High-Affinity Anti-DNA Antibody Parallels Clinical Course of Immunoabsorption Therapy for Systemic Lupus Erythematosus

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Anti-DNA antibody, especially high-affinity anti-DNA antibody (ADNA), is thought to have an important role in the pathogenesis of lupus nephritis. In this study, ADNA which binds to double-stranded DNA under a high concentration of sodium chloride was measured in patients who had received immunoabsorption (IA) therapy with a dextran-sulfate column. Titers of high-affinity ADNA in the cases with renal dysfunction tended to be higher than in those without renal dysfunction. The change in the titer of high-affinity ADNA paralleled the clinical course. These findings suggest that measurement of high-affinity ADNA is useful for follow-up of the clinical course of patients who have undergone IA therapy. (Internal Medicine 35: 367–372, 1996)

Key words: lupus nephritis, dextran-sulfate column, sodium chloride

Introduction

Systemic lupus erythematosus (SLE) is one of the autoimmune diseases characterized by multiple organ disorders caused by immune complexes (IC) which consist of anti-DNA antibody (ADNA) (1, 2). In particular, the ADNA responsible for the pathogenesis of SLE is believed to have a high affinity for double-stranded DNA (dsDNA) and to be a cationic IgG-subclass antibody (3, 4). SLE is currently treated with corticosteroids and immunosuppressive agents to inhibit the production of ADNA, however there is also a potential therapeutic effect in the removal of circulating ADNA and IC by plasmapheresis for drug-resistant cases. There have been several reports including ours describing that such severe cases of SLE have been successfully treated by selective removal of circulating ADNA by adsorption onto a dextran-sulfate column (5–8). In this paper, using the sera during this immunoabsorption (IA) therapy, we investigated whether the measurement of ADNA with high affinity for dsDNA in the serum is useful for follow-up of the patients who have undergone the treatment with this column.

Materials and Methods

Determination of ADNA

Conventional ADNA and high affinity antibody to dsDNA were determined using kits named RIA II (DPC, Japan) and Hi-A (DPC, Japan) respectively, both kits employing a modified Farr method (9). Briefly, a mixture of 25 µl of serum and 200 µl of ¹²⁵I-labeled dsDNA was incubated in the presence or absence of 125 mM NaCl in RIA II or Hi-A for 2 hours at 37°C. After precipitation by ammonium sulfate of complexes consisting of ADNA and dsDNA, the ratio of radioactivity of the complexes to the total radioactivity was calculated. The titer (U/ml) of ADNA in RIA II was determined using a standard curve made by standard sera of WHO (10), and the ADNA titer in Hi-A, the above ratio (%), was used as the titer of high affinity antibody to dsDNA according to the manufacturer's instructions.

Patients

Five patients with severe SLE, who met the criteria of ARA (11), were treated by IA therapy using a dextran-sulfate column. Their sera before and during the IA therapy were stored at 70°C until measurement of ADNA. The procedure of IA therapy is reported elsewhere (7). Briefly, plasma was separated from blood using a polysulfone column (FS-05, Nikiso, Japan) and

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it was passed through a dextran-sulfate column using an apheresis unit (MA0-01, Yokogawa Corp., Japan). Plasma of 60 ml/kg was processed during each IA session, which was repeated weekly a total of 4 to 6 times. Five patients, all female from 23 to 43 years of age (mean, 32.8), who were resistant to conventional drug therapies including oral prednisolone and cyclophosphamide which were administered during the preceding period from 2 to 14 years (mean, 6.8). Major symptoms and signs at the beginning of IA therapy were shown by Table 1. ADNA determined by RIA II in the sera from all patients was greater than 100 U/ml. Table 2 shows the clinical courses of the five cases. During the IA therapy, cases 2, 4, and 5 kept taking the same doses of prednisolone (10–25 mg/day). In order to suppress the rebound phenomenon of ADNA production, 500 mg of methylprednisolone (mPSL) in cases 1 and 3, and 600 mg of cyclophosphamide (CP) in case 2 was administered intravenously after IA session. In case 4, 4 mg/kg of cyclosporin A (CyA) was given everyday after the first IA session. The clinical course of each case was assessed by

comparing the disease activity and existence of proteinuria (more than 0.1 g/day) before and after the IA therapy as following. According to the criteria by Japanese Ministry of Health and Welfare, patients with three or more of the following items are regarded as having active disease; fever (more than 37°C), arthralgia, erythema, oral stomatitis or massive hair loss, leukopenia (less than 4,000/ μ l), hypo-complementemia (less than 20 CH50 U), accelerated erythrocyte sedimentation rate (more than 30 mm/h), LE cell phenomenon, hypoalbuminemia (less than 3.5 g/dl). Cases in whom either 4 or more items disappeared or 3 items as well as proteinuria disappeared were regarded as improved (I), cases in whom 2 items disappeared, as slightly improved (SI), or one or less item disappeared, as no change (NC), and if new items increased it was considered as worsened (W).

Statistical analysis

Significance of the correlation between two groups was evaluated by F-test.

Table 1. Patient Profile

Case	Age (yr)	Sex	Period after Dx ¹ (yr)	Chief complaint	WBC (/ μ l)	CH50 (U/ml)	Urinary protein (g/day)	Cellular cast	ADNA by RIA (μ /ml)
1	23	F ²	6	Skin rash	3,300	10.2	1.0	+	237
2	25	F	8	Proteinuria	4,800	14.7	2.0	+	134
3	34	F	2	Facial rash Arthralgia	1,900	40.8	0	–	214
4	43	F	4	Renal failure	3,600	8.0	ND ³	ND	106
5	39	F	14	Facial rash Psychosis	2,340	27.0	0	–	299

¹diagnosis, ²female, ³not done.

Table 2. Clinical Course

Case	IA ¹ therapy		PSL ² dose (mg/day)	Supportive therapy	Clinical course		
	Cycle	Plasma volume processed (<i>l</i>)			Change of		Evaluation ⁴
					Urinary protein	Activity score ³	
1	5	3.2	0	mPSL ⁵ 500 mg/W (i.v. ⁶)	+ → −	6 → 2	Improved
2	4	3.0	15	CPM ⁷ 600 mg/2W (i.v.)	+ → −	3 → 0	Improved
3	5	3.0	0	mPSL 500 mg/W (i.v.)	− → −	6 → 4	Slightly Improved
4	6	3.5	25	CyA ⁸ 4 mg/kg/day (oral)	ND → ND	2 → 1	No change
5	4	3.0	10	None	− → −	4 → 5	Worsened

¹immunoadsorption therapy, ²prednisolone, ³disease activity score described in "Materials and Methods", ⁴evaluation of clinical course described in "Materials and Methods", ⁵methyl prednisolone, ⁶intravenously, ⁷cyclophosphamide, ⁸cyclosporin A.

Results

Clinical courses

Cases 1 and 2 belonged to I; case 3, SI; case 4, who was complicated by chronic renal failure and was on hemodialysis, NC; case 5, took no supportive therapy (as described in Patients section), W (Table 2).

Relationship between the ADNA titers by RIA and Hi-A

ADNA was determined by RIA and Hi-A using serum samples drawn on the same day before IA therapy and just before the fourth IA session, and the relationship between the ADNA titers by the two methods are shown in Fig. 1. There was a positive correlation between titers by the two methods ($p < 0.05$). Before IA therapy, all samples showed a high titer of ADNA of over 100 U/ml by RIA, but there was a wide range of distribution from slightly to markedly elevated at Hi-A, although the total number of cases was not sufficient for statistical analysis (Fig. 2).

Relationship between renal dysfunction and ADNA by the two methods

By RIA, ADNA titers of the two cases (cases 3, 5) without renal dysfunction showed a high level of ADNA of over 200 U/ml, and had a tendency to be higher than the titers of those with proteinuria or chronic renal failure, although the difference was not significant. By Hi-A, in the 2 patients without renal dysfunction, one was high (case 5) and the other was low (case 3), while titers of the three cases with proteinuria or chronic renal failure were relatively high (Fig. 3).

Relationship between the titer of ADNA and clinical course

Serum ADNA titers before IA therapy measured by RIA and Hi-A were compared in association with clinical courses. The titer of case 5, who had worsened after IA therapy (W), was markedly high, and that of the case 4 (NC) was moderately high. The other three cases in SI and I showed moderate to high ADNA titers. On the other hand, the titers by Hi-A of the cases in W and NC were high, while that of case 3 in SI was slightly over the normal range, and the other 2 cases (cases 1, 2) in I showed high titers (Fig. 4).

Change of ADNA titer

Figure 5 shows the change of ADNA titer by RIA and Hi-A during the IA therapy. By RIA, the titer of case 5 (W) was high at the beginning and also through out the therapy. In cases 1 to 3 (I or SI), initial high titers were decreased during the IA therapy. In case 4 (NC), the titer increased markedly during the IA therapy. On the other hand, by Hi-A, the ADNA titers of the two cases in W and NC were high at the beginning and both increased during the therapy. The titers of cases 1 and 2 (I) showed high titers at the beginning and markedly decreased during the therapy, while that of case 3 (SI) was relatively low at the beginning and then normalized after IA therapy.

Figure 1. Relationship between the ADNA titers by RIA and Hi-A in all samples drawn at the beginning and just before the fourth session of IA therapy.

Figure 2. Relationship between the ADNA titers by RIA and Hi-A in the samples drawn only at the beginning. Hatched areas represent the cut off level of each method.

Discussion

Recently we demonstrated that IA therapy is useful for the treatment of patients with lupus nephritis resistant to conventional drugs (7, 8). Among the numerous patients with SLE who underwent IA therapy, we studied 5 cases with various clinical courses (improved, 2; slightly improved, 1; not changed, 1; worsened, 1). In case 5 (W), the ADNA titer was markedly high by both methods at the beginning, and remained still high just before the fourth session of IA, showing a correlation with the clinical course, while the titer just after each session of IA was decreased (data not shown). This was thought to be due to the fact that this case took no supportive drugs in addition to PSL;

Figure 3. Relationship between renal dysfunction and ADNA by the RIA and Hi-A. (+) cases with, and (-) cases without proteinuria or chronic renal failure. Hatched areas represent the cut off level of each method.

Figure 4. Relationship between the titer of ADNA at the beginning and during the clinical course. Hatched areas represent the cut off level of each method. I: improved, SI: slightly improved, NC: not changed, W: worsened. Details of the changes during the clinical course are described in text.

such is the case with plasmapheresis, in which a rebound phenomenon of immunoglobulin production often occurs.

It has been reported that the Farr assay of ADNA is a method

which can detect high affinity antibodies by making low affinity antibodies dissociate from DNA by using ammonium sulfate (12). On the other hand, a high concentration of sodium chloride

Figure 5. Change of ADNA titer at the beginning and just before the fourth session of IA therapy by the RIA and Hi-A. Triangles represent the worsened cases; open squares: case with no change, closed circles: improved cases, open circles: slightly improved case. 0, before IA therapy; 4, just before the fourth IA session.

is thought to make an environment in which ADNA further dissociates from DNA. Therefore, ADNA detected by the Hi-A kit we used is believed to consist of a large part of high-affinity ADNA (12–14). It has been reported by several investigators (14, 15) that this high-affinity ADNA binds to the basement membrane of the glomeruli, and that it is associated with renal dysfunction. In the present study, there was a weakly positive correlation between the ADNA titers by RIA and Hi-A of all samples before and during the IA therapy. However, it was more likely that titers of the cases with renal dysfunction had a tendency to be higher in Hi-A than in RIA, although this was not statistically significant.

Because various supportive drugs were given in the present cases, it seems difficult to select the patients with an indication of IA therapy only based on the levels of ADNA determined by either method. However, in the cases with high titers of ADNA by Hi-A, those who had a good clinical course showed a marked decrease of the titers, while those who had a bad clinical course maintained high titers. In addition, among the three cases in which IA therapy was effective, two with markedly elevated titers by Hi-A showed a high score of improvement and one with a slightly elevated titer did not. On the other hand, the level of the titers by RIA before IA therapy obviously did not parallel the clinical course.

These data suggest that ADNA detected in a high concentration of sodium chloride might be more closely associated with the pathogenesis of this disease than that detected by RIA. Further, it was more likely that the change of titers of ADNA measured by Hi-A paralleled the clinical course of IA therapy

than that measured by RIA. Therefore, measurement of high-affinity ADNA may be useful for the selection of patients for IA therapy. The correlation between the titer of high-affinity ADNA and the clinical outcome by IA therapy should be studied in more cases.

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