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Antiphospholipid Antibodies in Patients with Myasthenia Gravis

Ikuo KINOSHITA[1], Masakatsu MOTOMURA[1], Kunihiko NAGASATO[1], Katsuhiro ICHINOSE[1], Kiyosumi OHISHI[1], Gou TAKEO[1], Akira SATOH[1], Tatsufumi NAKAMURA[1], Toshiro YOSHIMURA[1], Mitsuhiro TSUJIHATA[2], and Shigenobu NAGATAKI[1]

1 The First Department of Internal Medicine, Nagasaki University School of Medicine
2 The School of Allied Medical Sciences, Nagasaki University, Nagasaki 852, Japan

We measured antiphospholipid antibodies in sera from 94 patients with myasthenia gravis (MG). We found lgG aCL in 14/94 (14.9％), lgM aCL in 6/94 (6.4％) and LA in 4/56 (7.1％) patients with MG. As a whole 21 of 94 (22.3％) patients with MG had some aPL. There was no correlation between the presence of aPL and the severity of MG, the presence of hyperplasia of thymus, titer of the anti-acetylcholine receptor antibodies or anti-single stranded DNA antibodies. Though the percentage of malignant thymoma with aPL were higher than that of malignant thymoma without aPL, we thought that aPL were not the specific antibody in malignant thymoma. In MG, aPL did not play as the aCL syndrome and seemed to be non-specific antibodies.

Keywords: Antiphospholipid antibody, Anticardiolipin antibody, Myasthenia gravis, Lupus anticoagulant, Anti-single stranded DNA antibody

Introduction

Anticardiolipin antibodies (aCA) belong to antibodies directed against anionic phospholipids[1], and have been reported in many clinical systemic syndromes. Most of these syndromes are related to lupus erythematosus[1)2)], but are not specific for this disease[3]. They include neurologic diseases which appear as ischemic thrombotic episodes affecting the arterial or venous system[4)5)]. Other non-stroke syndromes such as myelopathy[6], Guillain-Barre syndrome[7], migraine[8], chorea[9], seizures[10] and autoimmune neurological diseases[11], have been also described in association with raised aCL levels.

In myasthenia gravis (MG), an autoimmune response to the acetylcholine receptor (AChR) in postsynaptic membrane, neuromuscular transmission is impaired[12)13)]. Colaco et al. described that aCA were detected in sera of patients with multiple sclerosis, MG and Lambert-Eaton myasthenic syndrome[11]. But Rombos et al[14] reported that there was no significant difference of serum aCA level between MG and the controls. Therefore, relationship between aCA and MG is still not well defined.

In this report, measured antiphospholipid antibodies (aPL), aCA and lupus anticoagulant (LA) in sera of patients with MG and investigated the significance of these antibodies in MG.

Materials and methods

Ninety-four MG patients (31 men and 63 women) were studied. They were diagnosed according to the clinical findings, the electromyography (Harvay-Musland test), Tensilon test, the titer of anti-AChR antibodies[15] and deposition of immune complexes at the motor end-plates[16]. All blood samples were kept at -70℃ until examination. Control sera from 140 healthy subjects were available.

Anticaradiolipin antibody assay

The levels of lgG and lgM to aCL were determined by the solid phase enzyme-linked immunosorbent assay with some modifications. In brief, cardiolipin (Sigma, USA) was diluted in ethanol and used to coat microtiter plates with 100 μl (5 μg/ml). The plates were dried and washed three times with PBST (0.01MPBS, 0.05％ Tween) buffer. The antigen-coated plates were blocked with normal rabbit serum in PBS. The plates were incubated with test sera diluted to 1:50 in the same buffer for 90 minutes (100 m μl /well), and washed in PBST followed by a 90 minutes incubation with 100 μl of peroxidase conjugated goat anti-human lgG or lgM (DAKO, Japan). After 3 times of washing, color was developed using o-phenylenediamine (WAKO Pure Chem Indust, Japan) and the reaction was stopped by the addition of 2.5 M H_2SO_4. The optical density (O. D) was measured at 490 nm using an ELISA microtiter reader. The O.D values and positive values of lgG and lgM

Reprint request to : Ikuo Kinoshita, The First Department of Internal Medicine, Nagasaki University School of Medicine, 1-7-1 Sakamoto-machi, Nagasaki 852. Japan
FAX number: 418-649-5910

in healthy individuals were 0.105, 0.173 and 4.1 %, 2.7 % respectively. The ratio of the O.D of patients to the control was cut off index and the positive was defined as greater than 1.0. Comparisons were made with x^2 analysis.

Lupus anticoagulant activity

The tissue thromboplastin inhibition was performed[17]. The ratio of clotting times at 1:100 and 1:1000 dilutions of thromboplastin were determined. A ratio of 1.3 or greater was defined positive.

Autoantibody testing

Anti-AChR antibodies were assayed by an immunoprecipitation method using human junctional AChR as the antigen[15]. Antinuclear antibodies were detected by indirect immunofluorescence method. lgG-class antibodies to single-stranded (ss) and double-stranded (ds) DNA were detected by ELISA kit (MBL, Japan). 2 U/ml or greater was defined positive.

Results

Antiphospholipid antibodies in MG

Raised aCL activity was detected in 19/94 (20.2 %) patients with MG (Fig. 1). Positive of only lgG aCL was 12, only lgM was 4 and both of them was 2. LA was examined in 56 patients with MG. LA was detected in 4/56 (7.1 %). Two cases had aCL too. In this data, aPL was detected in 21/94 (22.3 %).

Features of 21 MG patients with antiphospholipid antibodies

In 21 MG patients with aPL, the clinical features and laboratory data were shown (Table 1, 2, 3). Three patients were ocular type and the others were generalized type. In their complications, rheumatoid arthritis, schizophrenia, nephrosis and malignant alopecia were observed. In the histology of the thymus, 5 patients had hyperplasia and 6 had thymoma (5 were malignent). The number of hyperplasia of thymus and thymoma were not significantly different in the two groups. But the number of malignant thymoma were higher in this group (5/21, 23.8 %) than in the patients without aPL (3/73, 4.1 %) ($P<0.005$). There were no patients who had the history of coagulopathy, thrombocytopenia and spontaneous abortion.
Titer of the anti-AChR antibodies were 13.8 ±28.7nM, and there were no significant differentce in comparison to another 73 MG patients. Two patients with LA had the prolongation of active partial thromboplastin time. Only one patient had a false-positive VDRL. Antinuclear antibodies were positive in 5/21 (23.8 %). Though anti-ssDNA antibodies were positive in 12/21 (57.1 %), there was no significant difference in two groups. Anti-ds DNA antibodies in all patients were negative.

Table 1. Clinical data for MG patients with aPL

case	age/sex	severity (Patten)	thymectomy (histology)	anti-AChR Ab (nM)	complication	steroid therapy
1	37/M	I	-	0.8	-	-
2	30/F	I	-	0.5	-	-
3	31/M	I	+(thymic tissue)*	2.2	-	-
4	26/F	II a	+(thymic tissue)	43.0	-	+
5	44/F	II a	+(hyperplasia)	35.6	-	-
6	47/F	II a	+(thymic tissue)	0.8	-	+
7	57/F	II a	-	20.0	-	-
8	40/M	II a	+(hyperplasia)	2.4	-	-
9	60/F	II a	+(thymoma)	11.2	schizophrenia	+
10	42/F	II a	-	26.0	-	-
11	55/F	II a	+(thymic tissue)	21.2	-	+
12	54/M	II a	-	12.7	rheumatoid arthritis	-
13	52/M	II a	+(malignant thymoma)	0.6	-	+
14	30/F	II a	+(hyperplasia)	89.2	nephrosis	+
15	46/F	II a	+(thymic tissue)	69.2	-	-
16	53/F	II a	-	68.0	-	+
17	46/F	II a	+(hyperplasia)	64.6	-	-
18	70/F	II b	+(malignant thymoma)	2.1	-	+
19	59/F	II c	-(malignant thymoma)**	11.7	-	+
20	67/M	II c	+(malignant thymoma)	31.3	-	+
21	45/M	II c	+(malignant thymoma)	72.4	malignant alopecia	+

* residual thymic tissue
** autopsy case

Table 2. Laboratory data for MG patients with aPL

case	BFP	ANA	LA	anti-DNA Ab(U/ml)		PT(%)	APTT(sec)
				ss	ds		
1	-	-	-	0.9	0.4		
2	-	-	+	0.6	0.3	103	36.7
3	-	-		0.7	0.3		
4	-	-	+	1.8	0.7	103	36.6
5	-	-	-	10.6	0.9		
6	-	-	-	0.5	0.3	106	25.0
7	-	-	-	25.3	1.8	81	34.4
8	-	40×	+	3.2	0.8		
9	-	-	-	0.7	0.6	119	29.6
10	-	-	-	3.0	0.7	115	30.3
11	-	-		1.8	1.0	79	33.8
12	-	-		4.3	0.6		
13	-	-		0.9	0.2		
14	-	160×		10.2	0.2		
15	-	-		0.7	0.4		
16	-	-		5.1	1.3		
17	-	-		70.1	1.5		
18	-	40×		14.0	1.7		
19	-	-		49.6	0.9		
20	-	-		2.2	0.9		
21	-	80×		15.9	0.8		

BFP: biological false positive for serological tests for syphilis
ANA: anti-nuclear antibody
LA: lupus anticoagulant
ssDNA: single-stranded DNA
dsDNA: double-stranded DNA
PT: prothrombin time
APTT: active partial thromboplastin time

Table 3. Correlation of clinical features and aPL in MG patients

	aPL(+) (n = 21)	aPL(-) (n = 73)
Type		
ocular	3	18
generalized	18	55
Thymus		
hyperplasia	5	10
thymoma	6	16
(malignant thymoma)*	(5)	(3)
Anti-AChR Ab(nM) [mean±SD]	27.9±28.7	25.2±91.4

* significantly different ($p<0.005$)

Discussion

ACL are found in various clinical conditions including infections and connective tissue disease[2)18)]. APL are associated with clinical complications such as thrombocytopenia, deep-vein thrombosis and spontaneous abortion in autoimmune disorders[19)]. These antibodies have been found in the patients with some neurological diseases, because they cross-react with the brain phospholipids cephalin and sphingomyelin[21)21)].

In the present study, we detected aPL in sera of MG patients; lgG aCL in 14/94 (14.9 %), lgM aCL in 6/94 (6.4 %) and LA in 4/56 (7.1 %). Therefore, 21 MG patients (22.3 %) had some aPL, and our data are not in agreement with those of Colaco et al. according to which, lgM aCL were more specific in MG than lgG aCL[11)]. There is relatively little cross-reactivity of the aCL with anti-DNA antibodies[22)]. This may be because the glyceride portion of the phospholipid is essential for antibody binding[23)24)]. These findings raise the possibility that membrane bound phospholipids in association with autologous or exogenous membrane antigens might play a role for the development of autoimmunity[11)]. But in our study, anti-ssDNA antibodies were positive in 12/21 (57.1 %) MG patients with aCL and in 26/73 (35.6 %) MG patients without aCL, and no significant difference in anti-ssDNA antibodies between these two groups. Though in the complication of thymoma these two groups had no significant difference, the percentage of the malignant thymoma was higher in aCL positive group than in negative group. Thymic hyperplasia and thymoma are thought to play a crucial role in the pathogenesis of MG and the antiskeletal muscle antibodies is reported to be specific or closely associated with thymoma[25)]. In the malignant thymoma with aPL, the patients were small number and 4 of them had anti-ssDNA antibodies. Though aPL were secondly detected by the tissue damage, we thought that aPL were not specific antibodies in the malignant thymoma. In MG, aPL were thought not to play as the aPL syndrome and had no correlation with severity of the MG and anti-AChR antibodies. In conclusion, aPL were detected in about 20 %

of MG patients but we could not observe the relation between these antibodies and MG. These data suggest that these antibodies had no activity of the aPL syndrome and were non-functional as polyclonal antibodies in MG.

Further studies are needed to confirm the relationship of these antibodies and pathogenesis of MG.

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