Clinical and immunological associations in myasthenia gravis 1: autoantibodies

H J SAGAR, K GELSTHORPE, A MILFORD-WARD
AND G A B DAVIES-JONES

From the Departments of Neurology and Immunology, The Royal Hallamshire Hospital and Tissue Typing Laboratory, Blood Transfusion Service, Sheffield

SUMMARY Associations between female sex, HLA B8, positive anti-thyroid microsomal antibody and, to a lesser extent, antinuclear antibody were seen in 34 patients with myasthenia gravis. This supports the concept that the disease is heterogeneous. Anti-DNA antibodies, which were present in 62% of the patients, did not show such associations.

Feltkamp¹ postulated the existence of two forms of myasthenia gravis on the basis of associations between certain clinical and immunological features. The first had an early age of disease onset, a female predominance, a high incidence of HLA B8 and thymic hyperplasia, with a low incidence of thymoma and antibodies to skeletal muscle. The second had a later onset, a male predominance, a high incidence of HLA A2 or A3, and a high incidence of thymoma and antibodies to skeletal muscle.²

This study is an attempt to confirm the heterogeneous nature of the disorder by an investigation of the relationship between HLA phenotype, autoantibody production, patient's sex and age of disease onset. This is a basis for an analysis of the cellular immune characteristics of the disorder to be described subsequently.

Methods

Patients

Thirty-four patients with myasthenia gravis were studied (mean age $\pm$ s.d. 44.9 years $\pm$ 15.2; 12 male). All had been diagnosed by consultant neurologists with the support of positive electro-physiological studies or Tensilon test. They represented all patients in the Sheffield area who fulfilled these criteria and who could be traced. Two patients had thymomas without thymectomy, and four others had had thymectomy but not for thymoma. Thymic histology in the remaining 28 patients was considered to be either hyperplasia or involution.

Address for reprint requests: Dr HJ Sagar, Department of Neurology, The Radcliffe Infirmary, Oxford.

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Myasthenic syndromes secondary to neoplasia or drugs were excluded, as were cases where doubt had been cast on the diagnosis. One patient had mild rheumatoid arthritis, and one had treated thyrotoxicosis, but none of the others had overt autoimmune or collagen vascular disease.

HLA typing

Lymphocytes were separated from heparinised blood samples by a carbonyl iron/Ficoll Triosil technique (3). Typing for the HLA-A and B antigens A1, A2, A3, A9, AW25, AW26, A11, A28, A29, AW30, AW31; B5, B7, B8, B12, B13, B14, B15, B16, B17, B18, BW35, BW40, BW21, BW22 and B37 was performed by the microlymphocytotoxicity method using 120 well-defined sera.⁴ The control population consisted of 3000 fully HLA A,B typed normal blood donors from the Sheffield region.

Autoantibodies

Antinuclear antibodies (ANF) and antibodies to mitochondria (AMA), gastric parietal cell (GPCA) and smooth muscle (SMA) were detected by indirect immunofluorescence using a composite tissue block of rat thymus, kidney and stomach as substrate and FITC conjugated swine antihuman immunoglobulin antiserum (Nordic Immunochemicals, Tilburg). Thyroid microsomal antibodies (TMA) were detected by a similar technique using toxic human thyroid as the substrate. All sera were examined at an initial dilution of 1:20; positive results were titred out at doubling dilutions, and the result expressed as the endpoint titre. Antibodies to native, double stranded DNA (dsDNA) were detected by passive haemagglutination using stabilised chicken erythrocytes coated with dsDNA (Fuzijoki Pharmaceutical Co., Japan).⁵ Serial dilutions were performed in microtitre plates and agglutination patterns recorded after six hours at 22°C. The incidence of these autoantibodies in the disease-free population under 60 years

of age, using these techniques, is ANF 0.5%, AMA 0.1%, GPCA 1%, SMA 0.5%, TMA 0.1% and dsDNA 0.1%.

Results

1 HLA

Fifteen patients, of 31 tested (48.5%), were positive for the HLA antigen B8, compared with 29.9% of controls. This difference is significant (relative risk 2.2, χ^2 4.75, $p < 0.05$), although the significance is not maintained if corrected for the number of antigens tested. The remaining antigens showed incidences similar to those of normal controls.

2 Autoantibodies

The incidence of positive autoantibodies in the group of myasthenic patients is shown in table 1.

Antinuclear antibodies (ANF) were detected in 41% of the total group and anti-thyroid microsomal antibodies in 32%. If weak positive results (titres less than 1:80) of these two tests are excluded, the incidence become 6/34 (18%) for ANF, and 7/34 (21%) for anti-thyroid antibodies. Anti-DNA antibodies (titres all greater than 1:80) were detected in an unexpected 62% of cases.

The incidence of autoantibodies in the few patients with thymoma or who had undergone thymectomy cannot be reliably assessed, but autoantibodies were sometimes detected in patients after thymectomy. Neither of the two patients with thymomas had antithyroid or anti-DNA antibodies and, although both showed positive ANF, this was only weakly so in both cases.

Table 2 shows the autoantibody incidence analysed according to the sex of the patient and the presence or absence of HLA B8. Thyroid

antibodies and to a lesser extent ANF were associated both with female sex and the HLA B8 antigen. All the 11 patients in whom there were antithyroid antibodies were female; no male myasthenic had this antibody. This sex difference is highly significant (χ^2 6.73, $p < 0.01$). The association between the presence of thyroid antibody and HLA B8 is also significant (χ^2 4.19, $p < 0.05$). There was a similar but weaker trend for antinuclear antibodies to be associated with B8 positive females, but this did not reach statistical significance. Nevertheless, 11 of the 14 ANF positive cases were female, and, of six cases with ANF titres more than 1:80, five were female and five were B8 positive.

Five cases were positive for both antithyroid antibody and ANF and all were female and B8 positive, but an association between ANF and antithyroid antibody was not otherwise seen.

Anti-DNA antibodies did not show any association with the patients' sex or any HLA phenotype.

Analysis of the autoantibody data with respect to age of disease onset showed a weak but insignificant association between early age of onset (under 35 years), female sex and the presence of thyroid antibodies (table 3). No difference was seen between early and late onset myasthenics in the incidence of ANF, anti-DNA antibodies or HLA phenotype.

Table 2 Autoantibodies in myasthenia gravis related to patient's sex and HLA B8

	B8 Positive (15)			B8 Negative (16)			Total (34)		
	ANF	Thyroid	DNA	ANF	Thyroid	DNA	ANF	Thyroid	DNA
Male (4)	1	0	4 (8)	2	0	5 (12)	3	0	9
Female (11)	7	8	6 (8)	3	2	4 (22)	11	11	12
Total (15)	8	8	10 (16)	5	2	9 (34)	14	11	21

Numbers indicate numbers of subjects.

Three of the total (all female) were not HLA-typed.

Table 3 Relationships of autoantibodies, patient's sex and HLA B8 to age of disease onset in myasthenia gravis

Age at onset (yr)	35 - (18)	35+ (16)
Thyroid+	7	3
ANF+	8	6
DNA+	10	11
B8+	8	7
B8+ female	8	3
Male: female	4:14	8:8

Numbers indicate numbers of subjects.

Table 1 Autoantibodies in myasthenia gravis

	ANF	Thyroid	DNA	AMA	SMA	GPCA
Intact thymus (28)	11 (39%)	9 (32%)	18 (64%)	3 (11%)	4 (14%)	1 (3.5%)
Thymoma (2)	2	0	0	0	1	0
Thymectomy (4)	1	2	3	0	0	0
Total (34)	14 (41%)	11 (32%)	21 (62%)	3 (9%)	5 (15%)	1 (3%)

Numbers indicate numbers of subjects

AMA = antimitochondrial antibody

SMA = anti-smooth muscle antibody

GPCA = anti-gastric parietal cell antibody

Discussion

This study has confirmed previously reported increases in the incidence of HLA B8,¹ and anti-thyroid and antinuclear antibodies^{6 7} in myasthenia gravis and supports the subdivision of the disease on this basis into at least two subgroups.² We found clear associations between female sex, HLA B8 and the presence of autoantibodies; there was a suggestion that thyroid antibodies and female sex were also associated with an early age of disease onset. No association could be seen, however, between HLA B8 and early age of onset but a previous study showing this association involved larger numbers of patients.²

We observed a surprisingly high incidence of anti-DNA antibodies. To our knowledge, only one other group have previously reported such antibodies in myasthenia gravis. These were detected using a similar haemagglutination method in 40% of cases.⁸ The significance of this finding remains at present uncertain. Viral involvement or an immune response to the release of nuclear material after tissue destruction could stimulate the production of such antibodies, but the high incidence and lack of relationship with any clinical or immunological parameter suggests caution should be exercised in its interpretation. Artefactual results produced by the haemagglutination method must be excluded by the confirmation of this finding using other methods. However our technique is not influenced by antibody to denatured (single-stranded) DNA.⁵

Considerable supporting evidence has accumulated for an autoimmune pathogenesis in myasthenia gravis since the first suggestion by Simpson.⁹ The disease overlaps with other putative autoimmune diseases⁹ and autoantibodies of varying kinds occur more frequently than expected.^{1 2 6 7} Systemic lupus erythematosus, which is characterised by the production of autoantibodies, shows a selective loss of suppressor cell function¹⁰; the clinical associations of autoantibody production in myasthenia gravis shown in this study suggest that a similar loss of suppressor cell function may be associated with the sub-group characterised by HLA B8, female sex and early age of onset.

Acetylcholine receptor antibodies show high specificity to the disease and occur in over 80% of cases.¹¹ They are pathogenic in rabbits¹² and immunoglobulins from human myasthenia have similar effects in mice.¹³ They show circulating levels in the human disease which correlate with the degree of muscular weakness.¹⁴ These

features suggest that it is one pathogenic mediator in most cases of the disease. The heterogeneity of myasthenia gravis shown here suggests that several unrelated mechanisms may independently act to lead to its abnormal production.

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