Differentiation of Specific DNA Binding Activity of SLE Sera from Non-Specific Binding by an Addition of Dextran Sulfate and Calcium Chloride to the Farr Assay System

Makoto Kashimura,* Akira Wakizaka,† Kazue Kurosaka,† Eiji Okuhara,† Tetsuo Akihama, Akira B. Miura and Akira Shibata*

The Third Department of Medicine and †Department of Biochemistry, Akita University School of Medicine, Akita 010


A new method for differentiation of specific DNA-binding by human sera from non-specific binding was evaluated with sera from patients with systemic lupus erythematosus in different stages of the disease. An addition of dextran sulfate or calcium chloride to Farr's radioimmunoassay mixture reduced non-specific binding of thermally denatured [3H]DNA of the patient sera without much effect on the specific binding. The measurement of the DNA-binding value by the sera in these addition systems provides accurate information with regard to the pathological state of the disease.—SLE; DNA-binding; anti-DNA antibody; RIA

Sera from healthy individuals occasionally give a high DNA binding value in Farr assay when highly polymerized thermally denatured radioactive DNA is used as the antigen. Several papers have reported the binding of DNA by normal sera in Farr assay (Hasselbacher and LeRoy 1974; Aarden et al. 1976; Izui et al. 1976; Wakizaka and Okuhara 1979). Estimation of the titer of anti-DNA antibodies in serum is essential for the clinical management of patients with SLE. Farr's radioimmunoassay is much more sensitive than the immunofluorescence technique, but above mentioned non-specific DNA binding activity of the serum sometimes masks a subtle increase of the titer for anti-DNA antibodies in the serum. Hence, the elimination of the effect of non-specific DNA binding from the value obtained by Farr assay appears to be a necessary procedure for measurement of actual DNA-antibody binding in the patient sera.

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* Present address: 1st Department of Medicine, Niigata University School of Medicine, Niigata 951.

Abbreviations: SLE, systemic lupus erythematosus; ESR, erythrocyte sedimentation rate; ANF, anti-nuclear factor; C₃ and C₄, the third and the fourth component of complement.
Wakizaka and Okuhara (1979) found that the non-specific DNA binding by normal serum can be reduced by an addition of dextran sulfate or calcium chloride to the assay mixture. Using this method, we determined the range of DNA binding value in sera from healthy individuals in different ages and sexes in the preceding paper (Wakizaka et al. 1981). In the present work, we measured the DNA binding activities of SLE sera in different stages of the disease by this modified Farr assay and found that this method is useful in differentiating the specific DNA binding by the sera from the non-specific one.

**Materials and Methods**

*Patients with SLE*

Fifty-one serum specimens from 10 patients with SLE were employed in this study. All patients manifested symptoms, which fulfilled four or more of the 14 criteria for SLE of American Rheumatism Association. Six patients were under our care in the 3rd Department of Medicine of Akita University Hospital, and the rest were under the care of Dr. S. Miura in Akita Red Cross Hospital.

Venous blood drawn from patients before breakfast was allowed to clot at room temperature for 2 hr. Serum was separated after centrifugation and stored below -25°C until use without any other treatment for immobilization of complement or elimination of cold hemagglutinin.

**Assay of DNA binding activity**

DNA binding activities by patient sera were measured by radioimmunoassay according to Steinberg's modification (1969) of Farr assay (Wold et al. 1978). Assay medium contained 1 µg of thermally denatured mouse embryo [3H]DNA (5,000–15,000 dpm), and 50 µl of a 10-fold diluted patient serum in 0.2 ml of 0.1 M borate buffer (pH 8.5). Precise procedures were described in the previous papers (Wakizaka and Okuhara 1979; Wakizaka et al. 1981). This assay system was designated as the non-addition system. In another system, 2.5 µg of dextran sulfate (Nakarai Chemicals, Kyoto, Japan) or 0.1 M calcium chloride was added to the assay mixture. The former was tentatively called the dextran sulfate system and the latter the calcium chloride system. All three systems were assayed simultaneously in duplicate for each serum. The normal range of the binding values obtained from healthy human sera, reported in our preceding paper (Wakizaka et al. 1981), was used as a standard for comparing the values obtained with patient sera in these three assay systems.

**Miscellaneous methods**

Contents of complement components C₃ and C₄ in sera were determined by immunodiffusion assay (Mancini et al. 1965) using a kit, M Partigen, from Behring, West Germany. Urinary protein was determined by the method of Kingsbury and Clark (1926). Antinuclear factor was measured by immunofluorescent technique (Friou 1958) using frozen rat liver section (6 µm) and the sera showing ANF at 10-fold dilution were taken as positive reaction. LE cell phenomenon was determined according to Mathis (1951). CRP was measured by precipitation method using a kit from Eiken Chemical Co. (Tokyo, Japan). All other chemicals used were of analytical grade or the best available.

**Results**

**DNA binding activity of SLE sera**

Fifty-one serum specimens from patients with SLE in different stages of the disease were tested for their DNA binding activities in the three Farr assay
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systems, non-addition, dextran sulfate addition and calcium chloride addition systems, using thermally denatured mouse embryo [³H] DNA. Only 14 specimens (27.5%) of these sera showed a DNA binding value exceeding the normal upper limit (mean + 2 s.d.) of the binding by healthy human sera in non-addition system.

The addition of dextran sulfate or calcium chloride to the assay medium resulted in change of the binding value as shown in Fig. 1. In the dextran sulfate system, 30 specimens (58.8%) of total 51 exhibited the binding value exceeding the mean + 2 s.d. limit of normal range. In calcium chloride system, 27 specimens (57.4%) of 47 exceeded the normal upper limit in DNA binding. 21 (44.7%) of 47 serum specimens showed the DNA binding values exceeding both normal limits.

Fig. 1. DNA binding activities of SLE patient sera in three different assay systems. 50 µl of a 10-fold diluted patient serum was added with 1 µg of thermally denatured mouse embryo [³H]DNA (5,000 dpm). The dextran sulfate system contained 2.5 µg of dextran sulfate and the calcium chloride system contained 0.1 M of calcium chloride in the assay mixture. Other conditions were as described in text. Bars by the side of plots indicate the normal DNA binding range (mean ± 2 s.d.) by healthy sera.

16 (43.2%) of 37 sera which showed the binding within the normal range in the non-addition system turned to exhibit a binding value exceeding the normal upper limit in the dextran sulfate system, and 14/33 (42.4%) turned to abnormal in the calcium chloride system. None of the sera which gave binding values exceeding the normal limit in the non-addition system showed a binding value in normal range in the dextran sulfate system. But in the calcium chloride system, one of 14 specimens turned to normal in DNA binding from the abnormal value in the nonaddition system.
Precise differentiation of DNA binding activity

The ratios of DNA binding value in the addition system to the value in the non-addition system with respective serum specimens were plotted against the binding value in the addition system as shown in Fig. 2.

In the dextran sulfate system (Fig. 2a), 26 (51.0%) out of 51 specimens exceeded the normal upper limit in both the ratio and the binding value. 8 sera (15.7%) exceeded one of the two limits and 17 (33.3%) were within the normal range in both indices.

In the calcium chloride system (Fig. 2b), 27 cases (57.4%) out of 47 specimens proved to be abnormally higher than the normal limits in both the ratio and the binding value, 3 (6.4%) were in the abnormal level only for the ratios and 17 (36.2%) showed both indices within the normal range.

After all, of 47 serum specimens from patients with SLE, 18 cases (38.3%) referred to a positive reaction for the antibodies to DNA in all of four indices (two ratios and two binding values), 5 cases (10.6%) showed abnormality in three of four indices, 9 (19.1%) were abnormal in two indices, and 5 (10.6%) were abnormal in one index. As a whole, 78.7% of tested patient sera showed a positive reaction for anti-DNA antibodies in one or more of these indices, while only 10 samples (21.3%) showed normal values in all indices.

Case Studies

DNA binding values obtained in the three different assay systems during the course of the disease were shown typically in the following serial studies:

Case 1

Fig. 3 shows a case of 53-year-old female (Ht), who had suffered from arthralgia
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for more than ten years and had been treated as a case of chronic rheumatoid arthritis. On December 24, 1976, she had a sudden onset of high fever and dyspnea and the next day she was admitted to the Akita University Hospital. Chest x-ray film examination revealed pleural and pericardial effusion. She was treated with prednisolone on a diagnosis of SLE.

Serum DNA binding 2 months before admission showed an abnormally high level in the addition system, but it remained within the normal range in the non-addition system. Two days before the acute exacerbation, the value exceeded the normal upper limit in all the three systems.

According to the treatment with steroids, the symptoms gradually subsided and the DNA binding values fell into a normal range together with other immunological markers. The DNA binding value continued to decrease and reached a much

Fig. 3. Serial studies on a patient (Ht) with SLE, showing an acute exacerbation with serositis during the course of the disease. (○) and (-----) in [3H]dDNA Binding indicate DNA binding value and its normal range (mean±2 s.d.) in the non-addition system; (○) and (------), DNA binding value and its upper normal limit (mean+2 s.d.) in the dextran sulfate system; (●) and (-----), those in the calcium chloride system. [3H]dDNA, tritiated thermally denatured DNA.
lower level than the normal lower limit (mean—2 s.d.) five and half months after admission. But unfortunately she died from Pneumocystis carinii pneumonia 6 months after admission.

Case 2

Fig. 4 shows a case of a 29-year-old female (Ya) with SLE which developed during pregnancy. However, her postpartal course was uneventful.

In the first trimester, she complained of pain in the knee- and elbow-joints. About 4 months later, she was admitted to Akita City Hospital for severe lassitude and dyspnea. Immediately after admission, developing a tonic cramp in the neck muscles she fell into unconsciousness. She was referred to the Akita University Hospital, on March 15, 1977. Examination of the blood disclosed 8.9 g/100 ml of hemoglobin, $254 \times 10^4$ red blood cells, 5,800 white blood cells and 78,000 thrombocytes. Urinalysis showed 2+ test for albumin; sediment contained many red cells and granular casts. She was treated with prednisolone on the diagnosis of
SLE. Renal biopsy done on Aug. 25 showed a mesangial proliferation in glomeruli.

She delivered a female child on May 12, but the child died of hyaline membrane disease. During perinatal period, DNA binding values of the sera in the non-addition system were apparently within the normal range. However, in the addition system those were proved to exceed the normal upper limit. In the cord blood, DNA binding value assayed in the non-addition system, dextran sulfate system and calcium chloride system was 23.4%, 1.3%, and 6.9% respectively, and C₃ was 28 mg/100 ml.

**Case 3**

Fig. 5 shows a case of 18-year old female (Nk) with SLE. She developed butterfly rash two months before admission. She noted a stiffness alternatively in the right and left 2nd fingers, accompanied with Raynaud phenomenon, paresthesia, joint pain, high fever (up to 39°C), and generalized skin rash. She was admitted to Akita Red Cross Hospital on February 13, 1976. Examination of the

![Graph](image_url)

**Fig. 5.** Serial studies on a patient (Nk) with SLE, showing a transient abnormally high level of serum lactic dehydrogenase activity with a coincidental decrease in hemoglobin level. Symbols are the same as in Fig. 3.
blood disclosed 11.4 g/100 ml hemoglobin, 350 × 10^4 red blood cells, 3,700 white blood cells and 21.6 × 10^4 thrombocytes. Urinary sediments contained many red cells and granular casts. Coombs test was negative. An abnormally high level of serum lactic dehydrogenase activity was found transiently coincidental with the decrease in hemoglobin level. On the diagnosis of SLE, she was treated with prednisolone.

DNA binding value in the non-addition system was decreased to a normal level in one month after admission. However, the value in the addition system still remained in the abnormally high level. The specific DNA binding value gradually decreased during the course of five months treatment, accompanying the increase of C₃ and the decrease of IgG contents in sera toward the normal level. The levels of IgM and IgA were unchanged during the course.

**DISCUSSION**

Many attempts have been made by several authors to eliminate the non-specific DNA binding by human sera. Aarden et al. (1976) described that non-specific DNA binding can be eliminated by heating the sera at 56°C for 30 min by immobilizing complement, which they expected to be the DNA binding component. And this non-specific binding was also eliminated by using phosphate buffer as the assay medium and by an addition of sodium chloride to the assay mixture. Izui et al. (1976), who considered some basic proteins from cells, such as histone, as the chief trigger of this phenomenon, demonstrated that an addition of sodium dodecyl sulfate to the mixture could eliminate the non-specific binding without any interference on the specific DNA binding by the antibodies. Wakizaka and Okuhara (1979) previously reported that serum gamma globulin should be a main component of this non-specific DNA binding and that the addition of dextran sulfate or divalent cations reduces the rate of this non-specific binding. Wakizaka et al. (1981) further investigated the normal level of the DNA binding by healthy human sera in these three Farr assay systems.

The present study revealed that the incidence of the positivity for anti-DNA antibodies in the patient serum seemed to be somewhat lower than those reported previously (Pincus et al. 1969; Koffler et al. 1971; Hughes et al. 1971; Lewis et al. 1973; Luciano and Rothfield 1973), when patient sera were assayed in the non-addition system. However, addition of dextran sulfate and calcium chloride to the medium sufficiently reduced the non-specific binding and increased the incidence of the positivity for anti-DNA antibodies in patient sera. Serial studies shown in Figs. 3, 4, and 5 proved that by our method would be obtained more precise data for anti-DNA antibodies contents in the patient serum. Furthermore, the ratios of DNA binding values between addition and non-addition systems seemed to be a good marker for detection of antibodies to DNA in the serum, as shown in Fig. 2.

It is remarkable that patient serum occasionally showed an extremely low binding activity in a certain stage of the disease as shown in Figs. 3 and 4. The
cause of this phenomenon is unknown, but it seems to have some relation with a
long-term administration of steroids. In any way, the non-specific DNA binding
activity of sera should be investigated further on its real nature and its relation
with a pathophysiological state of SLE.

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References

The influence of reaction conditions on the Farr assay as used for the detection of anti-
by a modified 125I-labelled DNA-binding test. Elimination of non-specific binding
of DNA to non-immunoglobulin basic proteins by using an anionic detergent. Clin.
8) Lewis, R.M., Stollar, D. & Goldberg, E.B. (1973) A rapid, sensitive test for the
9) Luciano, A. & Rothfield, N.F. (1973) Patterns of nuclear fluorescence and DNA-
of antigens by single radial immunodiffusion. Immunochem., 2, 235-254.
cells in peripheral blood. Blood, 6, 470-473.
281, 701-705.
13) Steinberg, A.D., Pincus, T. & Talal, N. (1969) DNA-binding assay for detection of
gamma globulin in Farr's radioimmunoassay by addition of dextran sulfate and
15) Wakizaka, A., Kurosaka, K., Okuhara, E., Kashimura, M., Akihama, T., Miura, A.B.
healthy human sera in Farr assay with or without addition of dextran sulfate and