml, gave weak precipitin lines with rabbit antisera or were unreactive in agar gel. An SLE serum (Rus) containing 125 μg/ml of SDNA as determined by hemagglutination inhibition, is shown in Fig. 5 *a* to precipitate with rabbit anti-A serum. The rabbit antiserum demonstrates a precipitin reaction with in vitro prepared SDNA. An additional precipitin line between the SLE serum and SDNA is observed. This appears to be due to the presence of excess anti-NDNA antibodies because, as shown in Fig. 5 *b*, absorption with NDNA eliminates the precipitin line. These as well as other studies indicate that this SLE serum contains both SDNA with reactive sites for the rabbit antiserum and anti-NDNA antibodies reactive with NDNA and SDNA.

Enzyme treatments. DNase treatment of serum resulted in a decrease of 50% or greater in hemagglutination inhibitory activity of serum. 12 sera with SDNA inhibitory titers which averaged 5.3 (log base 2) were reduced to inhibitory titers of 2.5 by DNase treatment. A similar effect was observed when NDNA or SDNA was added to a normal serum with no previous inhibitory activity. Ribonuclease incubation did not affect the inhibitory titers of any serum tested.

Diphenylamine determination. Selected sera were assayed for the presence of DNA by this method. Five sera with concentrations ranging from 31 to 250 μg/ml as determined by hemagglutination inhibition were found to

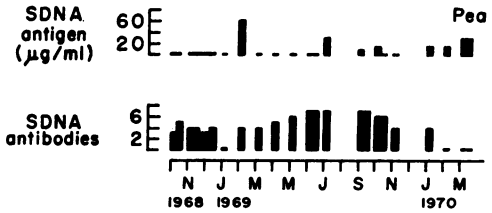

FIGURE 3 Pea. Patient with SLE and an episode of renal disease who manifested clinical activity throughout most of the period of observation. Anti-SDNA antibodies were found in most sera, including five of seven sera in which SDNA antigen was demonstrable.

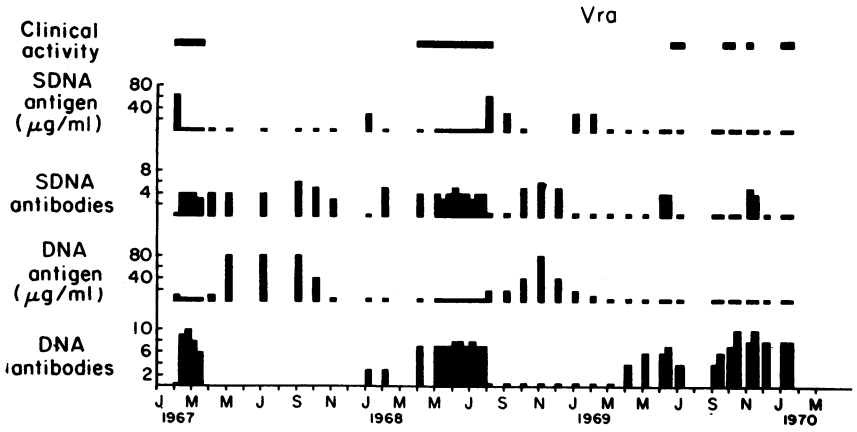

FIGURE 4 Vra. Patient with SLE showing multiple episodes of clinical exacerbation. Anti-SDNA antibody was present throughout most of the clinical course. SDNA antigen was demonstrable intermittently in a few sera, either before or after a peak of anti-SDNA antibody activity. The temporal relationship of SDNA antigen and antibody suggests that antigen-antibody complexes were formed during the first two clinical episodes. Note the occurrence of two periods when NDNA antigen alternates with the presence of anti-NDNA antibody in the absence of SDNA antigen.

have concentrations ranging from 19 to 232 μg/ml by chemical assay.

DISCUSSION

Single-stranded DNA antigen has been found in the sera of patients with several diseases. The highest levels of antigen were found in patients with SLE, especially when sera were assayed throughout the course of the disease in individual patients. Sera obtained from patients with rheumatoid arthritis had moderately elevated levels of SDNA, whereas all other patients had low levels of this antigen. The incidence of antigen detection was highest in SLE and rheumatoid arthritis patients, although a significant number of sera from patients with other diseases contained small amounts of SDNA. These results support the hypothesis that SDNA is immunogenic in patients with SLE and rheumatoid arthritis, in which a high incidence of anti-SDNA antibodies has been observed (1, 2). In contrast, anti-SDNA antibodies occur in low incidence in those groups of patients in which lesser amounts of SDNA were detectable.

The circulating SDNA antigen in SLE appears to have special significance in relation to the pathogenesis of the disease. The presence of antigen alternates with the appearance of anti-SDNA antibody in a fashion similar to that demonstrated for the NDNA-anti-NDNA

TABLE V

Incidence of SDNA Antigen and Antibody in Serial Studies of Rheumatoid Arthritis Patients

Patient	Duration of study	No. of sera tested	% of sera positive for SDNA antigen	Mean concn of SDNA antigen in sera	% of sera positive for SDNA antibody
	mo			*μg/ml*	
Joh	20	31	3	15.0	35
Los	37	37	3	31.0	86
Qui	46	16	6	3.8	12
Kov	12	9	11	15.0	45
Rom	8	17	47	14.3	29
Hil	26	29	48	16.5	0
Mar	18	12	75	12.5	17
Fal	58	17	76	15.1	12
Coo	5	9	78	7.5	22
Gar	30	15	100	11.5	0

DOCKET ALARM

Explore Litigation Insights

Docket Alarm provides insights to develop a more informed litigation strategy and the peace of mind of knowing you're on top of things.

Real-Time Litigation Alerts

Keep your litigation team up-to-date with **real-time alerts** and advanced team management tools built for the enterprise, all while greatly reducing PACER spend.

Our comprehensive service means we can handle Federal, State, and Administrative courts across the country.

Advanced Docket Research

With over 230 million records, Docket Alarm's cloud-native docket research platform finds what other services can't. Coverage includes Federal, State, plus PTAB, TTAB, ITC and NLRB decisions, all in one place.

Identify arguments that have been successful in the past with full text, pinpoint searching. Link to case law cited within any court document via Fastcase.

Analytics At Your Fingertips

Learn what happened the last time a particular judge, opposing counsel or company faced cases similar to yours.

Advanced out-of-the-box PTAB and TTAB analytics are always at your fingertips.

API

Docket Alarm offers a powerful API (application programming interface) to developers that want to integrate case filings into their apps.

LAW FIRMS
Build custom dashboards for your attorneys and clients with live data direct from the court.

Automate many repetitive legal tasks like conflict checks, document management, and marketing.

FINANCIAL INSTITUTIONS
Litigation and bankruptcy checks for companies and debtors.

E-DISCOVERY AND LEGAL VENDORS
Sync your system to PACER to automate legal marketing.