Relationship between Complement-Fixing (Hemolytic) Antibodies to Single-Stranded and Double-Stranded DNA and the Prognosis in Systemic Lupus Erythematosus

TAKESHI SASAKI, TAKEFUMI KADONO, FUMIO ENDO, YUKIO SEKIGUCHI, TAKAO SAITO, MASASHIRO ARAKAWA, TAKASHI FURUYAMA and KAORU YOSHINAGA

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SASAKI, T., KADONO, T., ENDO, F., SEKIGUCHI, Y., SAITO, T., ARAKAWA, M., FURUYAMA, T. and YOSHINAGA, K. Relationship between Complement-Fixing (Hemolytic) Antibodies to Single-Stranded and Double-Stranded DNA and the Prognosis in Systemic Lupus Erythematosus. Tohoku J. exp. Med., 1982, 138 (4), 341-355 — Hemolytic (complement-fixing) antibodies to single-stranded (ss) or double-stranded (ds) DNA, measured by recently developed PHL assay, occurred closely correlated with renal activity in patients with systemic lupus erythematosus (SLE). Approximately one third of the patients with renal disease had hemolytic antibodies to ss-DNA but never to ds-DNA. Hemolytic antibodies were scarcely detectable in patients with mild course. Serial studies also revealed that the estimation of the hemolytic antibodies to ds- and/or ss-DNA was particularly valuable in predicting the future course of the SLE. The emergence of hemolytic antibodies to DNA may be an ominous sign suggestive of grave prognosis in SLE.

SLE; hemolytic antibodies to DNA

The patients with systemic lupus erythematosus (SLE) show a variety of clinical manifestations and varying prognosis. In this respect, it has been tried to divide them into some subsets on the basis of their main clinical features, and renal, neurological or other visceral lesions (Feinglass et al. 1976; Miniter et al. 1979). Most of the patients with renal or neurological signs have a progressive course and, therefore, need to be vigorously treated, whereas others may have a relatively benign course and can be treated less intensively. Histological findings from renal biopsy also indicate the disease severity (Pollak et al. 1972; Baldwin et al. 1977; Decker et al. 1979); patients with diffuse proliferative glomerulonephritis have a worse course than those with focal proliferative changes. Therefore, the renal biopsy is an important procedure to decide the treatment. However, it is difficult to predict the future course of the disease at an early stage, especially before the onset of the renal or cerebral symptoms. Since early diagnosis or treatment improves the result of the disease, it is of therapeutic importance to
investigate which parameters, either clinical or laboratory, are specifically related to the future course of SLE.

Anti-double-stranded (ds)-DNA antibody is well known to correlate with the disease activity (Casals et al. 1964; Tan et al. 1966; Koffler et al. 1971; Bardana et al. 1975; Pennebaker et al. 1977). Nevertheless, it has not yet been demonstrated whether or not this parameter will give any information about the severity or prognosis of SLE. A number of reports agree on the close relation between the complement-fixing antibody to ds-DNA and the renal involvement, indicating the clinical importance of the complement-fixing ability of the antibody in the therapy of SLE (Tan et al. 1966; Tojo and Friou 1968; Schur and Sandon 1968; Sontheimer and Gilliam 1978). But, the usefulness of conventional complement fixation tests has been limited because of the presence of anticomplement activity in SLE sera. We have recently developed a simple and sensitive assay for complement-fixing (hemolytic) antibody to single-stranded (ss) and ds-DNA. The anticomplement activity was negligible in this system (Sasaki et al. 1978; Sasaki 1981). The purpose of this paper is to report the prognostic significance of the presence of the hemolytic antibodies to ss- and/or ds-DNA in patients with SLE.

**Patients and Methods**

**Patients**

One hundred and eleven patients with active SLE who fulfilled the ARA preliminary criteria for the diagnosis of SLE (Cohen et al. 1971) were divided into three groups as follows:

*Renal group (Group A).* Patients with abnormal urinalysis (persistent proteinuria, hematuria and cellular casts), and a reduced total hemolytic complement level. Renal biopsy was performed in 41 of 53 cases and showed active lupus nephritis (Pollak et al. 1972; Baldwin et al. 1977; Decker et al. 1979).

*CNS group (Group B)* (Kasscn and Lockshin 1979). Patients with disturbances of the central nervous system (CNS), i.e. episodes of seizure, unconsciousness, stroke or psychosis unrelated to corticosteroid. Included in this group are four patients with both CNS and renal lesions.

*Other group (Group C).* Patients with fever, an elevated erythrocyte sedimentation rate and typical multisystem involvement but normal urinalysis and no CNS sign.

**Sera**

All sera were inactivated before use by heating at 56°C for 30 min and then incubated with an equal volume of 20% washed sheep red blood cells (SRBC) at 37°C for 1 hr, followed by an incubation at 4°C overnight for anti-DNA antibody assays. In some studies, samples were further subdivided according to the presence of ss- or ds-DNA antibodies with hemolytic and agglutinating ability and the antibody profile was studied in relation to their prognosis or clinical picture.

**Anti-DNA antibody assay**

The levels of agglutinating and hemolytic (complement-fixing) antibodies to ds- and ss-DNA were determined by passive hemagglutination (PHA) and passive hemolysis (PHL) assays using DNA coated SRBC, which have been described in detail in other papers (Sasaki et al. 1978; Sasaki 1981). Briefly, ss-DNA was prepared by sonication and heat-denaturation of native calf thymus DNA. ds-DNA was obtained by sonication and
removing ss-regions in its structures using nuclease S1 (Shishido and Ando 1972). By use of chromium-chloride (CrCl₂•6H₂O) ss- or ds-DNA was coupled with SRBC as follows: One ml of ss-DNA (500 µg/ml) or ds-DNA (200 µg/ml) was mixed with 0.8 ml of 0.9 or 1.2% saline and 0.1 ml of CrCl₂•6H₂O (20 mg/ml). To this mixture was added 0.2 ml of a 50% SRBC. After 5 min, the suspensions were washed several times in PBS. PHA and PHL were carried out in microtitration plates as previously reported. The titer of anti-DNA antibody was expressed as the end point dilution of sera which gave a hemolytic or settling pattern in the well.

**Complement assays**

Total serum hemolytic complement activity was measured by the methods of Nelson (Nelson et al. 1966; Ellis and Clancy 1978). Namely, 25 µl of SRBC (1 X 10⁸/ml), sensitized with hemolysin, was incubated with two-fold diluted sera at 37°C for 60 min with shaking. After centrifugation for 10 min, the hemolytic pattern was read in each well. The end point (50% lysis) was recorded as a well number. The normal range of titer was 6–7 wells.

**Renal findings**

Renal histology was classified according to the criteria of Pollak et al. (1972), Baldwin et al. (1977) or Decker et al. (1979) to one of the five major groups: mesangial proliferative (mild), focal proliferative, diffuse proliferative (DPGN), membranoproliferative (MPGN) and membranous glomerulonephritis. A portion of each biopsy specimen was examined regarding the immunofluorescence for IgG, IgM, IgA or C₃.

**RESULTS**

**Anti-DNA antibody and disease activity**

One hundred and eleven patients with active SLE were studied in this series. The sera which were obtained on their first visit were classified into three groups depending on their clinical features as mentioned above.

The titers obtained in PHA tests were compared with those in PHL tests. Fig. 1 summarizes the data. Approximately two thirds of sera from the patients with active renal disease (Group A) contained both agglutinating and hemolytic antibodies to ss- and ds-DNA. The remainder in this group showed positive test for ss-determinant but negative for ds-DNA. All of the sera from Group A showed also a low level of total hemolytic complement activity. Of particular interest was that hemolytic and agglutinating antibodies to ss-DNA were detected in 7 of 8 sera from the patients with disturbances of CNS (Group B). All sera from Group C were positive for agglutinating antibodies to ds- and/or ss-DNA but scarcely for the hemolytic antibodies.

For a more detailed assessment of the clinical significance of the antibodies, these sera from the active SLE were divided into six subsets (Groups I–VI) on the basis of the presence or the absence of the antibodies determined by PHA and PHL tests, and the results were analyzed in relation to clinical signs, renal histological findings and total complement activity (Table 1). Most of the patients with both hemolytic and agglutinating antibodies to ds- and ss-DNA had renal or CNS disease. All of the sera showed low complement levels and renal biopsy demonstrated severe glomerular lesions such as DPGN or MPGN. The immunofluorescence study also revealed various kinds of deposition of IgG, IgM, IgA or C₃ in their glomeruli (data not shown).
The clinical and laboratory findings on the patients in Groups II and III, whose sera contained hemolytic antibodies to ss-DNA but never to ds-DNA, were analogous to those in Group I (Table 1). The incidence of active renal or CNS diseases in these groups increased significantly, as compared with that in others except Group IV \((p<0.005)\). There was no significant relationship between the mean titers of hemolytic antibodies to ss- or ds-DNA and histological findings of the renal specimen (data not shown).

In contrast to the flagrant symptoms in Group I, II or III, the clinical pictures in Groups IV and V, whose sera did not contain hemolytic antibodies to DNA in spite of the presence of agglutinating antibodies, were completely different; all of the patients in the latter showed mainly skin rash, Raynaud’s phenomenon, polyarthralgia or fever but no episodes of renal or CNS disease. The total hemolytic complement level was low only in one third of sera in these groups. It was notable that there were three patients with no anti-DNA antibody, who showed nephrotic syndrome or stroke (Group VI).

Serial study

The data described above show that the occurrence of hemolytic antibodies to DNA is closely related to the clinical picture, especially to renal or CNS involvement of SLE. In an attempt to know the predicting value of the antibodies, we investigated the relationship between the antibody profile and clinical course or prognosis by the follow-up observations. The serial studies were performed in all cases presented above. The mean observation period was 4.5 years (0.5–7 years).

Coincided with the clinical improvement, the levels of the antibodies dropped sharply in most of sera; they also decreased with the rise of total complement activity and the decrease in proteinuria. Finally the antibodies became undetectable in remission. In a small number of patients, however, agglutinating antibodies to ds- and/or ss-DNA persistently remained at high levels despite the clinical improvement. Thirty-one of the 111 patients were observed to relapse during the follow-up. It

### Table 1. The relationship between the antibody

<table>
<thead>
<tr>
<th>Group</th>
<th>ss-DNA Ab</th>
<th>ds-DNA Ab</th>
<th>Total cases</th>
<th>Renal cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>HA*</td>
<td>HL</td>
<td>37</td>
<td>29</td>
</tr>
<tr>
<td>II</td>
<td>+</td>
<td>+</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>III</td>
<td>+</td>
<td>+</td>
<td>20</td>
<td>11</td>
</tr>
<tr>
<td>IV</td>
<td>+</td>
<td>–</td>
<td>25</td>
<td>0</td>
</tr>
<tr>
<td>V</td>
<td>+</td>
<td>–</td>
<td>24</td>
<td>0</td>
</tr>
<tr>
<td>VI</td>
<td>–</td>
<td>–</td>
<td>4</td>
<td>3</td>
</tr>
</tbody>
</table>

* HA, agglutinating antibody; HL, hemolytic antibody.
† Mild, mesangial proliferative glomerulonephritis; FPGN, focal proliferative glomerulonephritis; DPGN, diffuse proliferative glomerulonephritis; MPGN, membranoproliferative glomerulonephritis; memb, membranous glomerulonephritis.
Hemolytic Antibody to DNA in Lupus Erythematosus

*profile and clinical manifestation on the first visit*

<table>
<thead>
<tr>
<th>Clinical type</th>
<th>Renal histology†</th>
<th>$C_{1-s}$ ‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>CNS Others</td>
<td>Mild FPGN DPGN MPGN Memb</td>
<td>4§ 8 3 0 7 13 0 3.6</td>
</tr>
<tr>
<td>0 1 0 0 2 6 1 3.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 25 0 0 2 6 1 5.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 24 0 0 2 6 1 6.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 0 1 0 0 2 0 4.0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

‡ Average titer of $C_{1-s}$ activity (normal range 6–7 well).
§ All of the patients showed CNS and renal signs.

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**Fig. 1. Relationship between anti-DNA antibody profile and clinical type of SLE.**

- A: Renal group.
- B: CNS group.
- C: Other group. ○, PHA; ●, PHL.

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**ss-DNA antibody**

**ds-DNA antibody**

**Antibody titer**

**$C_{1-s}$ activity**

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**$C_{1-s}$ activity**

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Fig. 2. Case 1 (M.A. 25 years old).

Fig. 3. Case 2 (K.O. 17 years old).
was noted that the hemolytic antibodies to DNA, accompanied by a decrease in total hemolytic complement activity, occurred preceding the relapse of renal manifestations in 8 of 10 patients as illustrated in Cases 1, 2 and 3 (Figs. 2 and 3). It has been known that the early stage of SLE goes not infrequently with no renal involvement. We could follow the longitudinal courses of such cases whose initial manifestation was one or two of the following symptoms: general malaise, fever, arthralgia, and Raynaud’s phenomenon or skin rash. Eight of the 10 patients whose sera showed positive test for hemolytic antibodies to ss- and/or ds-DNA at an early stage were observed to develop active renal or CNS disease later. The remainder showed general cutaneous angitis, skin rash and serositis 6 months later. On the other hand, of 6 patients with agglutinating but nonhemolytic antibodies to ds-DNA, four became subsequently worse. Their main clinical findings were butterfly erythema, joint involvement, oral ulcer and Raynaud’s phenomenon but no renal or CNS involvement. Some of the representative patients are described below.

Case 1. M.A. (Fig. 2). A 25-year-old woman was first hospitalized in July 1978 because of facial erythema and fever. Six months earlier she complained of transient arthralgia in fingers. Moderate anemia and leukopenia were revealed. At this time urinary protein was transiently positive. Anti-DNA antibody was complement-fixing and disappeared with her clinical improvement. The patient remained clinically well until 24 months later, when she developed massive proteinuria necessitating hospitalization. Preceding this exacerbation, hemolytic antibodies to ss-DNA had been detectable in her serum. Renal biopsy revealed MPGN.

Case 2. K.O. (Fig. 3). A 17-year-old female student complained of alopecia in December 1978. Physical examination revealed stomatitis and erythema of the hand. At that time, urinalysis was normal in spite of the presence of hemolytic antibodies to DNA and low complement activity in her serum. Six months later she developed renal disease and thrombophlebitis in the left lower extremity. Renal biopsy showed DPGN and multiple deposition of Ig and C3 in the glomeruli.

Case 3. T.M. A 20-year-old woman experienced transient polyarthralgia without swelling of her joints in October 1977. Two months later, butterfly erythema developed with slight fever. At that time urinalysis disclosed numerous red blood cells under high power microscope and urinary protein was slightly positive. Renal biopsy revealed minimal change in the glomeruli (Fig. 4A). Hemolytic antibodies to ss- and ds-DNA were positive. Above mentioned findings were improved by prednisolone 40 mg per day. However, 10 months later the hemolytic antibodies to ss- and ds-DNA became again detectable in her sera, followed by massive proteinuria and psychosis. Combination therapy of prednisolone and cyclophosphamide improved her state but induced severe pancytopenia and peptic ulcer which caused her death (Fig. 4B).

Case 4. M.T. (Fig. 5). A 48-year-old woman visited our hospital in May 1974 because of weight loss and general malaise. She had no other signs, but an elevated red cell sedimentation rate, moderate anemia, and hypergammaglobulinemia
Fig. 4. A: Renal biopsy specimen from Case 3. In the glomeruli only mild mesangial proliferation is seen. PAS stain, × 70.
B: Necropsy specimen of the kidney from Case 3 at three years after above renal biopsy. In the glomeruli conspicuous proliferative changes result in the obstruction of the capillary lumen. Tubular changes and interstitial proliferation are also remarkable. Azan-Mallory stain, × 70.
were noted. Serological examination revealed positive ANA, LE cells and anti-DNA antibodies. In August 1974 she had an episode of fever and swelling of the knee joints. Prednisolone therapy, 30 mg per day, was begun. However, in February 1975 facial erythema and mental disturbance appeared with fever. Finally she died of cerebrovascular accident in October 1975. Urinary protein was continuously negative.

Case 5. M.B. A 19-year-old woman complained of arthralgia in fingers and slight fever. Physical examinations gave normal results except for bilateral lymphadenopathy in her neck. The initial white cell count was 4,500/mm³. Direct Coomb's test and LE cells were positive. Hemolytic antibodies to ss- and ds-DNA were also positive. Urine showed no abnormal findings. Prednisolone therapy made her clinically well. However, a fatal cerebral stroke attacked her 9 months later.

Table 2 summarizes the relationship between the antibody profile in sera obtained at their first visit and the clinical course. Compared with the results in Table 1, it is more clearly demonstrated that the presence of the hemolytic antibodies to DNA is associated with the occurrence of the renal or CNS episodes. It should be noted that 10 of 14 patients from Groups I-III, who had shown relatively mild signs at the first visit (Table 1) developed active renal or CNS manifestations during the follow-up period, whereas only one of 49 patients from Groups IV and V showed massive proteinuria in their course.

Another approach concerning the prognosis is a survival study. During the follow-up periods up to 7 years, there were 10 deaths in our patients population.

![Fig. 5. Case 4 (M.T. 48 years old).](image-url)
Table 2. The relationship between the antibody profile and clinical manifestation during the follow-up study

<table>
<thead>
<tr>
<th>Group</th>
<th>ss-DNA Ab</th>
<th>ds-DNA Ab</th>
<th>Total cases</th>
<th>Clinical course</th>
<th>Death during the follow-up study</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HA*</td>
<td>HL</td>
<td>HA</td>
<td>HL</td>
<td>Renal</td>
</tr>
<tr>
<td>I</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>37</td>
</tr>
<tr>
<td>II</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td>III</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>20</td>
</tr>
<tr>
<td>IV</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>25</td>
</tr>
<tr>
<td>V</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>24</td>
</tr>
<tr>
<td>VI</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>4</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td></td>
<td>111</td>
<td>53</td>
</tr>
</tbody>
</table>

* HA, agglutinating antibody; HL, hemolytic antibody.  
† Seven of ten patients showed CNS and renal signs.  
‡ One of four patients showed CNS and renal signs.

Table 3. Cause of death and antibody profile

<table>
<thead>
<tr>
<th>Case</th>
<th>ss-DNA Ab</th>
<th>ds-DNA Ab</th>
<th>Renal disease</th>
<th>CNS</th>
<th>Duration from 1st visit to death</th>
<th>Main cause of death</th>
</tr>
</thead>
<tbody>
<tr>
<td>T.M.</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>4 yr. 2 mon. Esophageal perforation</td>
<td></td>
</tr>
<tr>
<td>M.S.</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>6 mon. Uremia</td>
<td></td>
</tr>
<tr>
<td>S.C.</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>11 yr. 5 mon. Uremia</td>
<td></td>
</tr>
<tr>
<td>Y.T.</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>3 yr.</td>
<td>Uremia</td>
</tr>
<tr>
<td>K.A.</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>6 yr. 6 mon. Hyperosmolar nonketotic coma</td>
<td></td>
</tr>
<tr>
<td>M.T.</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>1 yr. 4 mon. Stroke</td>
<td></td>
</tr>
<tr>
<td>M.A.</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>8 mon. Stroke</td>
<td></td>
</tr>
<tr>
<td>Y.S.</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>9 yr.</td>
<td>Infection</td>
</tr>
<tr>
<td>M.M.</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>10 yr.</td>
<td>Unconsciousness due to CNS</td>
</tr>
<tr>
<td>K.O.</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>14 yr.</td>
<td>Stroke</td>
</tr>
</tbody>
</table>

(Table 2). Causes of death are shown in Table 3. There were markedly significant differences in percent survival between the groups with and without hemolytic antibodies to DNA. Patients with fatal outcome had shown positive test for hemolytic antibodies to ss- and/or ds-DNA. The fatality rates were 19% and 21% in Groups I and III, respectively. Whereas, none of the patients in Group IV or V had died during the study. Long-term survival studies for Group II or VI are presumably unreliable because of such a small number of patients.

Discussion

It is of particular importance to know which parameter, either clinical or laboratory, would indicate the future progression of SLE. In the light of current
Hypothosis for the pathogenesis of lupus nephritis, DNA-anti-DNA complexes might reflect the disease activity of SLE. The facts that support the importance of immune complex in the pathogenesis are the demonstration of DNA or anti-DNA antibody in the glomeruli (Harbeck et al. 1973) and the detection of DNA-anti-DNA complexes in their sera (Davis et al. 1978). However, DNA-anti-DNA was demonstrated to occur only in a small number of SLE sera in spite of the high incidence of total immune complex, suggesting that the presence of a specific immune complex might not be used as an indicator of disease activity (Izui et al. 1977). Although serial measurements of anti-DNA antibody levels were useful in assessing the state of the disease (Tan et al. 1966; Koffler et al. 1971; Hughes et al. 1971), precipitating antibodies to ds-DNA did not reflect the severity of the renal lesions (Tron and Bah 1977; Hills et al. 1978; Appel et al. 1978). It has been proposed that a qualitative analysis of anti-DNA antibodies, such as antibody class (Koffler et al. 1967; Talal et al. 1976; Winfield et al. 1977), avidity (Pennebaker et al. 1977) or complement-fixing ability (Koffler et al. 1967; Aarden et al. 1975) is important in evaluating the clinical significance of the antibodies. IgG antibodies to ds-DNA were predominantly detectable in SLE with active renal disease, whereas IgM antibodies are said to be found in relatively mild cases (Koffler et al. 1967; Talal et al. 1976; Winfield et al. 1977) although this opinion is challenged by Clough and Valenzuela (1980). Many lines of evidence have been accumulated for the close relation between the presence of complement-fixing antibody to DNA, accompanied by the low total hemolytic complement activity, and the renal involvement, suggesting that the detection of complement-fixing activity of ds-DNA antibodies would be important as a guide to therapy (Tann et al. 1966; Tojo and Friou 1968; Schur and Sandon 1968; Sontheimer and Gilliam 1978). It should be noted, however, that the studies on the clinical significance of the antibody have been applied only to the antibodies to ds-DNA, and never to ss-DNA. In addition, the usefulness of conventional complement-fixing tests employed in these studies has been clinically limited because DNA, especially ss-DNA, was anticomplementary and some of SLE sera contained anticomplementary substances making the judgment of the reaction rather difficult. Such disadvantage was overcome by recently developed PHL assay (Sasaki et al. 1978; Sasaki 1981). PHL is a complement-mediated hemolysis test using ss- or ds-DNA-coated SRBC and anticomplement activity of SLE sera is negligible in this test probably because of the use of standard complement with high activity. Therefore, hemolytic (complement-fixing) antibodies to ss- or ds-DNA can be easily detected by use of this method.

The occurrence of ds-DNA antibody is said to be specific for SLE, but many studies (Friou 1967; Schur and Sandon 1968; Koffler et al. 1969; Miniter et al. 1979) agreed with the wide distribution of anti-ss-DNA antibody, determined mainly by RI assays. In addition, the presence of antibody to ss-DNA was suggested to correlate with the absence of renal involvement (Mulli and Cruchaud 1977). Subsequently, the antibody to ss-DNA has been thought to be less important in the diagnosis and treatment of the SLE.
Contrary to these observations, the present study indicates the clinical significance of hemolytic (complement-fixing) antibodies to ss-determinants as well as those to ds-DNA. This was shown by the following findings: 1) The hemolytic antibodies to ss-DNA were specifically detected in SLE sera as those to ds-DNA (Sasaki et al. 1978; Sasaki 1981). All of the patients with hemolytic antibodies to ss-DNA who had not fulfilled ARA criteria at one time became clinically ill and developed typical clinical manifestations of SLE in the later course (Cases 1, 2, 3, 4 and 5). 2) A remarkably high incidence of hemolytic antibodies to ss-DNA was found in patients with active renal or CNS disease. One third of patients with active renal disease had only ss-DNA antibodies with hemolytic ability but no ds-DNA antibodies. 3) Serial studies also confirmed the association of the antibodies with depressed C1-9 activity and with clinical severity in renal or CNS involvement. 4) The antibodies to ss-DNA have been shown to be specific for ss-determinants by the inhibition study using pure ds-DNA (Sasaki et al. 1978).

The discrepancy between the previous studies and ours may be mainly due to inherent difference in the antibody assay. Most of the estimations have been performed by radioimmunoassay, PHA or counterimmunoelectrosyneresis which can detect precipitating or agglutinating antibodies. A few reports (Schur and Sandon 1968; Miniter et al. 1979) suggested that the complement-fixing antibodies to ss-DNA were not specifically detected in SLE sera. However, they do not specify the precise data or serial studies. The conventional complement fixation test might be difficult to perform for estimation of anti-ss-DNA antibody because ss-DNA is more anticomplementary than ds-DNA (Tsutsui and Suzuki 1977). The close relationship between the anti-ss-DNA antibodies with complement-fixing ability and renal involvement may be reasonable because the antibodies eluted from the SLE kidney are complement-fixing, and the deposits in the glomeruli are predominantly antibodies to ss-DNA (Koffler et al. 1974), indicating the pathogenetic importance of the antibody to ss-DNA.

The incidence of hemolytic antibodies to ds-DNA was detected in approximately two thirds of the patients with renal lesions. This observation does not appear to be based on the lower sensitivity of this assay than that of radioimmunoassay (Miniter et al. 1979). The findings about the close relationship between the hemolytic antibodies to ds- and/or ss-DNA and renal involvement was quite consistent and statistically significant. The patients with hemolytic antibodies (Groups I, II, III) had a variety of renal lesions in terms of the histological and immunofluorescent findings. The incidence of DPGN or MPGN was significantly higher in both groups as compared with that in other categories. Furthermore, immunofluorescent studies of the biopsy specimen showed preferential deposition of IgG or other factors in their glomeruli. These results indicate the close correlation between the presence of hemolytic antibodies to ds- and/or ss-DNA and severity or activity of renal disease, although such antibodies were undetectable in 5% of patients with active renal disease (Group VI). An increased frequency of hemolytic antibodies to ss-DNA was also found in the patients with CNS
involvement. It should be kept in mind that 7 of 10 patients with CNS lesion in Group I had also renal lesions. Since anti-ds-DNA antibodies were infrequently detected in the CNS patients (Miniter et al. 1979), the hemolytic antibodies to ds-DNA in their sera might be associated with renal changes. In contrast to the close relationship between the hemolytic antibodies and the severity of the disease, the presence of agglutinating anti-DNA antibody without hemolytic ability occurred in mild cases of the disease (Table 2). Only one of the patients with nonhemolytic antibody to DNA had active renal involvement.

It is emphasized that the estimation of the hemolytic antibody to DNA is particularly valuable in evaluating the renal involvement and predicting the future course of the disease. Serial studies demonstrated that some patients with hemolytic antibodies, who had received a relatively mild therapy because of slight changes in the renal biopsy, developed nephrotic syndrome with renal involvement of DPGN or MPGN in later days (Case 3, Table 1). The hemolytic antibodies were also detected preceding the relapse of the renal disease in 8 of 10 patients. Of special interest was that most of the patients who had showed positive test for hemolytic antibody to DNA at an early stage of the disease developed active renal or CNS disease later (Cases 1, 2, 3, 4 and 5), which has been known to be associated with a severe prognosis (Susaki et al. 1978). Indeed, all deaths in our series were found in these groups with hemolytic antibodies to DNA. These indicate that the presence of the hemolytic antibodies to ss- and/or ds-DNA may serve as a warning sign predicting renal or CNS involvement. Whereas, that of agglutinating anti-DNA antibodies without hemolytic ability indicates a relatively benign nonprogressive course in SLE.

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References


