PAPERS AND ORIGINALS

Myasthenia Gravis, Autoantibodies, and HL-A Antigens

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Summary

The serum of 100 patients with myasthenia gravis and 441 of their first-degree relatives was studied for the presence of autoantibodies against several antigens. Antibodies to skeletal muscle were present in 22% of the patients and in 2% of the relatives. Both these frequencies were significantly higher than those in matched control subjects. Also, antinuclear antibodies were present more often both in the patients and in the relatives. Typing for HL-A antigens had shown a positive correlation between HL-A 8 and myasthenia gravis which was significantly higher in women than in men. Antibodies to skeletal muscle and thymomas were found to be much rarer in HL-A 8-positive patients than in HL-A 8-negative patients; HL-A 8-positive patients acquired the disease at an earlier age.

HL-A 2-positive patients more often had thymomas and antibodies to skeletal muscle than HL-A 2-negative patients; HL-A 2-positive patients acquired myasthenia gravis at a later age.

The fact that the clinical aspects of the HL-A 8-negative and HL-A 2-positive patients were different from those of the HL-A 8-positive and HL-A 2-negative patients justifies the hypothesis that there are two forms of myasthenia gravis.

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Introduction

Though there are some indications that myasthenia gravis occurs in families more often than can be explained by probability, definite conclusions about the role of heredity have been hampered by the low prevalence of the disease (Herrmann, 1971). Wright and Kerr (1967) found no antibodies to skeletal muscle in 86 first-degree relatives of 32 patients. Bundey *et al.* (1972) found these antibodies in 20 out of 149 first-degree relatives of 54 patients with juvenile myasthenia gravis. However, this figure was not significantly higher than that found in their control group. Van den Berg-Loonen *et al.* (1974) showed that people heterozygous for the histocompatibility antigen HL-A 8 have a five times greater chance of acquiring myasthenia gravis than those without the antigen, this chance being 25 times increased for homozygous persons.

This paper reports some results of a study of 100 patients with myasthenia gravis. The prevalence of antibodies to several autologous antigens was determined in the patients, 441 of their first-degree relatives, and 39 spouses. Furthermore, it was investigated whether the correlation of myasthenia gravis with antigens of the HL-A system, found in the group of patients as a whole (Feltkamp *et al.*, 1974; Van den Berg-Loonen *et al.*, 1974), would differ in patients with autoantibodies and a thymoma and in those without. Sex, age, and clinical conditions were taken into account in these correlation studies. The results possibly warrant the division of myasthenia gravis into two types.

Patients and Methods

The group of 100 patients with myasthenia gravis consisted of 76 women and 24 men; the mean age was 41 years. Four had only ocular symptoms, 64 had mild generalized symptoms, 25 had severe generalized symptoms with respiratory difficulties, and 7 had mild generalized symptoms with respiratory difficulties after thymectomy. A thymectomy was performed in 43 cases and a thymomectomy in eight. The diagnosis in all cases was confirmed by one of us (H.J.G.H. O.).

The serum from patients, relatives, and spouses were studied for the presence of autoantibodies to skeletal muscle, thyroid, gastric parietal cells, adrenocortex salivery duct cells, mitochondria, and nuclei by the indirect immunofluorescence technique (Lucas *et al.*, 1972; Feltkamp, 1974). The results were

compared with those obtained with the serum of 580 normal control subjects matched for sex and age. This group consisted of blood donors, inmates of homes for the elderly, and children typed for legitimacy.

The lymphocytes of the patients, relatives, and spouses were typed for HL-A with a standard microlymphocytotoxicity test (Van den Berg-Loonen *et al.*, 1974).

Results

Antibodies to skeletal muscle were found in 22 of the 100 patients and in none of the normal control subjects (table I). They were present in only 8 (2%) of the 441 first-degree relatives. Nevertheless, this was significantly more than the 0·2% of the 441 matched controls ($P<0·05$). None of the eight relatives with antibodies to skeletal muscle showed clinical signs of myasthenia gravis or of a thymoma. When tested on human thyroid tissue antinuclear antibodies were present in the serum of 24% of the patients and in 8% of the first-degree relatives. About the same prevalence of antinuclear antibodies was found when other tissues were used as the antigenic substrate. No antibodies to skeletal muscle were found in the serum of the spouses, nor was the prevalence of other autoantibodies significantly increased. Except for the expected relation between the presence of autoantibodies against parietal cells and antithyroid antibodies (Doniach and Roitt, 1964), no mutual relation between the presence of autoantibodies of other specificities was found.

TABLE I—*Prevalence of Autoantibodies in Patients with Myasthenia Gravis, their Relatives, and Matched Controls. Results expressed as a Percentage of Subjects*

	Patients (n = 100)	Controls (n = 100)	First-degree Relatives (n = 441)	Controls (n = 441)
Antibodies to:				
skeletal muscle	22*	0	2†	0·2
thyroid colloid	8	2	4	4
thyroid cytoplasm	9	3	6	4
thyroid colloid or cytoplasm	14	5	9	7
gastric parietal cells	6†	0	4	3
adrenocortex	3	0	0	0
salivary duct cells	2	1	0·2	0
smooth muscle	3	1	1	0·4
mitochondria	0	0	1	0·4
Antinuclear antibodies on:				
human thyroid	24*	2	8*	2
one or more substrates	44*	4	14*	4
all substrates	6	1	1	0

*Significantly increased ($P<0·001$) when compared with controls.
†Significantly increased ($P<0·05$) when compared with controls.

Some deviations from the normal distribution of HL-A antigens were found among the patients. HL-A 8 was present in only 33% of the 24 men but in 67% of the 76 women ($P<0·01$). HL-A 7, however, was found in 33% of the men and in only 9% of the women ($P<0·02$). The prevalence of HL-A 8 in the patients with antibodies to skeletal muscle or with a thymoma was significantly lower than that in patients without these symptoms. This was also the case in patients aged 40 years or over versus patients aged under 40 years (table II). In the older group the prevalence of HL-A 2 was significantly higher and that of HL-A 1 was significantly lower than in the younger group without antibodies to skeletal muscle or a thymoma.

Patients with myasthenia gravis and a thymoma nearly always have antibodies to skeletal muscle. In the present study they were found in 12 out of the 13 patients with a thymoma. The only patient with a thymoma whose serum did not react strongly enough with striated muscle to be called positive at the time of this study had had the antibodies five years previously. In only two out of 43 patients in whom the presence of a thymoma could be excluded by thymectomy were antibodies to skeletal muscle present.

The mean age of the 22 patients with antibodies to skeletal muscle was 52 years, that of the 13 patients with a thymoma was 50 years. Since the mean age of 77 patients without antibodies to skeletal muscle or a proved thymoma was only 38 years it was not clear whether the discrepant HL-A 8 and HL-A 2 distribution was linked to age or to the presence of autoantibodies or of a thymoma. Even in patients aged 40 or over without antibodies to skeletal muscle or a proved thymoma, however, HL-A 8 was present in only two out of the nine cases, in contrast to 27 out of 32 such patients aged under 40 ($P<0·01$). HL-A 2 was found in seven of these patients aged 40 or over and in only 10 aged u der 40 ($P<0·01$).

TABLE II—*Prevalence of HL-A 8 and HL-A 2 in Patients and in Subgroups of Patients with Myasthenia Gravis*

Patients and Subgroups	Number	HL-A 8		HL-A 2	
		%	P<	%	P<
M.G. patients	100	59	0·001	58	N.S.
Normal population	533	19		53	
M.G. with antibodies to muscle	22	27	0·01	82	0·05
M.G. without antibodies to muscle	78	68		51	
M.G. with thymoma	13	15	0·001	85	0·05
M.G. without thymoma	43*	72		44	
M.G. ⩾ 40 years	52	40	0·001	69	0·05
M.G. < 40 years	48	79		46	

*In 44 patients a thymoma could not be excluded.
N.S. = Not significant.

Since age at the time of study could be biased by several factors we also examined the possible relation between HL-A 8 and HL-A 2 prevalencies and the age of the patient at the onset of the disease (table III). This table shows that HL-A 8 is significantly more prevalent in patients in whom the disease began under the age of 40 years ($P<0·001$) and that HL-A 2 is significantly more prevalent in patients in whom the disease started after that age ($P<0·02$). The question whether the patients with autoantibodies other than those to skeletal muscle showed a prevalence for certain HL-A antigens was also studied. The results (table IV) present only the statistically significant relations ($P<0·05$). It should be noted, however, that a chance significance is not excluded. No such correlations could be found between HL-A antigens and certain clinical conditions—that is, thymus hyperplasia, muscle atrophy, and the duration of the disease.

TABLE III—*Age of Myasthenia Gravis at Onset of Disease in Relation to HL-A 8 and HL-A 2*

Age at Onset	Total	HL-A 8-Positive Patients	HL-A 2-Positive Patients
< 40 years	74	56*	37
⩾ 40 years	26	3	21†

*Significantly increased if compared to patients negative for this antigen ($P<<0·001$)
†Significantly increased if compared to patients negative for this antigen ($P<0·02$).

TABLE IV—*Statistically Significant* ($P<0.05$) *Relations Between HL-A Antigens and Autoantibodies in Myasthenia Gravis Patients*

Autoantibodies Present	Histocompatibility Antigens
Antibodies to:	
skeletal muscle	HL-A 2
parietal cells	W 10
Antinuclear antibodies on:	
rat kidney	HL-A 13 and W 14
rat stomach	HL-A 13 and W 14
human adrenal gland	W 15 and W 14

Because in the whole group of patients significant deviations from the normal prevalence of HL-A antigens were found to be secondary to the greatly increased prevalence of HL-A 8 the possibility that the deviations might also be secondary to the abnormal prevalence of HL-A 8 had to be investigated (Van den

Berg-Loonen *et al.*, 1974). We therefore looked to see whether the same relations were found if the χ^2 calculations were corrected for the effect of the excess of HL-A 8 (Aird *et al.*, 1954). A homogeneity of the distribution of HL-A 7 over the sexes was then still improbable ($P < 0{\cdot}1$). The same was found for HL-A 2 in patients with antibodies to skeletal muscle ($P < 0{\cdot}1$), with a thymoma ($P < 0{\cdot}1$), an age of 40 years or over ($P < 0{\cdot}2$), or an onset of the disease above that age ($P < 0{\cdot}1$), as well as for the relation between W 10 and antibodies to gastric parietal cells ($P < 0{\cdot}1$). The relations between the presence of antinuclear antibodies and the histocompatibility antigens HL-A 13, W 14, and W 15 ($P < 0{\cdot}01$) were not changed by such a correction procedure.

Discussion

Owing to the large number studied in the present investigation it was possible for the first time to show significantly increased prevalence of antibodies to skeletal muscle in first-degree relatives of myasthenia gravis patients. The correlation found between HL-A 8 and myasthenia gravis already pointed to the importance of the genetic constitution for the development of this disease (Pirskainen *et al.*, 1972; Van den Berg-Loonen *et al.*, 1974; Feltkamp *et al.*, 1974). The HL-A 8 influence is probably present mainly in women, since the occurrence of this antigen is significantly higher in female than in male patients.

It is striking that in patients with autoimmune phenomena—that is, antibodies to skeletal muscle or a thymoma or both—the prevalence of HL-A 8 was less, and that of HL-A 2 more than in patients lacking such phenomena. It might therefore be supposed that two forms of myasthenia gravis exist. Firstly, a form which develops especially in women who often have the HL-A 8 antigen, acquire the disease at an early age, and have no antibodies to skeletal muscle and no thymoma. Secondly, a form present in persons in whom HL-A 8 is less prevalent, in whom the onset of the disease is much later, and in whom antibodies to skeletal muscle and a thymoma are found. The HL-A 2 frequency in the second group of patients is much higher than in the first.

With respect to the observation by Kissmeyer-Nielsen *et al.* (1971) that HL-A 8 and HL-A 1 are associated with an increased transplant rejection, which probably indicates a sensitive immune system, it is significant that the myasthenia gravis patients with autoantibodies and a thymoma showed a normal prevalence of HL-A 8.

Idiopathic autoimmune diseases are possibly caused by viral infections (Van Loghem, 1973). The presence of certain HL-A antigens is also supposed to be linked to the degree of susceptibility to certain viral infections, either by providing a cell-membrane receptor site for viruses, or by an antigenic resemblance of certain viral and host cell-membrane antigens leading to a tolerance for the viral antigens (McDevitt and Bodmer, 1972). The concept of two forms of myasthenia gravis does not help to solve the problem of a supposed viral aetiology.

HL-A antigens are cell-membrane antigens, and myasthenia gravis is characterized by an impaired neuromuscular transmission. A certain genetic-determined cell-membrane composition may therefore be especially liable to quantitative shortcomings of neuromuscular transmitters. This proposition might stimulate further studies on the distribution of HL-A antigens present on the synapse and muscular end-plate membranes. The autoimmune traits of myasthenia gravis may be mere epiphenomena (Feltkamp, 1974). We hope that the new arguments for genetic factors being involved in the aetiology of myasthenia gravis may lead to a better understanding of the pathogenesis.

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