Circulating immune complexes in myasthenia gravis: a study in relation to thymectomy, clinical severity and thymus histology

CARLO BARTOLONI,* LUISA GUIDI,* CIRIACO SCOPPETTA,†
PIETRO TONALI,† EMANUELA BARTOCCIONI,‡ GIOVANNA FLAMINI,‡
GINO GAMBASSI,* TULLIO TERRANOVA‡

From the Istituto di Patologia Speciale Medica,* Istituto di Clinica Neurologica,† and Istituto di Patologia Generale,‡ Università Cattolica del Sacro Cuore, Rome, Italy

SUMMARY Circulating immune complexes were assayed employing the method recently described by Barnett and Chia in a group of patients with myasthenia gravis. The subjects were classified according to clinical severity and immune complexes were sought before and after thymectomy. The operated subjects were further divided into those with thymoma or thymic hyperplasia. Antigen-antibody complexes were higher before thymectomy than after, in hyperplasias than in thymomas, and in severe myasthenia gravis than in mild disease. Circulating immune complexes and anti-acetylcholine receptor antibodies did not correlate.

Myasthenia gravis (MG) is a disorder of neuromuscular transmission which is probably due to an autoimmune mechanism leading to reduction of the available nicotinic acetylcholine receptors (ACh-Rs) at the postsynaptic membrane of skeletal muscles. It is possible to induce experimental autoimmune myasthenia gravis employing purified ACh-R extracts (from *Electrophorus electricus* and *Torpedo californica* electric organs or from denervated mammalian muscle) in order to immunise NZW rabbits actively, which thereafter display a typical electrophysiological and clinical picture of myasthenia gravis.¹ Other investigators, by means of passive immunisation have been able to reproduce experimental autoimmune myasthenia gravis by injecting aliquots of myasthenic patients' sera into the mouse.² Drachman *et al*,³ employing IgG purified fractions of myasthenia gravis subject sera, discovered that the binding of antibody from myasthenic patients alters the ACh-R so that it could be preferentially degraded by the muscle cells. A serum globulin from myasthenia gravis patients is able to block the binding of alpha-bungarotoxin to the human neuromuscular junction,

as shown on a morphological basis by Bender and to denervated rat muscle ACh-R.⁵ Also, electron microscope studies have demonstrated the presence of immune complexes of IgG and C₃ at the motor end plate both in human and experimental autoimmune myasthenia gravis.⁶⁻⁸

In man many attempts have been made to detect circulating antibodies directed against the ACh-R protein and to establish a direct relation between their presence and the clinical evaluation of the myasthenic patient.⁹⁻¹¹ There is increasing evidence of an association between autoimmune diseases and myasthenia gravis, and that rheumatoid arthritis,¹² thyroid disorders,¹³ idiopathic thrombocytopenic purpura,^{14 15} and, perhaps, SLE¹⁶ occur more frequently. The possibility of a common pathogenesis of this neuromuscular disease and associated autoimmune conditions should be considered; the thymus could play a determining role¹⁷ and evidence of hidden autoimmune disease should be sought in every myasthenic patient.

There are few reports of circulating immune complexes in myasthenia gravis in the literature.¹⁸⁻²⁰ The possible presence of myasthenia gravis and immune complex-mediated disease in the same patient, and the suggested autoimmune pathogenesis of such a neuromuscular disorder, prompted us to assay immune complexes in the serum of several myasthenic patients in relation to clinical severity and thymus histology. The immune complex assay

Address for reprint requests: Dr Carlo Bartoloni, MD, Istituto di Patologia Speciale Medica, Università Cattolica del S. Cuore, Via della Pineta Sacchetti, 644 I-00168 Roma, Italy.

was performed before thymectomy and repeated at least one year after surgery.

Materials and methods

Patients

The myasthenic patients were classified according to clinical severity into mild (purely ocular and mild generalised) and severe (severe generalised with or without respiratory weakness) myasthenia gravis according to previously reported criteria.¹⁷ The operated subjects were further divided, according to thymus histology into thymomas and hyperplasias. Fifty-three subjects were studied before thymectomy. Forty-two out of 53 underwent operation and were divided histologically into thymic hyperplasias (34) or thymomas (8). Eleven patients were not treated surgically. Sixty blood samples were obtained from myasthenic patients one year or more after thymectomy. They were classified as 22 thymomas and 38 hyperplasias. Forty-two of these subjects had already been studied before the surgical approach and 18 of them underwent operation before we started the present study. None of the patients had autoimmune disease known to be associated with circulating antigen-antibody complexes. Every patient was included in the study concerning the assay of circulating IC in order to establish a possible relationship between clinical severity, thymic histology and response to surgical treatment (thymectomy). Twenty-one age-matched healthy blood bank donors were also simultaneously studied as a control group.

Immune complex assay

Blood samples were obtained by venipuncture and allowed to clot at room temperature; the serum was then frozen in 1 ml aliquots at -50°C until assayed. Serum immune complex levels were determined by the method recently described by Chia *et al.*²¹ Each assay was run in triplicate; the serum sample (0.1 ml) was mixed with 0.1 ml of 8% polyethyleneglycol (PEG) in phosphate buffered saline (0.01 M pH 7.4 PBS) and then incubated for 1 h at 4°C . Mixtures were centrifuged at 1000 g for 1 h at 4°C and the pellets were then washed with 0.5 ml of 4% PEG. The washed pellets were resuspended in 0.1 ml PBS by mechanical stirring until complete and permanent clearing of the solution. IgG level was measured by radial immunodiffusion (RID) according to Mancini²² both in the serum and in the resuspended pellet. The percentage of serum IgG precipitated by PEG was then calculated to compensate for the variability of the immunoglobulin in the serum; immune complex concentration, therefore, was expressed as per cent of 4% PEG-precipitable immunoglobulins G. All samples were run at once, control group included. Positive and known negative control sera were analysed to standardise the procedure. The normal value for circulating immune complexes obtained assaying their levels in the control group was 2.27 ± 0.58 . The percentage of IgG precipitated by 4% PEG in excess of 2 standard deviations from the normal mean was considered to be immune complexes; a value exceeding 3.45%, therefore, should be defined abnormal, as observed in those diseases known to be associated with immune complexes (vasculitis, R-A,

SLE, Behçet's syndrome) in agreement with previously reported data.²¹ Group means were compared by Student's *t* test. Linear regression equation was employed to look for possible correlation between anti Ach-R antibody titre and immune complex levels (see under Results).

Results

The results obtained in the subjects studied before and after one year or more from thymectomy divided according to thymic histology and compared to controls, directly expressed as immune complex concentrations, are shown in tables 1 and 2. Actual IgG values (mg/100 ml) were: thymoma MG: 1261 ± 75.3 (serum), 59.8 ± 3.37 (complexed) before thymectomy and 1522.4 ± 57.8 (serum), 55.3 ± 4 (complexed) after the operation; hyperplasia myasthenia gravis: 1567.2 ± 56.8 (serum), 93.7 ± 9.1 (complexed) before surgery and 1347.6 ± 31 (serum), 76 ± 4.7 (complexed) after thymectomy. It should be emphasised that, for each patient, the calculation of immune complex concentration as per cent PEG-precipitable immunoglobulins G was directly performed from a triplicate assay (both in the serum and in the resuspended pellet) and not from the actual IgG mean values representing the whole groups. Circulating immune complex levels were always higher in hyperplasias than in thymomas and, in almost all the studied groups, they displayed higher values before thymectomy than after, even if the patients were subgrouped in relation to clinical severity. In hyperplasia myasthenia gravis patients, although the decrease in immune complex concentration after the operation does reach a border line statistically significant level only, the general trend following thymectomy was confirmed. The unique group in which the immune complex levels were higher after thymectomy, when compared with their value before the operation, were those with mild myasthenia gravis, with thymoma, but this may be explained by the small number of patients. When the myasthenic patients were classified according to clinical severity, immune complexes were generally found to be significantly higher in the severe form of myasthenia gravis than in the mild one.

In the control population the mean concentration of antigen-antibody complexes was lower than in every myasthenic patient group; in the latter, anti Ach-R antibodies have been assayed as described elsewhere.¹¹ No correlation was found between anti Ach-R antibody titre and circulating immune complex levels.

Discussion

Previous reports from other investigators have

Table 1 Circulating immune complexes in myasthenia gravis patients

	Patients		% PEG-precipitable IgG		Comparison between control and MG patient group <i>p</i> value
	No		Mean	± SD	
Before thymectomy					
Thymoma MG (all)	8		4.33	0.232	< 0.001
Hyperplasia MG (all)	34		5.98	0.5	< 0.001
All MG	53		5.68	0.468	< 0.001
Normal Subjects	21		2.27	0.58	—
After thymectomy					
Thymoma MG (all)	22		3.81	0.19	< 0.001
Hyperplasia MG (all)	38		5.77	0.39	< 0.001
All MG	60		5.05	0.343	< 0.001

Table 2 Comparison between IC levels in MG patient groups analysed by Student's *t* test

Before thymectomy	<i>p</i> value	After thymectomy	<i>p</i> value
Mild MG < Severe MG	< 0.001	Mild MG < Severe MG	< 0.001
Hyperplasia MG > Thymoma MG	< 0.001	Hyperplasia MG > Thymoma MG	< 0.001
<i>IC levels compared before vs after thymectomy</i>			
MG before thymectomy > MG after thymectomy			< 0.001
Mild MG before thymectomy > Mild MG after thymectomy			< 0.001
Severe MG before thymectomy > Severe MG after thymectomy			< 0.001
Hyperplasia MG before thymectomy > Hyperplasia MG after thymectomy			0.0496*
Thymoma MG before thymectomy > Thymoma MG after thymectomy			< 0.001

*Border line significant level.

emphasised the pathogenetic role of complement both in human and in experimental myasthenia gravis. The first investigations were made by Nastuk and associates more than 20 years ago;²³⁻²⁵ more recently, this view has been confirmed experimentally in passively induced myasthenia gravis by myasthenic serum in mice,² and in experimental autoimmune myasthenia gravis which could not occur in complement depleted rats employing cobra venom factor.²⁶ Immunoelectronmicroscopic studies have shown the presence of deposits of IgG and C₃ at the post-synaptic membrane level of the motor endplates in myasthenia patients⁶ and in experimental autoimmune myasthenia gravis.⁷ These reports and the finding of anti Ach-R antibodies in most of the myasthenia gravis patients⁹⁻¹¹ and the good correlation between reduction of C₄ and C_{1q}-binding activity, which is an expression of circulating immune complexes, observed in seven of eight subjects with myasthenia gravis by Casali and associates,¹⁸ have prompted us to seek antigen-antibody complexes in the serum of our myasthenic patients. Although the published works on myasthenia gravis and circulating immune complexes are yet few, our data do not seem to be in agreement with the results obtained by Behan and Behan²⁰ who reported a conspicuous association between decreased C₄ concentrations (and presence of immune complexes)

and mild or moderate disease; in addition, Tachovsky *et al*,¹⁹ employing the Raji-cell radio-immune assay, found no serum circulating immune complex in five myasthenic patients, although by the use of a different method, the same kind of immune complex could not be detected.²⁷

In myasthenic patients with thymoma we found lower values of circulating immune complexes than in those with hyperplasia, as also in those with severe myasthenia gravis when compared with those with mild disease. Evaluating the patients one year or more after the operation, a decrease in immune complex levels followed thymectomy but a difference persisted between hyperplasias and thymomas. In the light of previously reported data concerning anti Ach-R antibodies and cell-mediated immunity,^{29, 30} the results we obtained seem to confirm that the immune system plays an important part in the pathogenesis of myasthenia gravis, although its complete role is not clear yet.

Our data indicate that, in myasthenia gravis, circulating immune complex levels are generally higher before thymectomy than after and they are always more elevated in thymic hyperplasias than in thymomas. This might reflect a different functional activity between hyperplastic and neoplastic thymic cells. In addition, immune complex levels appear to be higher in the severe form of myasthenia gravis

than in the mild one, suggesting the possibility of a modulation of the antigenic stimulus and of the antibody responses in the different clinical forms of myasthenia gravis.

The concentration of anti Ach-R antibodies decreased after surgical excision of the thymus¹⁰ and a positive correlation has been described between the severity of the disease and the amount of the receptor antibody.²⁸ The level of circulating immune complex and the titre of anti Ach-R antibodies evaluated in our patient groups, however, did not correlate. This finding could mean that either the Ach-R antibody assay is only able to recognise uncomplexed antibodies (excess of antibody) or, more likely, that the possible antigen involved in the immune complex formation represents immunologically active Ach-R degradation products. These hypotheses are supported by Drachman's,³ Engel's⁶ and Sahashi's⁷ investigations but further studies are needed in order to evaluate the exact composition of circulating immune complexes detected in myasthenia gravis patients.

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